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(54) Title: TREATMENT OF A DISEASE OF THE GASTROINTESTINAL TRACT WITH LIVE BIOTHERAPEUTICS

(57) Abrégé/Abstract:
This disclosure features methods and compositions for treating diseases of the gastrointestinal tract with a live biotherapeutic.

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(54) **Title:** TREATMENT OF A DISEASE OF THE GASTROINTESTINAL TRACT WITH LIVE BIOTHERAPEUTICS

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TREATMENT OF A DISEASE OF THE GASTROINTESTINAL TRACT WITH LIVE BIOTHERAPEUTICS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 62/479,027, filed March 30, 2017; U.S. Provisional Patent Application Serial No. 62/545,297, filed August 14, 2017; U.S. Provisional Patent Application Serial No. 62/583,932, filed November 9, 2017; U.S. Provisional Patent Application Serial No. 62/596,040, filed December 7, 2017; and U.S. Provisional Patent Application Serial No. 62/598,948, filed December 14, 2017; each of which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

This disclosure features methods and compositions for treating diseases of the gastrointestinal tract with live biotherapeutics such as populations of live bacteria and/or live yeast, bacteriophages or prophages, stem cells or a medium conditioned by stem cells, or organoids aproduced from stem cells.

BACKGROUND

The gastrointestinal (GI) tract generally provides a therapeutic medium for an individual's body. At times, therapeutic drugs may need to be dispensed to specified locations within the small intestine or large intestine, which is more effective than oral administration of the therapeutic drugs to cure or alleviate the symptoms of some medical conditions. For example, therapeutic drugs dispensed directly within the small intestine would not be contaminated, digested or otherwise compromised in the stomach, and thus allow a higher dose to be delivered at a specific location within the small intestine. However, dispensing therapeutic drugs directly within the small intestine inside a human body (e.g., the cecum, the ascending colon) can be difficult, because a device or mechanism (e.g., special formulation) would be needed to transport a therapeutically effective dose of drug to a desired location within the small intestine and then automatically deliver the therapeutic drug at the desired location. Dispensing therapeutic drugs directly within other locations in the GI tract of the human body can be similarly difficult. Such a device or mechanism also would also need to be operated in a safe manner in that the device or mechanism needs to physically enter the human body.

In sum, there remains a significant unmet medical need for improved treatment regimens for gastrointestinal diseases, such as inflammatory bowel disease (IBD), including a need for

regimens which can dispense therapeutics to specific locations within the GI tract, thereby reducing or avoiding the drawbacks of oral or other forms of systemic administration.

As described herein, diseases of the gastrointestinal (GI) tract can be treated with live cell therapies that include populations of live bacteria and/or live yeast, bacteriophages or prophages, stem cells or a medium conditioned by stem cells, or organoids produced from stem cells.

SUMMARY

The present disclosure provides novel treatment paradigms for inflammatory conditions of the gastrointestinal tract. The methods and compositions described herein allow for the regio-specific release of therapeutic drugs at or near the site of disease in the gastrointestinal tract. By releasing a therapeutic drug locally instead of systemically, the bioavailability of the drug can be increased at the site of injury and/or decreased in the systemic circulation, thereby resulting in improved overall safety and/or efficacy and fewer adverse side effects. Advantages may include one or more of increased drug engagement at the target, leading to new and more efficacious treatment regimens, and/or lower systemic drug levels, which can translate to reduced toxicity and reduced immunogenicity, e.g., in the case of biologics. In some instances, releasing a therapeutic drug locally also provides for new modes of action that may be unique to local delivery in the GI tract as opposed to systemic administration. For patients and clinicians, this can mean an easier or simpler route of administration, fewer co-medicaments (e.g., immunomodulators), fewer side effects, and/or better outcomes.

For example, a patient may present to a physician with one or more symptoms of a disorder of the GI tract (e.g., inflamatory bowel disease), and the physician can determine the specific discrete location(s) of diseased tissue (e.g., inflamed tissue or a lesion) in the patient's GI tract, and then use any of the devices described herein to administer a therapeutically effective amount of a live biotherapeutic proximate to, proximal to, or directly onto the specific discrete location(s) of diseased tissue in the patient.

In other examples, a patient may present to a physician with one or more symptoms of a disorder of the GI tract (e.g., inflamatory bowel disease) and the physician can use any of the devices provided herein to identify the specific discrete location(s) of diseased tissue (e.g., inflamed tissue or a lesion) in the pateint's GI tract, and then use the same device or a different device (e.g., any of the devices described herein) to administer a therapeutically effective amount of a live biotherapeutic proximate to, proximal to, or directly onto the specific discrete locations of diseased tissue in the patient.

As can be appreciated by those in the art, these methods may be performed periodically on a patient at periodic intervals, e.g., approximately twice a month, approximately once a month, approximately every two months, approximately every three months, approximately four months, approximately five months, or approximately every six months. In some examples, these methods can provide for increased efficacy of treatment (e.g., reduced negative side effects and/or increased reduction in the severity, frequency, or number of symptoms) as compared to a patient which is administered an oral dosage form of the same live biotherapeutic. In some embodiments, the dosage of the live biotherapeutic administered using any of the devices described herein can differ between the different clinical visits based on an observation or measurement of the severity of disease at the specific discrete location(s) of diseased tissue (e.g., inflamed tissue or a lesion) in the pateint's GI tract at the time of each clinical visit, or based on one or more observations or measurements of systemic disease markers (e.g., inflammatory markers in the blood) or markers in stool (e.g., calprotectin and lactoferrin). In some examples, over time, new specific discrete location(s) of diseased tissue may be detected or observed in the patient, and any of the devices described herein can be used to administer a therapeutically effective amount of a live biotherapeutic onto or proximal to the new specific discrete location(s) of diseased tissue in the patient's GI tract.

In some examples, the identification of the specific discrete location(s) of diseased tissue (e.g., inflamed tissue or a lesion) in the patient's GI tract and the administration of a therapeutically effective amount of a live biotherapeutic using any of the devices described herein can be performed in a single clinical visit.

In some examples, the diagnosis of a disorder of the GI tract (e.g., irritable bowel syndrome), the identification of the specific discrete location(s) of diseased tissue (e.g., inflamed tissue or a lesion) in the pateint's GI tract, and the administration of a therapeutically effective amount of a live biotherapeutic proximate to, proximal, to or directly onto the specific discrete locations of diseased tissue in the patient using any of the devices described herein, can be performed in a single clinical visit.

Accordingly, described herein are methods for treating disorders of the gastrointestinal (GI) tract. The methods can include one or more of:

- diagnosing a GI disease in a subject; and/or
- mapping, sampling, and/or assessing the site, severity, pathology, and extent of a GI disease in the GI tract of a subject and/or mapping, sampling, and/or assessing a patient response to a therapeutic agent, e.g., in the patient's GI tract; and/or

- identifying, quantifying, and/or monitoring one or more markers of a GI disease in the GI tract of the subject and/or one or more markers of patient response to a therapeutic agent, e.g., in the patient's GI tract; and/or
- releasing a therapeutic agent, e.g., proximate to the site of a GI disease.

The present disclosure accordingly provides patients and physicians more personalized treatment options for GI disorders by facilitating regimens which can release a therapeutic agent according to desired (e.g., customized or optimized) dosage, timing, and/or location parameters. In some cases, the treatment methods can employ one or more ingestible devices to achieve the benefits disclosed herein.

In some embodiments, provided herein is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

administering to the subject a pharmaceutical formulation that comprises a live biotherapeutic such as a stem cell or other therapeutic described herein such as an enzyme, glycoprotein, or ErbB4 receptor tyrosine kinase agonist,

wherein the pharmaceutical formulation is released at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In some embodiments, provided herein the pharmaceutical formulation is administered in an ingestible device. In some embodiments, the pharmaceutical formulation is released from an ingestible device. In some embodiments, the ingestible device comprises a housing, a reservoir containing the pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device,

wherein the reservoir is releasably or permanently attached to the exterior of the housing or internal to the housing.

In some embodiments, provided herein is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

administering to the subject an ingestible device comprising a housing, a reservoir containing a pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device,

wherein the reservoir is releasably or permanently attached to the exterior of the housing or internal to the housing;

wherein the pharmaceutical formulation comprises a live biotherapeutic such as a stem cell or other therapeutic described herein such as an enzyme, glycoprotein, or ErbB4 receptor tyrosine kinase agonist, and

the ingestible device releases the pharmaceutical formulation at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In some embodiments, the housing is non-biodegradable in the GI tract.

In some embodiments, the release of the formulation is triggered autonomously. In some embodiments, the device is programmed to release the formulation with one or more release profiles that may be the same or different at one or more locations. In some embodiments, the device is programmed to release the formulation at a location proximate to one or more sites of disease. In some embodiments, the location of one or more sites of disease is predetermined.

In some embodiments, the reservoir is made of a material that allows the formulation to leave the reservoir, such as a biodegradable material.

In some embodiments, the release of the formulation is triggered by a pre-programmed algorithm. In some embodiments, the release of the formulation is triggered by data from a sensor or detector to identify the location of the device. In some more particular embodiments, the data is not based solely on a physiological parameter (such as pH, temperature, and/or transit time).

In some embodiments, the device comprises a detector configured to detect light reflectance from an environment external to the housing. In some more particular embodiments, the release is triggered autonomously or based on the detected reflectance.

In some embodiments, the device releases the formulation at substantially the same time as one or more sites of disease are detected. In some embodiments, the one or more sites of disease are detected by the device (e.g., by imaging the GI tract).

In some embodiments, the release mechanism is an actuation system. In some embodiments, the release mechanism is a chemical actuation system. In some embodiments, the release mechanism is a mechanical actuation system. In some embodiments, the release mechanism is an electrical actuation system. In some embodiments, the actuation system comprises a pump and releasing the formulation comprises pumping the formulation out of the reservoir. In some embodiments, the actuation system comprises a gas generating cell.

In some embodiments, the device further comprises an anchoring mechanism. In some embodiments, the formulation comprises a therapeutically effective amount of the live biotherapeutic such as a stem cell. In some embodiments, the formulation comprises a human equivalent dose (HED) of the live biotherapeutic such as a stem cell.

In some embodiments, the device is a device capable of releasing a solid formulation comprising the live biotherapeutic such as a stem cell. In some embodiments, the device is a

device capable of releasing a liquid formulation comprising the live biotherapeutic such as a stem cell. Accordingly, in some embodiments of the methods herein, the pharmaceutical formulation released from the device is a solid formulation. Accordingly, in some embodiments of the methods herein, the pharmaceutical formulation released from the device is a liquid formulation.

The devices disclosed herein are capable of releasing a live biotherapeutic such as a stem cell or a formulation comprising the live biotherapeutic such as a stem cell irrespective of the particular type of live biotherapeutic such as a stem cell.

In some embodiments, provided herein is a method of releasing a live biotherapeutic such as a stem cell into the gastrointestinal tract of a subject for treating one or more sites of disease within the gastrointestinal tract, the method comprising:

administering to the subject a therapeutically effective amount of the live biotherapeutic such as a stem cell housed in an ingestible device, wherein the ingestible device comprises

a detector configured to detect the presence of the one or more sites of disease, and

a controller or processor configured to trigger the release of the live biotherapeutic such as a stem cell proximate to the one or more sites of disease in response to the detector detecting the presence of the one or more sites of disease.

In some embodiments, provided herein is a method of releasing a live biotherapeutic such as a stem cell into the gastrointestinal tract of a subject for treating one or more pre-determined sites of disease within the gastrointestinal tract, the method comprising:

administering to the subject a therapeutically effective amount of the live biotherapeutic such as a stem cell contained in an ingestible device, wherein the ingestible device comprises

a detector configured to detect the location of the device within the gastrointestinal tract, and

a controller or processor configured to trigger the release of the live biotherapeutic such as a stem cell proximate to the one or more predetermined sites of disease in response to the detector detecting a location of the device that corresponds to the location of the one or more pre-determined sites of disease.

In some embodiments, provided herein is a method of releasing a live biotherapeutic such as a stem cell into the gastrointestinal tract of a subject for treating one or more sites of disease within the gastrointestinal tract, the method comprising:

administering to the subject a therapeutically effective amount of the live biotherapeutic such as a stem cell contained in an ingestible device;

receiving at an external receiver from the device a signal transmitting environmental data;

assessing the environmental data to confirm the presence of the one or more sites of disease; and

when the presence of the one or more sites of disease is confirmed, sending from an external transmitter to the device a signal triggering the release of the live biotherapeutic such as a stem cell proximate to the one or more sites of disease.

In some embodiments, provided herein is a method of releasing a live biotherapeutic such as a stem cell into the gastrointestinal tract of a subject for treating one or more sites of disease within the gastrointestinal tract, the method comprising:

administering to the subject a therapeutically effective amount of the live biotherapeutic such as a stem cell contained in an ingestible device;

receiving at an external receiver from the device a signal transmitting environmental or optical data;

assessing the environmental or optical data to confirm the location of the device within the gastrointestinal tract; and

when the location of the device is confirmed, sending from an external transmitter to the device a signal triggering the release of the live biotherapeutic such as a stem cell proximate to the one or more sites of disease.

Provided herein in one embodiment is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

delivering a live biotherapeutic such as a stem cell at a location in the gastrointestinal tract of the subject,

wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic such as the stem cell.

Provided herein in one embodiment is a method of treating a disease of the large intestine in a subject, comprising:

delivering a live biotherapeutic such as a stem cell at a location in the proximal portion of the large intestine of the subject,

wherein the method comprises administering endoscopically to the subject a therapeutically effective amount of the live biotherapeutic such as the stem cell.

Provided herein in one embodiment is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic such as a stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease,

wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic such as the stem cell.

Provided herein in one embodiment is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic such as a stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease,

wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic such as the stem cell, wherein the pharmaceutical composition is an ingestible device. and the method comprises administering orally to the subject the pharmaceutical composition.

Provided herein in one embodiment is a method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic such as a stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic such as the stem cell, wherein the method provides a concentration of the live biotherapeutic such as the stem cell in the plasma of the subject that is less than 3 μg/ml.

Provided herein in one embodiment is a method of treating a disease of the large intestine in a subject, comprising:

releasing a live biotherapeutic such as a stem cell at a location in the proximal portion of the large intestine of the subject that is proximate to one or more sites of disease, wherein the method comprises administering endoscopically to the subject a therapeutically effective amount of the live biotherapeutic such as the stem cell.

In another aspect of the present invention, there is provided a live biotherapeutic such as a stem cell for use in a method of treating a disease of the gastrointestinal tract in a subject, wherein the method comprises orally administering to the subject an ingestible device loaded with the live biotherapeutic such as the stem cell, wherein the live biotherapeutic is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In another aspect, the present invention provides a composition comprising or consisting of an ingestible device loaded with a therapeutically effective amount of a live biotherapeutic such as a stem cell, for use in a method of treatment, wherein the method comprises orally

administering the composition to the subject, wherein the live biotherapeutic such as the stem cell is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In another aspect, the present invention provides an ingestible device loaded with a therapeutically effective amount of a live biotherapeutic such as a stem cell, wherein the device is controllable to release the live biotherapeutic such as the stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease. The device may be for use in a method of treatment of the human or animal body, for example, any method as described herein.

In still another aspect, the present invention provides an ingestible device for use in a method of treating a disease of the gastrointestinal tract in a subject, wherein the method comprises orally administering to the subject the ingestible device loaded with a therapeutically effective amount of a live biotherapeutic such as stem cell, wherein the live biotherapeutic such as the stem cell is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

An ingestible device as used in the present invention may comprise one or more mechanical and/or electrical mechanisms which actively control release of the live biotherapeutic such as a stem cell. For example, in any of the above aspects and embodiments, the ingestible device as used in the present invention may comprise a release mechanism for release of the live biotherapeutic such as a stem cell (e.g., from a reservoir comprising the stem cell) and an actuator controlling the release mechanism.

In one embodiment, the ingestible device comprises:

an ingestible housing comprising a reservoir having a therapeutically effective amount of the live biotherapeutic such as the stem cell stored therein;

a release mechanism having a closed state which retains the live biotherapeutic such as the stem cell in the reservoir and an open state which releases the live biotherapeutic from the reservoir to the exterior of the device; and

an actuator which changes the state of the release mechanism from the closed to the open state.

In one embodiment, the ingestible device comprises

a housing defined by a first end, a second end substantially opposite from the first end;

a reservoir located within the housing and containing the live biotherapeutic such as the stem cell wherein a first end of the reservoir is attached to the first end of the housing;

a mechanism for releasing the live biotherapeutic such as the stem cell from the reservoir; and

an exit valve configured to allow the live biotherapeutic such as the stem cell to be released out of the housing from the reservoir.

Here, the exit valve can be considered as the release mechanism having a closed state which retains the live biotherapeutic such as the stem cell in the reservoir and an open state which releases the live biotherapeutic such as the stem cell from the reservoir to the exterior of the device, and the mechanism for releasing the live biotherapeutic such as the stem cell from the reservoir can be considered as the actuator.

In some embodiments of methods of treatment as described herein, the one or more disease sites may have been pre-determined (e.g., determined in a step preceding the administration of the composition of the present invention). The disease site(s) may have been determined by imaging the gastrointestinal tract. For example, the disease site(s) may have been pre-determined by endoscopy (e.g., a step of colonoscopy, enteroscopy, or using a capsule endoscope). Determination that the device is proximate to the disease site may therefore comprise a determining that the device is in a location corresponding to this previously-determined disease site.

In some embodiments, the location of the device in the gut may be detected by tracking the device. For example, the device may comprise a localization mechanism which may be a communication system for transmitting localization data, e.g., by radiofrequency transmission. The device may additionally or alternatively comprise a communication system for receiving a signal remotely triggering the actuator and thus causing release of the stem cell. The signal may be sent when it is determined that the device is in the correct location in the gut.

Thus, the ingestible device may comprise:

an ingestible housing comprising a reservoir having a therapeutically effective amount of the live biotherapeutic such as the stem cell stored therein;

a release mechanism having a closed state which retains the live biotherapeutic such as the stem cell in the reservoir and an open state which releases the live biotherapeutic such as the stem cell from the reservoir to the exterior of the device;

a communication system for transmitting localization data to an external receiver and for receiving a signal from an external transmitter; and

an actuator which changes the state of the release mechanism from the closed to the open state and which can be triggered by the signal.

In other embodiments, the ingestible device as used in the present invention may comprise an environmental sensor for detecting the location of the device in the gut and/or for detecting the presence of disease in the GI tract. For example, the environment sensor may be an image sensor for obtaining images *in vivo*.

Detecting the presence of disease may comprise, for example, detecting the presence of inflamed tissue, and/or lesions such as ulceration e.g., aphthoid ulcerations, "punched-out ulcers" and/or superficial ulcers of the mucosa, cobblestoning, stenosis, granulomas, crypt abscesses, fissures, e.g., extensive linear fissures, villous atrophy, fibrosis, and/or bleeding.

Detecting the presence of disease may also comprise molecular sensing, such as detecting the amount of an inflammatory cytokine or other marker of inflammation. Such a marker can be measured locally from a biopsy or systemically in the serum.

Where the ingestible device comprises an environmental sensor, actuation of the release mechanism may be triggered by a processor or controller communicably coupled to the environmental sensor. Thus, in some embodiments, the device may not require any external signal or control in order to release the drug.

In one embodiment, the ingestible device may comprise:

an ingestible housing comprising a reservoir having a therapeutically effective amount of the live biotherapeutic such as the stem cell stored therein;

a release mechanism having a closed state which retains the live biotherapeutic such as the stem cell in the reservoir and an open state which releases the live biotherapeutic such as the stem cell from the reservoir to the exterior of the device;

an actuator which controls the transition of the release mechanism from the closed to the open state;

a detector for detecting the location of the device in the gut and/or the presence of diseased tissue; and

a processor or controller which is coupled to the detector and to the actuator and which triggers the actuator to cause the release mechanism to transition from its closed state to its open state when it is determined that the device is in the presence of diseased tissue and/or in a location in the gut that has been predetermined to be proximal to diseased tissue.

In another embodiment, there is provided:

an ingestible housing comprising a reservoir having a therapeutically effective amount of the live biotherapeutic such as the stem cell stored therein;

a detector coupled to the ingestible housing, the detector configured to detect when the ingestible housing is proximate to a respective disease site of the one of the one or more sites of disease;

a valve system in fluid communication with the reservoir system; and

a controller communicably coupled to the valve system and the detector, the controller configured to cause the valve system to open in response to the detector detecting that the ingestible housing is proximate to the respective disease site so as to release the therapeutically effective amount of the live biotherapeutic such as the stem cell at the respective disease site.

As above, detection that the ingestible housing is proximate to the respective disease site may be based on environmental data indicating the location of the device in the GI tract (and reference to a pre-determined disease site) or on environmental data directly indicating the presence of diseased tissue.

Additionally, or alternatively, the device may further comprise a communication system adapted to transmit the environment data to an external receiver (e.g., outside of the body). This data may be used, for example, for diagnostic purposes. The external receiver may comprise means for displaying the data.

In some embodiments, this data may be analyzed externally to the device and used to determine when the drug should be released: an external signal may then be sent to the device to trigger release of the drug. Thus, the communication system may further be adapted to receive a signal remotely triggering the actuator and thus causing release of the live biotherapeutic such as the stem cell. The signal may be sent from an external transmitter in response to receipt/analysis and/or assessment of the environmental data, e.g., data indicating that the device has reached the desired location of the gut (where the location of the diseased tissue has been pre-determined) and/or data indicating the presence of diseased tissue. "External" may be "outside of the body".

Thus, in another embodiment, the ingestible device may comprise:

an ingestible housing comprising a reservoir having a therapeutically effective amount of the live biotherapeutic such as the stem cell stored therein;

a release mechanism having a closed state which retains the live biotherapeutic such as the stem cell in the reservoir and an open state which releases the live biotherapeutic such as the stem cell from the reservoir to the exterior of the device;

an environmental detector for detecting environmental data indicating the location of the device in the gut and/or the presence of diseased tissue;

a communication system for transmitting the environmental data to an external receiver and for receiving a signal from an external transmitter; and

an actuator which controls the transition of the release mechanism from the closed to the open state in response to the signal.

It will be understood from the above that when the device comprises one or more environmental detectors, e.g., comprises an image detector, the compositions may be used both for disease detection and for disease treatment.

Accordingly, in a further embodiment, there is provided a live biotherapeutic such as a stem cell for use in a method of detecting and treating a disease of the gastrointestinal tract in a subject, wherein the method comprises orally administering to the subject an ingestible device loaded with the live biotherapeutic such as the stem cell, wherein the ingestible device comprises an environmental sensor for determining the presence of diseased tissue in the GI tract, and wherein the live biotherapeutic such as the stem cell is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, as detected by the environmental sensor. The device may be according to any of the embodiments described herein.

In another embodiment, there is provided a composition for use in a method of detecting and treating a disease of the gastrointestinal tract in a subject, wherein the composition comprises or consists of an ingestible device loaded with a therapeutically effective amount of a live biotherapeutic such as a stem cell, wherein the ingestible device comprises an environmental sensor for determining the presence of diseased tissue in the GI tract, and wherein the live biotherapeutic such as a stem cell is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, as detected by the environmental sensor. Again, the device may be according to any of the embodiments described herein.

In some embodiments, where the ingestible device as used in the present invention comprises an environmental sensor for detecting the presence of disease in the GI tract and a communication system as described above, the method of treatment may comprise:

i) receiving at an external receiver from the ingestible device a signal transmitting the environmental data;

ii) assessing the environmental data to confirm the presence of the disease; and

iii) when the presence of the disease is confirmed, sending from an external transmitter to the ingestible device a signal triggering release of the live biotherapeutic such as a stem cell.

For example, the presence of disease may be confirmed based on the presence of inflamed tissue and/or lesions associated with any of the disease states referred to herein. For example, the presence of disease may be confirmed based on the presence of inflammation, ulceration e.g., aphthoid ulcerations, "punched-out ulcers" and/or superficial ulcers of the mucosa, cobblestoning, stenosis, granulomas, crypt abscesses, fissures, e.g., extensive linear fissures, villous atrophy, fibrosis, and/or bleeding.

In some embodiments, the present invention may relate to a system comprising:

an ingestible device loaded with a therapeutically effective amount of a live biotherapeutic such as a stem cell, a release mechanism for release of the live biotherapeutic such as the stem cell (e.g., from a reservoir comprising the stem cell), an actuator controlling the release mechanism, an environmental sensor for determining the location of the device in the gut and/or for detecting the presence of diseased tissue and a communication system adapted to transmit the environment data and receive a signal triggering the actuator;

a receiver and display module for receiving and displaying outside of the body the environment data from the ingestible device;

a transmitter for sending to the ingestible device a signal triggering the actuator.

In any of the above embodiments, the ingestible device may further comprise an anchoring system for anchoring the device or a portion thereof in a location and an actuator for the anchoring system. This may be triggered in response to a determination that the device is at a location in the gastrointestinal tract of the subject proximate to one or more sites of disease. For instance, this may be detected by the environmental sensor. The triggering may be controlled by a processor in the device, that is, autonomously. A device where the triggering is controlled by a processor in the device is said to be an autonomous device. Alternatively, it may be controlled by a signal sent from outside of the body, as described above.

In any of the above aspects and embodiments, disease of the GI tract may be an inflammatory bowel disease.

In some embodiments, the disease of the GI tract is ulcerative colitis.

In some embodiments, the disease of the GI tract is Crohn's disease.

In general, apparatuses, compositions, and methods disclosed herein are useful in the treatment of diseases of the gastrointestinal tract. Exemplary gastrointestinal tract diseases that can be treated include, without limitation, inflammatory bowel disease (IBD), Crohn's disease (e.g., active Crohn's disease, refractory Crohn's disease, or fistulizing Crohn's disease), ulcerative colitis, indeterminate colitis, microscopic colitis, infectious colitis, drug or chemical-

induced colitis, diverticulitis, and ischemic colitis, gastritis, peptic ulcers, stress ulcers, bleeding ulcers, gastric hyperacidity, dyspepsia, gastroparesis, Zollinger-Ellison syndrome, gastroesophageal reflux disease, short-bowel (anastomosis) syndrome, a hypersecretory state associated with systemic mastocytosis or basophilic leukemia or hyperhistaminemia, Celiac disease (e.g., nontropical Sprue), enteropathy associated with seronegative arthropathies, microscopic colitis, collagenous colitis, eosinophilic gastroenteritis, colitis associated with radiotherapy or chemotherapy, colitis associated with disorders of innate immunity as in leukocyte adhesion deficiency-1, chronic granulomatous disease, food allergies, gastritis, infectious gastritis or enterocolitis (e.g., Helicobacter pylori-infected chronic active gastritis), other forms of gastrointestinal inflammation caused by an infectious agent, pseudomembranous colitis, hemorrhagic colitis, hemolytic-uremic syndrome colitis, diversion colitis, irritable bowel syndrome, irritable colon syndrome, and pouchitis.

In some embodiments, apparatuses, compositions, and methods disclosed herein are used to treat one gastrointestinal disease. In some embodiments, apparatuses, compositions, and methods disclosed herein are used to treat more than one gastrointestinal disease. In some embodiments, apparatuses, compositions, and methods disclosed herein are used to treat multiple gastrointestinal diseases that occur in the same area of the gastrointestinal tract (e.g., each disease can occur in the small intestine, large intestine, colon, or any sub-region thereof). In some embodiments, apparatuses, compositions, and methods disclosed herein are used to treat multiple gastrointestinal diseases that occur in different areas of the gastrointestinal tract. In some embodiments, administration (e.g., local administration to the gastrointestinal tract) of live biotherapeutic such as a stem cell is useful in the treatment of gastrointestinal diseases including, but not limited to, inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, or any of the other gastrointestinal diseases described herein.

Aspects and embodiments as described herein are intended to be freely combinable. For example, any details or embodiments described herein for methods of treatment apply equally to a live biotherapeutic such as a stem cell, composition or ingestible device for use in said treatment. Any details or embodiments described for a device apply equally to methods of treatment using the device, or to a live biotherapeutic such as a stem cell or composition for use in a method of treatment involving the device.

Brief Description of the Drawings

FIG. 1 is a view of an example embodiment of an ingestible device, in accordance with some embodiments of the disclosure;

FIG. 2 is an exploded view of the ingestible device of FIG. 1, in accordance with some embodiments of the disclosure;

FIG. 3 is a diagram of an ingestible device during an example transit through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 4 is a diagram of an ingestible device during an example transit through a jejunum, in accordance with some embodiments of the disclosure;

FIG. 5 is a flowchart of illustrative steps for determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 6 is a flowchart of illustrative steps for detecting transitions from a stomach to a duodenum and from a duodenum back to a stomach, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 7 is a plot illustrating data collected during an example operation of an ingestible device, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 8 is another plot illustrating data collected during an example operation of an ingestible device, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 9 is a flowchart of illustrative steps for detecting a transition from a duodenum to a jejunum, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 10 is a plot illustrating data collected during an example operation of an ingestible device, which may be used when detecting a transition from a duodenum to a jejunum, in accordance with some embodiments of the disclosure;

FIG. 11 is a plot illustrating muscle contractions detected by an ingestible device over time, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 12 is a flowchart of illustrative steps for detecting a transition from a jejenum to an ileum, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 13 is a flowchart of illustrative steps for detecting a transition from a jejenum to an ileum, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 14 is a flowchart of illustrative steps for detecting a transition from an ileum to a cecum, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 15 is a flowchart of illustrative steps for detecting a transition from a cecum to a colon, which may be used when determining a location of an ingestible device as it transits through a GI tract, in accordance with some embodiments of the disclosure;

FIG. 16 illustrates an ingestible device for delivering a substance in the GI tract;

FIG. 17 illustrates aspects of a mechanism for an ingestible device with a gas generating cell configured to generate a gas to dispense a substance;

FIG. 18 illustrates an ingestible device having a piston to push for drug delivery;

FIG. 19 illustrates an ingestible device having a bellow structure for a storage reservoir of dispensable substances;

FIG. 20 illustrates an ingestible device having a flexible diaphragm to deform for drug delivery;

FIG. 21 shows an illustrative embodiment of an ingestible device with multiple openings in the housing;

FIG. 22 shows a highly cross-section of an ingestible device including a valve system and a sampling system;

FIG. 23 illustrates a valve system;

FIGs. 24A and 24B illustrate a portion of a two-stage valve system in its first and second stages, respectively;

FIGs. 25A and 25B illustrate a portion of a two-stage valve system in its first and second stages, respectively;

FIGs. 26A and 26B illustrate a portion of a two-stage valve system in its first and second stages, respectively;

FIG. 27 illustrates a more detailed view of an ingestible device including a valve system and a sampling system;

FIG. 28 illustrates a portion of an ingestible device including a sampling system and a two-stage valve system in its second stage; and

FIG. 29 is a highly schematic illustrate of an ingestible device.

FIG. 30 is a graph showing the percentage (%) change in body weight at day 14 (± SEM) for DSS mice treated with anti-IL-12 p40 antibody intraperitoneally (10 mg/kg) every third day (Q3D) or intracecally (10 mg/kg or 1 mg/kg) daily (QD), when compared to mice treated with anti-IL-12 p40 antibody intraperitoneally (10 mg/kg) every third day (Q3D) and vehicle control (Vehicle). Mann-Whitney's U¬- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 31 is a graph showing the concentration of anti-IL-12 p40 rat IgG2A (µg/mL) in plasma of anti-IL-12 p40 intraperitoneally (10 mg/kg) and intracecally (10 mg/kg and 1 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D) when compared to vehicle control (Vehicle) and when IP is compared to IC. ELISA analysis was used to determine the concentration of anti-IL-12 p40 (IgG2A). Data presented as mean ± SEM. Mann-Whitney's U¬- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 32 is a graph showing the concentration of anti-IL-12 p40 antibody (IgG2A) (µg/mL) in the cecum and colon content of anti-IL-12 p40 antibody intraperitoneally (10 mg/kg) and intracecally (10 mg/kg and 1 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D), when compared to vehicle control (Vehicle) and when IP is compared to IC. ELISA analysis was used to determine the concentration of rat IgG2A. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 33 is a graph showing the mean overall tissue immunolabel scores (intensity and extent) in acute DSS colitis mouse colon of anti-IL-12 p40 antibody intracecally-treated versus vehicle control-treated DSS mice. Data presented as mean ± SEM.

FIG. 34 is a graph showing the mean location-specific immunolabel scores in acute DSS colitis mouse colon of anti-IL-12 p40 intracecally-treated versus vehicle control-treated DSS mice. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 35 is a graph showing the ratio of anti-IL-12 p40 antibody in the colon tissue to the plasma concentration of the anti-IL-12 p40 antibody in mice treated with the anti-IL-12 p40

antibody on day 0 (Q0) or day 3 (Q3D) of the study, when measured at the same time point after the initial dosing. An outlier animal was removed from Group 5.

FIG. 36 is a graph showing the concentration of Il-1β (μg/mL) in colon tissue lysate of acute DSS colitis mice treated with anti-IL-12 p40 intraperitoneally (10 mg/kg) every third day (Q3D) or intracecally (10 mg/kg or 1 mg/kg) administered daily (QD), when compared to vehicle control (Vehicle). Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 37 is a graph showing the concentration of Il-6 (μg/mL) in colon tissue lysate of acute DSS colitis mice treated with anti-IL-12 p40 intraperitoneally (10 mg/kg) every third day (Q3D) or intracecally (10 mg/kg or 1 mg/kg) administered daily (QD), when compared to vehicle control (Vehicle). Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.

FIG. 38 is a graph showing the concentration of Il-17A (μg/mL) in colon tissue lysate of acute DSS colitis mice treated with anti-IL-12 p40 intraperitoneally (10 mg/kg) every third day (Q3D) or intracecally (10 mg/kg and 1 mg/kg) administered daily (QD), when compared to vehicle control (Vehicle). Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 39 is a graph showing the percentage (%) change in body weight at day 14 (± SEM) for DSS mice treated with DATK32 (anti-α4β7) antibody intraperitoneally (25 mg/kg) every third day (Q3D) or intracecally (25 mg/kg or 5 mg/kg) administered daily (QD), when compared to vehicle control (Vehicle) and when IC is compared to IP. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 40 is a graph showing the plasma concentration of DATK32 rat IgG2A (μg/mL) of intraperitoneally (25mg/kg) and intracecally (25 mg/kg and 5 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D), where IP is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 41 is a graph showing the concentration of DATK32 rat IgG2A antibody (μg/mL) in cecum and colon content of intraperitoneally (25mg/kg) or intracecally (25 mg/kg and 5 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D), where IP is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 42 is a graph showing the concentration of DATK32 rat IgG2A (μg/mL) in the colon content of intraperitoneally (25mg/kg) or intracecally (25 mg/kg and 5 mg/kg) administered treatment groups given daily (QD), and concentration over time (1, 2 ,4, 24, and 48 hours), where IP is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p<0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 43 is a graph showing the concentration of DATK32 rat IgG2A (μg/g) in colon tissue of intraperitoneally (25mg/kg) or intracecally (25 mg/kg and 5 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D), where IP is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p<0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 44 is a graph showing the concentration of DATK32 rat IgG2A (μg/g) in the colon tissue of intraperitoneally (25mg/kg) or intracecally (25 mg/kg and 5 mg/kg) administered treatment groups given daily (QD), and the concentration over time (1, 2, 4, 24, and 48 hours) was determined, where IP is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 45 is a graph showing the mean overall tissue immunolabel scores (intensity and extent) in acute DSS colitis mouse colon of DATK32 (anti-α4β7) antibody treated versus vehicle control (Vehicle) treated DSS mice. The data are presented as mean ± SEM.

FIG. 46 is a graph showing the mean location-specific immunolabel scores in acute DSS colitis mouse colon of DATK32 (anti-α4β7) antibody-treated versus vehicle control (Vehicle)-treated DSS mice. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 47 is a graph showing the ratio of the DATK-32 antibody in the colon tissue to the plasma concentration of the DATK-32 antibody in mice treated with the DATK-32 antibody on day 0 (Q0) or day 3 (Q3D) of the study (Groups 9-12), when measured after initial dosing.

FIG. 48 is a graph showing the mean percentage of Th memory cells (mean ± SEM) in blood for DATK32 (anti-α4β7) antibody intraperitoneally (25mg/kg) or intracecally (25 mg/kg or 5 mg/kg) administered treatment groups given daily (QD) or every third day (Q3D), when compared to vehicle control (Vehicle) and when IP is compared to IC. Mean percentage Th memory cells were measured using FACS analysis. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 49 is an exemplary image of a histological section of a distal transverse colon of Animal 1501 showing no significant lesions (i.e., normal colon).

FIG. 50 is an exemplary image of a histological section of a distal transverse colon of Animal 2501 (treated with TNBS) showing areas of necrosis and inflammation.

FIG. 51 is a representative graph of plasma adalimumab concentrations over time following a single subcutaneous (SQ) or topical administration of adalimumab. The plasma concentrations of adalimumab were determined 6, 12, 24, and 48 hours after administration of adalimumab. N/D = not detectable.

FIG. 52 is a representative table of the plasma adalimumab concentrations (μg/mL) as shown in Figure 4.6.

FIG. 53 is a graph showing the concentration of TNFα (pg/mL per mg of total protein) in non-inflamed and inflamed colon tissue after intracecal administration of adalimumab, as measured 6, 12, 24, and 24 hours after the initial dosing.

FIG. 54 is a graph showing the concentration of TNFα (pg/mL per mg of total protein) in colon tissue after subcutaneous or intracecal (topical) administration of adalimumab, as measured 48 hours after the initial dosing.

FIG. 55 is a graph showing the percentage (%) change in body weight at day 14 (± SEM) in acute DSS colitis mice treated with cyclosporine A orally (10 mg/kg) every third day (Q3D) or intracecally (10 mg/kg or 3 mg/kg) daily (QD), when compared to vehicle control (Vehicle). Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 56 is a graph showing the plasma cyclosporine A (CsA) (ng/mL) concentration over time (1 h, 2 h, 4 h, and 24 h) in acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA. Data presented as mean ± SEM.

FIG. 57 is a graph showing the colon tissue cyclosporine A (CsA) (ng/g) concentration over time (1 h, 2 h ,4 h and 24 h) in acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA. Data presented as mean ± SEM.

FIG. 58 is a graph showing the peak colon tissue cyclosporine A (CsA) (ng/g) concentration in acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA. Data presented as mean ± SEM.

FIG. 59 is a graph showing the trough tissue concentration of cyclosporine (CsA) (ng/g) in colon of acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA. Data presented as mean ± SEM.

FIG. 60 is a graph showing the interleukin-2 (Il-2) concentration (μg/mL) in colon tissue of acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA, where PO is compared to IC. Data presented as mean ± SEM. Mann-Whitney's *U*- test and Student's t-test were used for statistical analysis on non-Gaussian and Gaussian data respectively. A value of $p < 0.05$ was considered significant (Graph Pad Software, Inc.).

FIG. 61 is a graph showing the interleukin-6 (Il-6) concentration (μg/mL) in colon tissue of acute DSS colitis mice treated daily (QD) with orally (PO) (10 mg/kg) or intracecally (IC) (10 mg/kg or 3 mg/kg) administered CsA. Data presented as mean ± SEM.

FIG. 62 illustrates a nonlimiting example of a system for collecting, communicating and/or analyzing data about a subject, using an ingestible device.

FIGs. 63A-F are graphs showing rat IgG2A concentration as measured in (A) colon homogenate, (B) mLN homogenate, (C) small intestine homogenate, (D) cecum contents, (E) colon contents, and (F) plasma by ELISA. Standards were prepared with plasma matrix. Samples were diluted 1:50 before analysis. Sample 20 was removed from cecum contents analysis graph (outlier). *p<0.05; **p<0.01; ****p<0.001 were determined using the unpaired t test.

FIG. 64 illustrates a tapered silicon bellows.

FIG. 65 illustrates a tapered silicone bellows in the simulated device jig.

FIG. 66 illustrates a smooth PVC bellows.

FIG. 67 illustrates a smooth PVC bellows in the simulated device jig.

FIG. 68 demonstrates a principle of a competition assay performed in an experiment.

FIG. 69 shows AlphaLISA data.

FIG. 70 shows AlphaLISA data.

FIG. 71 shows AlphaLISA data.

FIG. 72 is a flowchart of illustrative steps of a clinical protocol, in accordance with some embodiments of the disclosure.

FIG. 73 is a graph showing the level of FAM-SMAD7-AS oligonucleotide in the cecum tissue of DSS-induced colitis mice at 12-hours. The bars represent from left to right, Groups 2 through 5 in the experiment described in Example 9.

FIG. 74 is a graph showing the level of FAM-SMAD7-AS oligonucleotide in the colon tissue of DSS-induced colitis mice at 12-hours. The bars represent from left to right, Groups 2 through 5 in the experiment described in Example 9.

FIG. 75 is a graph showing the level of FAM-SMAD7-AS oligonucleotide in the cecum contents of DSS-induced colitis mice at 12-hours. The bars represent from left to right, Groups 2 through 5 in the experiment described in Example 9.

FIG. 76 is a graph showing the mean concentration of tacrolimus in the cecum tissue and the proximal colon tissue 12 hours after intra-cecal or oral administration of tacrolimus to swine as described in Example 10.

FIG. 77 is a graph showing the mean concentration of tacrolimus in the blood 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 12 hours after intra-cecal (IC) or oral administration (PO) of tacrolimus to swine as described in Example 13.

FIG. 78 is a graph showing the $AUC_{0\text{-}12\ hours}$ of tacrolimus in the blood after intra-cecal (IC) or oral administration (PO) of tacrolimus in swine as described in Example 13.

FIG. 79 is a graph showing the mean concentration of tacrolimus in the cecum tissue, the proximal colon tissue, the spiral colon tissue, the transverse colon tissue, and the distal colon tissue after intra-cecal (IC) or oral administration (PO) of tacrolimus in swine as described in Example 13. **** $P<0.0001$, *** $P<0.001$.

FIG. 80 is a graph showing the mean concentation of tacrolimus in the cecum lumen, the proximal lumen, the spiral colon lumen, the transverse colon lumen, and the distal colon lumen in swine after intra-cecal (IC) or oral administration (PO) of tacrolimus in swine as described in Example 13. **** $P<0.0001$, *** $P<0.001$

FIG. 81 is a bar graph showing the mean concentration of tacrolimus in the rectal content 1 hour, 3 hours, 6 hours, and 12 hours after intra-cecal (IC) or oral administration (PO) of tacrolimus to swine as described in Example 13.

FIG. 82 is a line graph showing the mean concentration of tacrolimus in the rectal content 1 hour, 3 hours, 6 hours, and 12 hours after intra-cecal (IC) or oral administration (PO) of tacrolimus to swine as described in Example 13.

FIG. 83 is a graph showing the mean concentration of a SMAD7 antisense molecuile (SMAD7-AS-FAM) in the cecum tissue in untreated swine or in swine after intra-cecal (IC) or oral administration(PO) of SMAD7-AS-FAM as described in Example 9.

FIG. 84 is a graph showing the mean concentration of SMAD7-AS-FAM in the colon tissue in untreated swine or in swine after intra-cecal (IC) or oral administration(PO) of SMAD7-AS-FAM as described in Example 9.

FIG. 85 is a graph showing the mean concentration of SMAD7-AS-FAM in the colon contents in untreated swine or in swine after intra-cecal (IC) or oral administration(PO) of SMAD7-AS-FAM as described in Example 9.

FIG. 86 is a graph showing the mean concentration of SMAD7-AS-FAM in the cecum contents in untreated swine or in swine after intra-cecal (IC) or oral administration(PO) of SMAD7-AS-FAM as described in Example 9.

FIG. 87 is a graph showing the mean concentration of tacrolimus in the blood of swine 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 12 hours after intra-cecal (IC) or oral administration (PO) of tacrolimus as described in Example 10.

FIG. 88 is a graph showing the $AUC_{0\text{-}12\ hours}$ of tacrolimus in the blood of swine after intra-cecal (IC) or oral administration (PO) of tacrolimus as described in Example 10.

FIG. 89 is a representative table showing the Tmax, Cmax, trough (at 12 hours post-administration), and $AUC_{0\text{-}12\ hours}$ of tacrolimus in swine after intra-cecal (IC) or oral administration (PO) as described in Example 10.

FIG. 90 is a graph showing the mean concentration of tacrolimus in the cecum, the proximal colon, the spiral colon, the transverse colon, and the distal colon of swine after intra-cecal (IC) or oral administration (PO) of tacrolimus as described in Example 10.

FIG. 91 is a graph showing the mean concentration of tacrolimus in the cecum lumen, the proximal colon lumen, the spiral colon lumen, the transverse colon lumen, and the distal colon lumen of swine after intra-cecal (IC) or oral administration (PO) of tacrolimus as described in Example 10.

FIG. 92 is a graph showing the mean concentration of tacrolimus in the rectal content of swine at 1 hour, 3 hours, 6 hours, and 12 hours after intra-cecal (IC) or oral administration (PO) of tacrolimus as described in Example 10.

FIG. 93 is a representative table showing the quantitative histological grading of colitis as described in Example 11.

FIG. 94 is a graph showing the histopathological scores of two slides for animal 1502 (healthy control swine treated with placebo), animal 2501 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab), animal 2503 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab), and animal 2504 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab) at the placebo or adalimumab administration site prior to administration of placebo or adalimumab, respect tively. Absence of a bar for a particular parameter indicates that the value for this parameter was 0.

FIG. 95 is a representative hematoxylin- and eosin-stained image of the transverse colon of animal 1501 (healthy control swine). M, mucosa; SM, submucosa; TM, tunica muscularis. Numerous intestinal crypts (asterisks) are present and the surface epithelium (top two arrows) is intact. Mononuclear inflammatory cells are prominent in the lamina propria (light arrows) of the mucosa and extend a short distance into the submucosa (bottom two arrows). This amount of inflammatory cell infiltrate was expected background change and considered unrelated to the experimental protocol.

FIG. 96 is a representative hematoxylin- and eosin-stained image of the transverse colon of animal 2504 (8.5% DSS-induced colitis swine administered 1.86 mg/kg adalimumab) prior to administration of adalimumab. M, mucosa; SM, submucosa; TM, tunica muscularis. Extensive loss (light asterisks) of intestinal crypts is present in the mucosa. Scattered crypts remain (dark asterisks) and are often dilated and filled with inflammatory cell debris and mucus. The luminal epithelium persists in some areas (upper left arrow), but is absent in others (erosion; top middle and top right arrows). Inflammatory cells in the mucosa (light arrow) are abundant and extend into the submucosa (bottom left and bottom middle arrows).

FIG. 97 is a representative immunohistochemistry micrograph of the transverse colon of animal 1501 (healthy control swine) stained for human IgG. M, mucosa; SM, submucosa; TM, tunica muscularis. Serosal surface (arrows) and loose connective mesentery tissue (asterisks) are indicated. Faint 3,3-diaminobenzidine (DAB) staining in this tissue was considered a background effect and not indicative of human IgG.

FIG. 98 is a representative immunohistochemistry micrograph of the transverse colon of animal 2504 (8.5% DSS-induced colitis swine treated with 1.86 mg/kg dose of adsalimumab) stained for human IgG. M, mucosa; SM, submucosa; TM, tunica muscularis. DAB staining demonstrates the presence of human IgG at the surface of luminal epithelium (two top right arrows) and at the luminal surface of an area of inflammation and erosion (top two left arrows). Intense staining is also present in the loose connective mesentery tissue (asterisks) and extends a short distance into the outer edge of the tunica muscularis (bottom left two arrows). This type of staining was considered strong (grade 4) or very strong (grade 5).

FIG. 99 is a representative immunohistochemistry micrograph of the large intestine of animal 2504 (8.5% DSS-induced colitis swine treated with 1.86 mg/kg adalimumab) stained for human IgG. M, mucosa; SM, submucosa; TM, tunica muscularis. Lesions of DSS-induced colitis are present in this section. The luminal epithelium is absent (erosion) and diffuse loss of crypts (glands) is seen (top two asterisks). Very strong (grade 5) DAB (brown) staining demonstrates the presence of human IgG in the loose mesentery connective tissue (bottom two arterisks) and extending a short distance into the outer edge of the tunica muscularis (bottom two arrows). Strong (grade 4) staining for human IgG is seen at the eroded luminal surface (top two arrows pointing down) and within the inflammatory exudate. Weak (grade 2) staining for human IgG extends into the lamina propria (top two arrows pointing up) near the luminal surface.

FIG. 100 is a graph showing the presence of human IgG (adalimumab) at the specified locations (lumen/superficial mucosa, lamina propria, and tunica muscularis- outer/serosa) (scored level) in two slides from each of animal 1502 (placebo-treated healthy control swine), animal 2501 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab), animal 2503 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab) and animal 2504 (swine with 8.5% DSS-induced colitis treated with 1.86 mg/kg adalimumab) at the placebo or adalimumab administration site. Absence of a bar for a particular location indicates that the value for this location was 0. Scoring: 0 = not present; 1 = minimal; 2 = weak; 3 = moderate; 4 = strong; and 5=very strong immunolabel.

DETAILED DESCRIPTION

The present disclosure is directed to various methods and formulations for treating diseases of the gastrointestinal tract with a live biotherapeutic such as a stem cell, populations of live bacteria and/or live yeast, bacteriophages or prophages, a medium conditioned by stem cells, or organoids produced from stem cells, glycoproteins, enzymes, or ErbB4 receptor tyrosine

kinase agonists. For example, in an embodiment, a method of treating a disease of the gastrointestinal tract in a subject comprises administering to the subject a pharmaceutical formulation comprising a live biotherapeutic such as a stem cell wherein the pharmaceutical formulation is released in the subject's gastrointestinal tract proximate to one or more sites of disease. For example, in an embodiment, the pharmaceutical formulation comprises a therapeutically effective amount of a live biotherapeutic such as a stem cell.

In some embodiments, the formulation is contained in an ingestible device, and the device releases the formulation at a location proximate to the site of disease. The location of the site of disease may be predetermined. For example, an ingestible device, the location of which within the GI tract can be accurately determined as disclosed herein, may be used to sample one or more locations in the GI tract and to detect one or more analytes, including markers of the disease, in the GI tract of the subject. A pharmaceutical formulation may be then administered via an ingestible device and released at a location proximate to the predetermined site of disease. The release of the formulation may be triggered autonomously, as further described herein.

The following disclosure illustrates aspects of the formulations and methods embodied in the claims.

Formulations, including Pharmaceutical Formulations

As used herein, a "formulation" of a live biotherapeutic such as a stem cell may refer to either the live biotherapeutic such as a stem cell in pure form, such as, for example, a lyophilized live biotherapeutic such as a stem cell, or a mixture of the live biotherapeutic such as a stem cell with one or more physiologically acceptable carriers, excipients or stabilizers. Thus, therapeutic formulations or medicaments can be prepared by mixing the live biotherapeutic such as a stem cell having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) antibody; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers

such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt- forming counter-ions such as sodium; metal complexes (e.g., Zn- protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX<®>, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases. Exemplary lyophilized formulations are described in US Patent No. 6,267,958. Aqueous formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

A formulation of a live biotherapeutic such as a stem cell as disclosed herein, e.g., sustained-release formulations, can further include a mucoadhesive agent, e.g., one or more of polyvinyl pyrolidine, methyl cellulose, sodium carboxyl methyl cellulose, hydroxyl propyl cellulose, carbopol, a polyacrylate, chitosan, a eudragit analogue, a polymer, and a thiomer. Additional examples of mucoadhesive agents that can be included in a formulation with a live biotherapeutic such as a stem cell are described in, e.g., Peppas et al., *Biomaterials* 17(16):1553-1561, 1996; Kharenko et al., *Pharmaceutical Chemistry J.* 43(4):200-208, 2009; Salamat-Miller et al., *Adv. Drug Deliv. Reviews* 57(11):1666-1691, 2005; Bernkop-Schnurch, *Adv. Drug Deliv. Rev.* 57(11):1569-1582, 2005; and Harding et al., *Biotechnol. Genet. Eng. News* 16(1):41-86, 1999.

In some embodiments, components of a formulation may include any one of the following components, or any combination thereof:

Acacia, Alginate, Alginic Acid, Aluminum Acetate, an antiseptic, Benzyl Alcohol, Butyl Paraben, Butylated Hydroxy Toluene, an antioxidant. Citric acid, Calcium carbonate, Candelilla wax, a binder, Croscarmellose sodium, Confectioner sugar, Colloidal silicone dioxide, Cellulose, Carnuba wax, Corn starch, Carboxymethylcellulose calcium, Calcium stearate, Calcium disodium EDTA, Chelation agents, Copolyvidone, Castor oil hydrogenated, Calcium hydrogen phosphate dehydrate, Cetylpyridine chloride, Cysteine HCl, Crosspovidone, Dibasic Calcium

Phosphate, Disodium hydrogen phosphate, Dimethicone, Erythrosine Sodium, Ethyl Cellulose, Gelatin, Glyceryl monooleate, Glycerin, Glycine, Glyceryl monostearate, Glyceryl behenate, Hydroxy propyl cellulose, Hydroxyl propyl methyl cellulose, Hypromellose, HPMC Pthalate, Iron oxides or ferric oxide, Iron oxide yellow, Iron oxide red or ferric oxide, Lactose (hydrous or anhydrous or monohydrate or spray dried), Magnesium stearate, Microcrystalline cellulose, Mannitol, Methyl cellulose,, Magnesium carbonate, Mineral oil, Methacrylic acid copolymer, Magnesium oxide, Methyl paraben, PEG, Polysorbate 80, Propylene glycol, Polyethylene oxide, Propylene paraben, Polaxamer 407 or 188 or plain, Potassium bicarbonate, Potassium sorbate, Potato starch, Phosphoric acid, Polyoxy140 stearate, Sodium starch glycolate, Starch pregelatinized, Sodium crossmellose, Sodium lauryl sulfate, Starch, Silicon dioxide, Sodium benzoate,, Stearic acid, Sucrose base for medicated confectionery, a granulating agent, Sorbic acid, Sodium carbonate, Saccharin sodium, Sodium alginate, Silica gel, Sorbiton monooleate, Sodium stearyl fumarate, Sodium chloride, Sodium metabisulfite, Sodium citrate dehydrate, Sodium starch, Sodium carboxy methyl cellulose, Succinic acid, Sodium propionate, Titanium dioxide, Talc, Triacetin, Triethyl citrate.

Accordingly, in some embodiments of the method of treating a disease as disclosed herein, the method comprises administering to the subject a pharmaceutical composition that is a formulation as disclosed herein. In some embodiments the formulation is a dosage form, which may be, as an example, a solid form such as, for example, a capsule, a tablet, a sachet, or a lozenge; or which may be, as an example, a liquid form such as, for example, a solution, a suspension, an emulsion, a foam, or a syrup.

In some embodiments, the formulation is not comprised in an ingestible device. In some embodiments wherein the formulation is not comprised in an ingestible device, the formulation may be suitable for oral administration. The formulation may be, for example, a solid dosage form or a liquid dosage form as disclosed herein. In some embodiments wherein the formulation is not comprised in an ingestible device, the formulation may be suitable for rectal administration. The formulation may be, for example, a dosage form such as a suppository or an enema. In embodiments where the formulation is not comprised in an ingestible device, the formulation releases the live biotherapeutic such as a stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease. Such localized release may be achieved, for example, with a formulation comprising an enteric coating. Such localized release may be achieved, as another example, with a formulation comprising a core comprising one or more polymers suitable for controlled release of an active substance. A non-limiting list

of such polymers includes: poly(2-(diethylamino)ethyl methacrylate, 2-(dimethylamino)ethyl methacrylate, poly(ethylene glycol), poly(2-aminoethyl methacrylate), (2-hydroxypropyl)methacrylamide, poly(β-benzyl-l-aspartate), poly(N-isopropylacrylamide), and cellulose derivatives.

In some embodiments, the formulation is comprised in an ingestible device as disclosed herein. In some embodiments wherein the formulation is comprised in an ingestible device, the formulation may be suitable for oral administration. The formulation may be, for example, a solid dosage form or a liquid dosage form as disclosed herein. In some embodiments the formulation is suitable for introduction and optionally for storage in the device. In some embodiments the formulation is suitable for introduction and optionally for storage in a reservoir comprised in the device. In some embodiments the formulation is suitable for introduction and optionally for storage in a reservoir comprised in the device. Thus, in some embodiments, provided herein is a reservoir comprising a therapeutically effective amount of a live biotherapeutic such as a stem cell, wherein the reservoir is configured to fit into an ingestible device. In some embodiments, the reservoir comprising a therapeutically effective amount of a live biotherapeutic such as a stem cell is attachable to an ingestible device. In some embodiments, the reservoir comprising a therapeutically effective amount of a live biotherapeutic such as a stem cell is capable of anchoring itself to the subject's tissue. As an example, the reservoir capable of anchoring itself to the subject's tissue comprises silicone. As an example, the reservoir capable of anchoring itself to the subject's tissue comprises polyvinyl chloride.

In some embodiments the formulation is suitable for introduction in a spray catheter, as disclosed herein.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, for example, those with complementary activities that do not adversely affect each other. For instance, the formulation may further comprise another live biotherapeutic such as a stem cell or a chemotherapeutic agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained- release preparations include semipermeable matrices of solid hydrophobic polymers containing the live biotherapeutic such as a stem cell, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2- hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated live biotherapeutic such as a stem cells remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Pharmaceutical formulations may contain one or more live biotherapeutics such as a stem cells, populations of live bacteria and/or live yeast, bacteriophages or prophages, a medium conditioned by stem cells, or organoids produced from stem cells, or other therapeutics such as glycoproteins, enzymes, or ErbB4 receptor tyrosine kinase agonists. The pharmaceutical formulations may be formulated in any manner known in the art. In some embodiments the formulations include one or more of the following components: a sterile diluent (e.g., sterile water or saline), a fixed oil, polyethylene glycol, glycerin, propylene glycol, or other synthetic solvents, antibacterial or antifungal agents, such as benzyl alcohol or methyl parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like, antioxidants, such as ascorbic acid or sodium bisulfite, chelating agents, such as ethylenediaminetetraacetic acid, buffers, such as acetates, citrates, or phosphates, and isotonic agents, such as sugars (e.g., dextrose), polyalcohols (e.g., mannitol or sorbitol), or salts (e.g., sodium chloride), or any combination thereof. Liposomal suspensions can also be used as pharmaceutically acceptable carriers (see, e.g., U.S.

Patent No. 4,522,811, incorporated by reference herein in its entirety). The formulations can be formulated and enclosed in ampules, disposable syringes, or multiple dose vials. Where required, proper fluidity can be maintained by, for example, the use of a coating, such as lecithin, or a surfactant. Controlled release of the live biotherapeutic such as a stem cell can be achieved by implants and microencapsulated delivery systems, which can include biodegradable, biocompatible polymers (e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid; Alza Corporation and Nova Pharmaceutical, Inc.).

In some embodiments, the live biotherapeutic such as a stem cell is present in a pharmaceutical formulation within the device.

In some embodiments, the live biotherapeutic such as a stem cell is present in solution within the device.

In some embodiments, the live biotherapeutic such as a stem cell is present in a suspension in a liquid medium within the device.

In some embodiments, the live biotherapeutic such as a stem cell is present as a pure, powder (e.g., lyophilized) form of the live biotherapeutic such as a stem cell.

Definitions

By “ingestible”, it is meant that the device can be swallowed whole.

"Gastrointestinal inflammatory disorders" are a group of chronic disorders that cause inflammation and/or ulceration in the mucous membrane. These disorders include, for example, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, indeterminate colitis and infectious colitis), mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis and proctitis), necrotizing enterocolitis, and esophagitis.

"Inflammatory Bowel Disease" or "IBD" is a chronic inflammatory autoimmune condition of the gastrointestinal (GI) tract. The GI tract is a large organ that extends from the buccal cavity to the anus. The primary function of the GI tract is to digest food, absorb nutrients and eliminated any waste. The GI tract can be divided into four main different sections, the esophagus, stomach, small intestine and large intestine or colon. The small intestine possesses three main subcompartments: the duodenum, jejunum and ileum. Similarly, the large intestine consists of six sections: the cecum, ascending colon, transverse colon, ascending colon, sigmoid colon, and the rectum. The small intestine is about 6 m long, its diameter is 2.5 to 3 cm and the transit time through it is typically 3 hours. The duodenum has a C-shape, and is 30 cm long. Due to its direct connection with the stomach, it is physically more stable than the jejunum and ileum,

which are sections that can freely move. The jejunum is 2.4 m in length and the ileum is 3.6 m in length and their surface areas are 180 m^2 and 280 m^2 respectively. The large intestine is 1.5 m long, its diameter is between 6.3 and 6.5 cm, the transit time though this section is 20 hours and has a reduced surface area of approximately 150 m^2. The higher surface area of the small intestine enhances its capacity for systemic drug absorption.

Over 1000 different microbial species have been identified that can live in the human GI tract, e.g., *Actinobacteria*, *Bifidobacterium* spp., *Coriobacteriales*, *Eggerthella, Slackia* spp., *Actinomycetales*, *Bacteroidetes*, *Firmicutes, Gemella, Clostridia, Lachnospiraceae, Negativicutes, Fusobacteria,* and fungi (e.g., *Eukarya*) . See, e.g., Rajilic-Stojanovic and de Vos (2014) *FEMS Microbiol. Rev.* 38(5): 996-1047; and Carroll et al. (2015) *Mamm. Genome* 20(7): 395-403. Whereas the small intestine contains very few bacteria, the colon comprises between 10^{13} and 10^{14} commensal bacteria (Johansson et al. (2013) *Nat. Rev. Gastroenterol. Hepatol.* 10(6): 352-361).

The etiology of IBD is complex, and many aspects of the pathogenesis remain unclear. The treatment of moderate to severe IBD poses significant challenges to treating physicians, because conventional therapy with corticosteroids and immunomodulator therapy (e.g., azathioprine, 6 mercaptopurine, and methotrexate administered via traditional routes such as tablet form, oral suspension, or intravenously) is associated with side effects and intolerance and has not shown proven benefit in maintenance therapy (steroids). Monoclonal antibodies targeting tumor necrosis factor alpha (TNF-a), such as infliximab (a chimeric antibody) and adalimumab (a fully human antibody), are currently used in the management of CD. Infliximab has also shown efficacy and has been approved for use in UC. However, approximately 10%-20% of patients with CD are primary non-responders to anti-TNF therapy, and another ~20%-30% of CD patients lose response over time (Schnitzler *et al.*, *Gut* 58:492-500 (2009)). Other adverse events (AEs) associated with anti TNFs include elevated rates of bacterial infection, including tuberculosis, and, more rarely, lymphoma and demyelination (Chang *et al., Nat Clin Pract Gastroenterol Hepatology* 3:220 (2006); Hoentjen *et al., World J. Gastroenterol.* 15(17):2067 (2009)). No currently available therapy achieves sustained remission in more than 20%-30% of IBD patients with chronic disease (Hanauer *et al.*, *Lancet* 359: 1541-49 (2002); Sandborn *et al.*, *N Engl J Med* 353: 1912-25 (2005)). In addition, most patients do not achieve sustained steroid-free remission and mucosal healing, clinical outcomes that correlate with true disease modification.

Although the cause of IBD remains unknown, several factors such as genetic, infectious and immunologic susceptibility have been implicated. IBD is much more common in Caucasians,

especially those of Jewish descent. The chronic inflammatory nature of the condition has prompted an intense search for a possible infectious cause. Although agents have been found which stimulate acute inflammation, none has been found to cause the chronic inflammation associated with IBD. The hypothesis that IBD is an autoimmune disease is supported by the previously mentioned extraintestinal manifestation of IBD as joint arthritis, and the known positive response to IBD by treatment with therapeutic agents such as adrenal glucocorticoids, cyclosporine and azathioprine, which are known to suppress immune response. In addition, the GI tract, more than any other organ of the body, is continuously exposed to potential antigenic substances such as proteins from food, bacterial byproducts (LPS), etc.

A chronic inflammatory autoimmune condition of the gastrointestinal (GI) tract presents clinically as either ulcerative colitis (UC) or Crohn's disease (CD). Both IBD conditions are associated with an increased risk for malignancy of the GI tract.

"Crohn's disease" ("CD") is a chronic transmural inflammatory disease with the potential to affect any part of the entire GI tract, and UC is a mucosal inflammation of the colon. Both conditions are characterized clinically by frequent bowel motions, malnutrition, and dehydration, with disruption in the activities of daily living.

CD is frequently complicated by the development of malabsorption, strictures, and fistulae and may require repeated surgery. UC, less frequently, may be complicated by severe bloody diarrhea and toxic megacolon, also requiring surgery. The most prominent feature Crohn's disease is the granular, reddish-purple edematous thickening of the bowel wall. With the development of inflammation, these granulomas often lose their circumscribed borders and integrate with the surrounding tissue. Diarrhea and obstruction of the bowel are the predominant clinical features. As with ulcerative colitis, the course of Crohn's disease may be continuous or relapsing, mild or severe, but unlike ulcerative colitis, Crohn's disease is not curable by resection of the involved segment of bowel. Most patients with Crohn's disease require surgery at some point, but subsequent relapse is common and continuous medical treatment is usual. Crohn's disease may involve any part of the alimentary tract from the mouth to the anus, although typically it appears in the ileocolic, small-intestinal or colonic-anorectal regions. Histopathologically, the disease manifests by discontinuous granulomatomas, crypt abscesses, fissures and aphthous ulcers. The inflammatory infiltrate is mixed, consisting of lymphocytes (both T and B cells), plasma cells, macrophages, and neutrophils. There is a disproportionate increase in IgM- and IgG-secreting plasma cells, macrophages and neutrophils.

To date, the primary outcome measure in Crohn's Disease clinical trials is the Crohn's Disease Activity Index (CDAI), which has served as the basis for approval of multiple drug treatments, including for example, vedolizumab and natalizumab. The CDAI was developed by regressing clinician global assessment of disease activity on eighteen potential items representing patient reported outcomes (PROs) (i.e., abdominal pain, pain awakening patient from sleep, appetite), physical signs (i.e., average daily temperature, abdominal mass), medication use (i.e., loperamide or opiate use for diarrhea) and a laboratory test (i.e. hematocrit). Backward stepwise regression analysis identified eight independent predictors which are the number of liquid or soft stools, severity of abdominal pain, general well-being, occurrence of extra-intestinal symptoms, need for anti-diarrheal drugs, presence of an abdominal mass, hematocrit, and body weight. The final score is a composite of these eight items, adjusted using regression coefficients and standardization to construct an overall CDAI score, ranging from 0 to 600 with higher score indicating greater disease activity. Widely used benchmarks are: CDAI <150 is defined as clinical remission, 150 to 219 is defined as mildly active disease, 220 to 450 is defined as moderately active disease, and above 450 is defined as very severe disease (Best WR, et al., Gastroenterology 77:843-6, 1979). Vedolizumab and natalizumab have been approved on the basis of demonstrated clinical remission, i.e. CDAI < 150.

Although the CDAI has been in use for over 40 years, and has served as the basis for drug approval, it has several limitations as an outcome measure for clinical trials. For example, most of the overall score comes from the patient diary card items (pain, number of liquid bowel movements, and general well-being), which are vaguely defined and not standardized terms (Sandler *et al.*, *J. Clin. Epidemiol* 41:451-8, 1988; Thia *et al.*, *Inflamm Bowel Dis* 17: 105-11, 2011). In addition, measurement of pain is based on a four-point scale rather than an updated seven-point scale. The remaining 5 index items contribute very little to identifying an efficacy signal and may be a source of measurement noise. Furthermore, concerns have been raised about poor criterion validity for the CDAI, a reported lack of correlation between the CDAI and endoscopic measures of inflammation (which may render the CDAI as a poor discriminator of active CD and irritable bowel syndrome) and high reported placebo rates (Korzenik *et al.*, *N Engl J Med.* 352:2193-201, 2005; Sandborn WJ, *et al.*, *N Engl J Med* 353: 1912-25, 2005; Sandborn WJ, *et al.*, Ann Intern 19; 146:829-38, 2007, Epub 2007 Apr 30; Kim *et al.*, *Gastroenterology* 146: (5 supplement 1) S-368, 2014).

It is, thus, generally recognized that additional or alternative measures of CD symptoms are needed, such as new PRO tools or adaptations of the CDAI to derive a new PRO. The PRO2

and PRO3 tools are such adaptations of the CDAI and have been recently described in Khanna *et al.*, *Aliment Pharmacol. Ther.* 41: 77-86, 2015. The PRO2 evaluates the frequency of loose/liquid stools and abdominal pain (Id). These items are derived and weighted accordingly from the CDAI and are the CDAI diary card items, along with general well-being, that contribute most to the observed clinical benefit measured by CDAI (Sandler *et al.*, *J. Clin. Epidemiol* 41 :451-8, 1988; Thia *et al.*, *Inflamm Bowel Dis* 17: 105-11, 2011; Kim *et al.*, *Gastroenterology* 146: (5 supplement 1) S-368, 2014). The remission score of < 11 is the CDAI-weighted sum of the average stool frequency and pain scores in a 7-day period, which yielded optimum sensitivity and specificity for identification of CDAI remission (score of < 150) in a retrospective data analysis of ustekinumab induction treatment for moderate to severe CD in a Phase II clinical study (Gasink C, *et al.*, abstract, ACG Annual Meeting 2014). The PRO2 was shown to be sensitive and responsive when used as a continuous outcome measure in a retrospective data analysis of MTX treatment in active CD (Khanna R, *et al.*, *Inflamm Bowel Dis* 20: 1850-61, 2014) measured by CDAI. Additional outcome measures include the Mayo Clinic Score, the Crohn disease endoscopic index of severity (CDEIS), and the Ulcerative colitis endoscopic index of severity (UCEIS). Additional outcome measures include Clinical remission, Mucosal healing, Histological healing (transmural), MRI or ultrasound for measurement or evaluation of bowel wall thickness, abscesses, fistula and histology.

An additional means of assessing the extent and severity of Crohn's Disease is endoscopy. Endoscopic lesions typical of Crohn's disease have been described in numerous studies and include, e.g., aphthoid ulcerations, "punched-out ulcers," cobblestoning and stenosis. Endoscopic evaluation of such lesions was used to develop the first validated endoscopic score, the Crohn's Disease Endoscopic Index of Severity (CDEIS) (Mary *et al.*, *Gut* 39:983-9, 1989). More recently, because the CDEIS is time-consuming, complicated and impractical for routine use, a Simplified Endoscopic Activity Score for Crohn's Disease (SES- CD) was developed and validated (Daperno *et al.*, *Gastrointest. Endosc.* 60(4):505-12, 2004). The SES-CD consists of four endoscopic variables (size of ulcers, proportion of surface covered by ulcers, proportion of surface with any other lesions (e.g., inflammation), and presence of narrowings [stenosis]) that are scored in five ileocolonic segments, with each variable, or assessment, rated from 0 to 3.

To date, there is no cure for CD. Accordingly, the current treatment goals for CD are to induce and maintain symptom improvement, induce mucosal healing, avoid surgery, and improve quality of life (Lichtenstein GR, *et al.*, *Am J Gastroenterol* 104:465-83, 2009; Van Assche G, *et*

al., *J Crohns Colitis.* 4:63-101, 2010). The current therapy of IBD usually involves the administration of anti-inflammatory or immunosuppressive agents, such as sulfasalazine, corticosteroids, 6- mercaptopurine/azathioprine, or cyclosporine, all of which are not typically delivered by localized release of a drug at the site or location of disease. More recently, biologics like TNF-alpha inhibitors and IL-12/IL-23 blockers, are used to treat IBD. If anti-inflammatory/immunosuppressive/biologic therapies fail, colectomies are the last line of defense. The typical operation for CD not involving the rectum is resection (removal of a diseased segment of bowel) and anastomosis (reconnection) without an ostomy. Sections of the small or large intestine may be removed. About 30% of CD patients will need surgery within the first year after diagnosis. In the subsequent years, the rate is about 5% per year. Unfortunately, CD is characterized by a high rate of recurrence; about 5% of patients need a second surgery each year after initial surgery.

Refining a diagnosis of inflammatory bowel disease involves evaluating the progression status of the diseases using standard classification criteria. The classification systems used in IBD include the Truelove and Witts Index (Truelove S. C. and Witts, L.J. *Br Med J.* 1955;2: 1041-1048), which classifies colitis as mild, moderate, or severe, as well as Lennard- Jones. (Lennard-Jones JE. Scand J, *Gastroenterol Suppl* 1989; 170:2-6) and the simple clinical colitis activity index (SCCAI). (Walmsley *et. al.*, *Gut.* 1998; 43:29-32) These systems track such variables as daily bowel movements, rectal bleeding, temperature, heart rate, hemoglobin levels, erythrocyte sedimentation rate, weight, hematocrit score, and the level of serum albumin.

There is sufficient overlap in the diagnostic criteria for UC and CD that it is sometimes impossible to say which a given patient has; however, the type of lesion typically seen is different, as is the localization. UC mostly appears in the colon, proximal to the rectum, and the characteristic lesion is a superficial ulcer of the mucosa; CD can appear anywhere in the bowel, with occasional involvement of stomach, esophagus and duodenum, and the lesions are usually described as extensive linear fissures.

In approximately 10-15% of cases, a definitive diagnosis of ulcerative colitis or Crohn's disease cannot be made and such cases are often referred to as "indeterminate colitis." Two antibody detection tests are available that can help the diagnosis, each of which assays for antibodies in the blood. The antibodies are "perinuclear anti-neutrophil antibody" (pANCA) and "anti-*Saccharomyces cerevisiae* antibody" (ASCA). Most patients with ulcerative colitis have the pANCA antibody but not the ASCA antibody, while most patients with Crohn's disease have the ASCA antibody but not the pANCA antibody. However, these two tests have shortcomings as

some patients have neither antibody and some Crohn's disease patients may have only the pANCA antibody. A third test, which measures the presence and accumulation of circulating anti-microbial antibodies – particularly flagellin antibodies, has proven to be useful for detecting susceptibility to Crohn's Disease before disease development. See Choung, R. S., *et al.* "Serologic microbial associated markers can predict Crohn's disease behaviour years before disease diagnosis." *Alimentary pharmacology & therapeutics* 43.12 (2016): 1300-1310.

"Ulcerative colitis (UC)" afflicts the large intestine. The course of the disease may be continuous or relapsing, mild or severe. The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkuhn. Coalescence of these distended and ruptured crypts tends to separate the overlying mucosa from its blood supply, leading to ulceration. Symptoms of the disease include cramping, lower abdominal pain, rectal bleeding, and frequent, loose discharges consisting mainly of blood, pus and mucus with scanty fecal particles. A total colectomy may be required for acute, severe or chronic, unremitting ulcerative colitis.

The clinical features of UC are highly variable, and the onset may be insidious or abrupt, and may include diarrhea, tenesmus and relapsing rectal bleeding. With fulminant involvement of the entire colon, toxic megacolon, a life-threatening emergency, may occur. Extraintestinal manifestations include arthritis, pyoderma gangrenoum, uveitis, and erythema nodosum.

In certain embodiments, the GI tract disorder is small intestinal bacterial overgrowth (SIBO). The small intestine houses less than 10^3 bacteria/mL under healthy conditions. When the homeostasis of the gut microbiome is disrupted or aberrant, various functions of the gut microbiota are uncontrolled. See, e.g., Shreiner *et al.* (2016) *Curr. Opin. Gastroenterol.* 31(1): 69-75; Bures *et al.* (2010) *World J. Gastroenterol.* 16(24): 2978-2990. Excessive levels of bacteria (over 10^5 bacteria /mL) and abnormal types of bacteria in the small intestine leads to the development of SIBO. SIBO is associated with chronic diarrhea, abdominal discomfort, bloating, malabsorption, flatulence, and unintentional weight loss. While Gram-positive bacteria are typically found in the small intestine, subjects suffering from SIBO have a variety of bacteria in the small intestine including Gram-negative bacteria which are normally only present in very small numbers or not at all within the small intestine. For example, bacteria present in SIBO may secrete mucosal damaging toxins or metabolize bile salts, which can lead to malabsorption and bloating. A study comparing the prevalence of SIBO in subjects aged 24 to 50 and in subjects aged 61 or older found that SIBO was more prevalent in older subjects as compared to younger subjects (15.6% and 5.9% respectively) (Parlesak *et al.* (2003) *J. Am. Geriatr. Soc.*

51(6): 768-773). SIBO was also seen more frequently in subjects with reduced body weight. Risk factors for developing SIBO include: metabolic disorders (e.g., diabetes, hypochloryhydria), malnutrition, irritable bowel syndrome (IBS), Celiac disease, Crohn's disease, cirrhosis, renal failure, gastroparesis, small bowel dysmotility, structural abnormalities of the GI tract (e.g., jejunal diverticula), gastric resection and immuno-deficiency. Additional risk factors include the use of certain medications (e.g., antibiotics, gastric acid secretion inhibitors). See, e.g., Dukowicz *et al.* (2007) *Gastronenterol. Hepatol.* 3(2): 112-122. In some embodiments, subjects having SIBO have delayed intestinal transit times (Cuoco *et al.* (2002) *Hepatogastroenterology* 49: 1582-1586). In some embodiments, subjects having SIBO have accelerated intestinal transit times (Van Citters and Lin (2006) Clin. Nutrition in Gastrointestinal Disease. Thorofare: Slack Inc; 2006; 271-280).

As used herein, a subject has or is at risk of having SIBO if the subject has intestinal bacteria levels that are greater than 10^3 colony forming units (CFU)/ mL, e.g., greater than 10^4 CFU/ mL, greater than 10^5 CFU/ mL, greater than 10^6 CFU/ mL, greater than 10^7 CFU/ mL, greater than 10^8 CFU/ mL, greater than 10^9 CFU/ mL, greater than 10^{10} CFU/ mL.

The prevalence of SIBO in healthy individuals varies from about 0-20% (see, e.g., Lombardo *et al.* (2010) Clin. Gastroenterol. Hepatol. 8: 504–8; Sabaté *et al.* (2008) *Obes. Surg.* 18: 371–7; Posserud *et al.* (2007) *Gut* 56: 802-8; Teo (2004) *J. Gastroenterol. Hepatol.* 19: 904–9; Lewis *et al.* (1999) *Age Ageing* 28: 181–5; Pimentel *et al.* (2003) *Am. J. Gastroenterol.* 98: 412-9; Rana *et al.* (2011) *Diabetes Technol. Ther.* 13: 1115–20; Bratten *et al.* (2008) *Am. J. Gastroenterol.* 103: 958-63; and Scarpellini *et al.* (2009) *J. Pediatr.* 155: 416-20). Several clinical conditions are associated with SIBO and are referred to herein as "SIBO-related conditions." Exemplary SIBO-related conditions include, but are not limited to, coeliac disease (see, e.g., Rana *et al.* (2007) *Trop. Gastroenterol.* 28: 159-61; Rubio-Tapia *et al.* (2009) *J. Clin. Gastroenterol.* 43: 157-61; and Tursi *et al.* (2003) *Am. J. Gastroenterol.* 98: 839–43), connective tissue diseases such as scleroderma (see, e.g., Levesque *et al.* (2009) *Rheumatology* 48: 1314–9; and Parodi *et al.* (2008) *Am. J. Gastroenterol.* 103: 1257–62), Crohn's disease (see, *e.g.*, Fukushima *et al.* (1999) *Dis. Colon Rectum* 42: 1072-7; Klaus *et al.* (2009) *Gastroenterol.* 9: 61; and U.S. Publication No. 2002/0039599), diabetes mellitus (see, e.g., Rana *et al.* (2011) *Diabetes Technol Ther* 13: 1115–20, and Zaccardi *et al.* (2009) *Eur. Rev. Med. Pharmacol. Sci.* 13: 419–23), hypothyroidism (see, e.g., Lauritano *et al.* (2007) *J. Clin. Endocr. Metab.* 92: 4180–4), nonspecific dysmotility (see, e.g., Jacobs *et al.* (2013) *Aliment. Pharmacol. Ther.* 37: 1103–11), radiation enteropathy (see, e.g., Wedlake *et al.* (2008) *Eur. J Cancer* 44: 2212–7), ulcerative

colitis (see, e.g., Ibanez *et al*. (2008) *Gastroenterology* 134: A-350), chronic fatigue syndrome (see, e.g., Ojetti *et al.* (2009) *Eur. Rev. Med. Pharmacol. Sci.* 13: 419–23), chronic pancreatitis (see, e.g., Mancilla *et al*. (2008) 136: 976-80; and Trespi *et* al (1999) *Curr. Med. Res. Opin.* 15: 47–52), drug-induced inhibition of acid secretion (see, e.g., Jacobs (2013) *Aliment. Pharmacol. Ther.* 37: 1103–11; Compare *et al.* (2010) *Eur. J. Clin. Invest.* 41: 380-6; and Lombardo *et al.* (2010) *Clin. Gastroenterol. Hepatol.* 8: 504–8), end-stage renal failure (see, e.g., Strid *et al.* (2003) *Digestion* 67: 129-37), fibromyalgia (see, e.g., U.S. Publication No. 2002/0039599), irritable bowel syndrome (Posserud *et al*. (2007) *Gut* 56: 802-8; Bratten *et al.* (2008) *Am. J. Gastroenterol.* 103: 958–63; 30. Pimentel *et al*. (2000) *Am. J. Gastroenterol.* 95: 3503-6; Nucera *et al.* (2005) *Aliment. Pharmacol. Ther.* 21: 1391-5; Lupascu *et al*. (2005) *Aliment. Pharmacol. Ther.* 22: 1157-60; and Grover *et al*. (2008) *Neurogastroenterol. Motil.* 20: 998-1008), immunodeficiency syndromes such as HIV-infection and chronic lymphocytic leukaemia (see, e.g., Chave *et al. Am. J. Gastroenterol.* 89: 2168-71; and Smith *et al*. (1990) *J. Clin. Pathol.* 43: 57-9), liver cirrhosis (see, e.g., Yang *et al*. (1998) *Scand. J. Gastroenterol.* 33: 867-71; and Gunnarsdottir (2003) *Am. J. Gastroenterol.* 98: 1362-70), obesity (see, e.g., Sabaté *et al*. (2008) Obes. Surg. 18: 371-7; and Madrid *et al.* (2011) *Dig. Dis. Sci.* 56: 155–60), parenteral nutrition (see, e.g., Gutierrez *et al*. (2012) *J. Pediatr. Surg.* 47: 1150-4), rosacea (Parodi *et al*. Clin. *Gastroenterol. Hepatol.* 6: 759-64), muscular dystrophy (see, e.g., Tarnopolsky *et al.* (2010) *Muscle Nerve* 42: 853-5), and Parkinson's disease (see, e.g., Gabrielli (2011) *Movement Disord.* 26: 889-92). Thus, in some embodiments of any of the methods described herein, the subject has a SIBO-related condition selected from the group consisting of coeliac disease, a connective tissue disease (e.g., scleroderma), Crohn's disease, diabetes mellitus, hypothyroidism, nonspecific dysmotility, radiation enteropathy, ulcerative colitis, chronic fatigue syndrome, chronic pancreatitis, drug-induced inhibition of acid secretion, end-stage renal failure, fibromyalgia, irritable bowel syndrome, an immunodeficiency syndrome (e.g., HIV-infection and chronic lymphocytic leukaemia), obesity, parenteral nutrition, rosacea, muscular dystrophy, and Parkinson's disease.

In certain embodiments, a non-GI tract disorder such as liver disease can result in one or more symptoms in the GI tract. For example, bile acids are products of cholesterol synthesis that are synthesized in the liver, conjugated to taurine or glycine, and stored in the gallbladder until released into the small intestine. The primary bile acids are cholic acid, and chenodeoxycholic acid, which are deconjugated and dehydroxylated by instestinal bacteria to form the secondary bile acids deoxycholic acid and lithocholic acid, respectively. The majority of bile acids (about

95%) are reabsorbed in the distal ileum and returned to the liver (see, e.g., U.S. Publication No. 2017/0343535, incorporated herein by reference). Impaired absorption of bile acids in the ileum can lead to excess bile acids in the colon which can cause symptoms of bile acid malabsorption (BAM; also known as bile acid diarrhea), including watery stool and fecal incontinence. Interestingly, up to 50% of patients with irritable bowel syndrome with diarrhea (IBS-D) also have BAM (see, e.g., Camilleri *et al.* (2009) *Neurogastroeterol. Motil.* 21(7): 734-43). A non-exhaustive list of liver diseases and disorders, include, but are not limited to fibrosis, cirrhosis, alcoholic lever disease, fatty liver disease (hepatic steatosis), non-alcoholic fatty liver disease (NASH), cholestatic liver disease, liver parenchyma, an inherited metabolic disorder of the liver, PFIC (progressive familial intrahepatic cholestasis), autoimmune hepatitis, primary biliary cirrhosis (PBC), liver fibrosis, NAFLD, chronic autoimmune liver disease leading to progressive cholestasis, pruritus of cholestatic liver disease, inflammation of the liver, and liver fibrosis.

The terms "antibody" and "immunoglobulin" are used interchangeably in the broadest sense and include monoclonal antibodies (for example, full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific, trispecific etc. antibodies so long as they exhibit the desired biological activity) and may also include certain antibody fragments (as described in greater detail herein). An antibody can be human, humanized and/or affinity matured.

"Antibody fragments" comprise only a portion of an intact antibody, where in certain embodiments, the portion retains at least one, and typically most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an *in vivo* half-life substantially similar to an intact antibody. For example, such an antibody fragment may comprise on antigen binding arm linked to an Fc sequence capable of conferring in vivo stability to the fragment.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single

antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al, Proc. Natl. Acad. Sci. USA 81 :6851-6855 (1984)).

"Treatment regimen" refers to a combination of dosage, frequency of administration, or duration of treatment, with or without addition of a second medication.

"Effective treatment regimen" refers to a treatment regimen that will offer beneficial response to a patient receiving the treatment.

"Effective amount" refers to an amount of drug that offers beneficial response to a patient receiving the treatment. For example, an effective amount may be a Human Equivalent Dose (HED).

“Dispensable”, with reference to any substance, refers to any substance that may be released from an ingestible device as disclosed herein, or from a component of the device such as a reservoir. For example, a dispensable substance may be a live biotherapeutic such as a stem cell, and/or a formulation comprising a live biotherapeutic such as a stem cell.

"Patient response" or "patient responsiveness" can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression, including slowing down and complete arrest; (2) reduction in the number of disease episodes and/or symptoms; (3) reduction in lesional size; (4) inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; (5) inhibition (i.e., reduction, slowing down or complete stopping) of disease spread; (6) decrease of auto-immune response, which may, but does not have to, result in the regression or ablation of the disease lesion; (7) relief, to some extent, of one or more symptoms associated with the disorder; (8) increase in the length of disease-free presentation following treatment; and/or (9) decreased mortality at a given point of time following treatment. The term

"responsiveness" refers to a measurable response, including complete response (CR) and partial response (PR).

As used herein, "complete response" or "CR" means the disappearance of all signs of inflammation or remission in response to treatment. This does not necessarily mean the disease has been cured.

"Partial response" or "PR" refers to a decrease of at least 50% in the severity of inflammation, in response to treatment.

A "beneficial response" of a patient to treatment with a therapeutic agent and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for or suffering from a gastrointestinal inflammatory disorder from or as a result of the treatment with the agent. Such benefit includes cellular or biological responses, a complete response, a partial response, a stable disease (without progression or relapse), or a response with a later relapse of the patient from or as a result of the treatment with the agent.

As used herein, "non-response" or "lack of response" or similar wording means an absence of a complete response, a partial response, or a beneficial response to treatment with a therapeutic agent.

"A patient maintains responsiveness to a treatment" when the patient's responsiveness does not decrease with time during the course of a treatment.

A "symptom" of a disease or disorder (e.g., inflammatory bowel disease, e.g., ulcerative colitis or Crohn's disease) is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by a subject and indicative of disease.

Live Biotherapeutics

In some embodiments, the live biotherapeutic (also can be referred to as live cell therapy) includes populations of live bacteria and/or yeast, optionally in combination with a prebiotic such as a non-digestible carbohydrate, oligosaccharide, or short polysaccharide (e.g., one or more of inulin, oligofructose, galactofructose, a galacto-oligosaccharides, or a xylo-oligosaccharide) and/or an antibiotic or antifungal agent, or both an antibiotic and antifungal agent. The bacteria or the yeast can be recombinant. The populations of live bacteria and/or yeast can be used to selectively alter beneficial species within the GI tract and/or to reduce detrimental species within the GI tract of the subject. See, for example, U.S. Patent Publication No. 20070258953; U.S. Patent Publication No. 20080003207; WO2007076534; WO2007136719; and WO2010099824.

In some embodiments, the live biotherapeutic includes one or more species of bacteria (e.g., two or more, three or more, four or more, five or more, six or more, or seven or more species) that are underrepresented in patients with IBD. The microbiotas of Crohn's disease (CD) and ulcerative colitis (UC) patients have statistically significant differences from those of non-inflammatory bowel disease controls, including a reduction in beneficial commensal bacteria in IBD patients relative to non-inflammatory bowel disease patients. For example, members of the phyla Firmicutes (e.g., *Clostridium* clusters XIVa and IV), Bacteroidetes (e.g., *Bacteroides fragilis* or *Bacteroides vulgatus*), and Actinobacteria (e.g., *Coriobacteriaceae* spp. or *Bifidobacterium adolescentis*) are reduced in CD and UC patients. See, e.g., Frank, *et al., Proc Natl Acad Sci U S A*, 2007, 104:13780–13785; Forbes, *et al., Front Microbiol.*, 2016; 7: 1081, and Nagao-Kitamoto and Kamada, *Immune Netw.* 2017 17(1): 1–12. *Clostridium* cluster XIVa includes species belonging to, for example, the *Clostridium*, *Ruminococcus*, *Lachnospira*, *Roseburia*, *Eubacterium*, *Coprococcus*, *Dorea*, and *Butyrivibrio* genera. *Clostridium* cluster IV includes species belonging to, for example, the *Clostridium*, *Ruminococcus*, *Eubacterium* and *Anaerofilum* genera. For example, *Faecalibacterium prausnitzii* (also referred to as *Bacteroides praussnitzii*), *Roseburia hominis*, *Eubacterium rectale*, *Dialister invisus*, *Ruminococcus albus*, *Ruminococcus callidus*, and *Ruminococcus bromii* are less abundant in CD or UC patients. See, e.g., Nagao-Kitamoto and Kamada, 2017, *supra.*

In some embodiments, the live biotherapeutic includes one or more species of bacteria (e.g., two or more, three or more, four or more, five or more, six or more, or seven or more species) that produce a desired product such as a short chain fatty acid (SCFA) (e.g., butyrate, acetate, or propionate) or induce production (e.g., *Clostridium butyricum* or *F. prausnitzii*) of an anti-inflammatory agent such as interleukin-10 in host cells. See, e.g., Hayashi, *et al.*, *Cell Host Microbe* (2013) 13:711–722.

In some embodiments, a live biotherapeutic can be used to reduce detrimental species in the GI tract, including SIBO, and/or otherwise positively modify the composition of the gut flora, using, for example, probiotics (i.e., live nonpathogenic organisms) or prebiotics, optionally in combination with an antibiotic and/or an antifungal agent. The prebiotics can be a non-digestible carbohydrate, oligosaccharide or short polysaccharide such as inulin, oligofructose, galactofructose, a galacto-oligosaccharide, or a xylo-oligosaccharide, or combinations thereof. Non-limiting examples of probiotics include *Lactobacillus* spp. such as *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *L. reuteri*, *L. plantarum*, or *L. casei*, *Bifidobacterium* spp. such as *B. animalis, B. infanti, B lactis, and B longum*, *Escherichia coli* strain Nissle 1917, or yeast such as

Saccharomyces boulardii or *Saccharomyces cerevisiae*. See, e.g., Kruis, *et al.*, *Gut*, 2004, 53:1617–1623, Islam, *Medicine*, 2016, 95(5):e2658; and Sokol, *et al.*, *Gut*, 2016, 1–10. In some embodiments, a probiotic includes a combination of two or more bacterial species (e.g., two or more strains of Lactobacilli such as *L. acidophilus* and *L. bulgaricus*, or *L. rhamnosus* and *L. reuteri*; or a combination of Lactobacilli and Bifodobacterium species such as *L. acidophilus* and *B. lactis* or *L. bulgaricus*). For example, a probiotic can include two or more species of Lactobacilli (e.g., two, three, four, or five species of Lactobacilli), two or more species of Bifidobacteria (e.g., two, three, or four strains of Bifidobacteria), and one or more optional bacteria such as *Streptococcus thermophilus*. For example, a probiotic can be the VSL#3 formulation and include *B. infanti, B lactis, B longum*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, and *Streptococcus thermophilus*. See, e.g., Gionchetti, *et al.*, *Gastroenterology*, 2000, 119:305–309.

In embodiments in which an antibiotic and/or antifungal agent are administered, the antibiotic and/or antifungal agent can be administered before or after the populations of live bacteria and/or yeast, or before or after the prebiotics or probiotics.

In some embodiments, the live biotherapeutic includes one or more species of bacteria (e.g., two or more, three or more, four or more, five or more, six or more, or seven or more species) that are underrepresented in patients with IBD and one or more probiotics (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, or eight or more probiotics).

In some embodiments, the live biotherapeutic is FIN-524 (Finch Therapeutics, Somerville, MA), a cocktail of cultured microbial strains that are linked to positive outcomes among IBD patients.

In some embodiments, the live biotherapeutic includes one or more species of bacteria from a healthy donor (e.g., as collected from a stool sample). See, e.g., Vermeire, *J Crohns Colitis*, 2016, 10(4): 387-394. For example, the live biotherapeutic can be FIN-403 (Finch Therapeutics, Somerville, MA), a candidate for *Clostridium difficile* treatment.

For live biotherapeutics containing bacteria or yeast, the bacteria or yeast can be lyophilized, or can be in saline or other pharmaceutical carrier. The ingestible device can deliver from about 1×10^6 to about 1×10^{12} colony forming units (CFU) of bacteria or yeast (e.g., about 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11}, 1×10^{12} CFU) to the GI tract of the subject. See e.g., Islam, 2016, *supra.* Depending on the volume of the cells, multiple doses of the ingestible device may be required to deliver the desired number of cells to the GI tract.

In some embodiments, the live biotherapeutic can include bacteria or yeast that are genetically modified to produce: one or more products that are anti-inflammatory and/or that can enhance intestinal barrier function, including, for example, interleukin-10, glucagon-like peptide 2 (GLP-2), GLP-1, a short chain fatty acid such as butyrate, propionate, or acetate, IL-2, IL-22, superoxide dismutase (SOD), kynurenine, IL-27, TGF-βI, TGF-2, N-acylphosphatidylethanolamines (NAPEs), elafin (also called peptidase inhibitor 3 or SKALP), trefoil factor, melatonin, PGD2, or kynurenic acid; or one or more products that can inhibit or neutralize (e.g., an antibody or fragment thereof such as a Fab fragment, single-chain antibody, or single domain antibody such as a nanobody, an antisense RNA, a small interfering RNA (siRNA), a short hairpin RNA (shRNA, or a protein)) pro-inflammatory molecules such as TNFα, interleukin 6 receptor (IL-6R), IL-13, IL-18, or p40. See, e.g., WO 2016141108; Neurath, *Nature*, 2014, 7(1): 6-19; Braat, *et al.*, *Clin. Gastroenterol. Hepatol.*, 2006, 4:754–759; and Vandenbroucke, *et al.*, *Mucosal Immunology*, 2010, 3:49–56.

In some embodiments, one or more agents for inhibiting the growth of a fungus (e.g., a yeast such as a species of *Candida*) can be included in the locally released therapeutic. In some subjects with Crohn's disease, the bacterial species of *E. coli* and *Serratia marcescens* and the yeast species *Candida tropicalis* are found at higher concentrations versus that of healthy relatives, indicating that the bacteria and fungus may interact in the intestines. In some embodiments, the agent inhibiting the growth of a fungus (i.e., an anti-fungal agent) is amphotericin B, an echinocandin such as Caspofungin, Micafungin, or Anidulafungin, or an extended-spectrum triazole. In some embodiments, the therapeutic includes about 2.5 mg/L of Amphotericin B.

In some embodiments, the live biotherapeutic can deliver bacteriophage or prophage (i.e., the genetic material of a bacteriophage incorporated into the genome of a bacterium or existing as an extrachromosomal plasmid of the bacterium, and able to produce phages if specifically activated) to the GI tract of the subject. The bacteriophage can be lytic or lysogenic. In some embodiments, the bacteriophage can infect bacteria commonly found in the GI tract. For example, the bacteriophage can infect one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more species of bacteria within the GI tract. See, for example, Wang, *et al.*, *Inflamm Bowel Dis.* 2015; 21(6): 1419–1427. In some embodiments, the bacteriophage can be lytic bacteriophage and infect one or more detrimental bacterial species in the GI tract to reduce the detrimental species in the GI tract. For example, the bacteriophage can infect two or more, three or more, four or more, five or

more, six or more, or seven or more detrimental bacterial species. In some embodiments, bacteriophage can be a member of the families from the order Caudovirales such as *Siphoviridae*, *Myroviridae*, *Podoviridae*, or *Microviridae*. See, e.g., Babickova and Gardlik, *World J. Gastroentrol.* 2015; 21(40):11321-11330. In some embodiments, the bacteriophage can include one or more of bacteriophage K (such as ATCC strain 19685- B l), bacteriophage 17 (such as ATCC strain 23361-B l), and Stab8. See, e.g., WO2016172380A1. In some embodiments, one or more bacteriophages, and one or more probiotics or prebiotics, optionally in combination with an antibiotic, are used to reduce detrimental bacterial species.

In some embodiments, the live biotherapeutic can include bacteriophage or prophage that are genetically modified to produce one or more products that are anti-inflammatory and/or that can enhance intestinal barrier function.

In some embodiments, the ingestible device includes a targeted antimicrobial in which RNA-guided nucleases (RGNs) targeting specific DNA sequences within a target bacteria can be efficiently delivered to microbial populations using bacteriophage or bacteria carrying plasmids. For example, a targeted antimicrobial can couple a phage vector with the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas system (e.g., the biological nanobots from Eligo Bioscience (Eligobiotics)). The biological nanobots can be composed of a capsid from a bacteriophage virus (modified to not multiply) that can deliver the CRISPR/Cas9 system into the targeted bacteria, resulting in the targeted bacteria being killed by cleavage of the bacterial genome by Cas9 enzyme within a predetermined pathogenic sequence. See, for example, WO2017009399A1 and Citorik, *et al.*, *Nat Biotechnol.*, 2014, 32(11): 1141–1145.

For live biotherapeutics containing bacteriophage or prophage, the bacteriophage or prophage can be lyophilized, or can be in saline or other pharmaceutical carrier. Lyophilized phage can be incorporated into tablets or pills without materially reducing efficiency, and can be temperature stable up to 55°C with a shelf life of 14 months. Liquid formulations of phage can be stored at 4°C.

The ingestible device can deliver from about 1×10^4 to about 1×10^{12} plaque forming units (PFU) of bacteriophage (e.g., about 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}, 1×10^{11}, 1×10^{12} PFU) to the GI tract of the subject. For example, between 1×10^6 and 1×10^{10} or between 1×10^7 and 1×10^{11} PFU of a lytic bacteriophage can be delivered to the GI tract. Depending on the volume, multiple doses of the ingestible device may be required to deliver the desired number of cells to the GI tract.

In some embodiments, the live biotherapeutic includes regulatory T cells (Treg cells). For example, from about 10^6 to about 10^9 (e.g., 10^6, 10^7, 10^8, or 10^9) autologous Treg cells (e.g., ova-specific T cells) can be delivered to the GI tract of the subject. Autologous Treg cells can be prepared by isolating peripheral blood mononuclear cells (PBMCs) from the subject's blood and then expanding ova-specific T cells by culturing the PBMCs in the presence of ovalbumin using *Drosophila* derived artificial antigen presenting cells transfected with specific stimulatory molecules. See, e.g., Brun, *et al.*, *Int Immunopharmacol.*, 2009, 9(5):609-13. T cells can be cloned and Ova-Treg clones can be selected based on an ovalbumin-specific IL-10 production. A phase 1/2a study in 20 patients showed that a single injection of antigen-specific (ovalbumin) Treg cells was safe in CD and about 40% of the patients show a clinical response after treatment. See, e.g., Neurath, 2014, *supra*; and Desreumaux, *et al.*, *Gastroenterology*, 2012, 143:1207–1217.

In some embodiments, the live biotherapeutic can deliver stem cells to the GI tract of the subject. The delivery of stem cells into the GI tract can offer measurable therapeutic benefit as the stem cells have the ability to differentiate into numerous different cell types, and rejuvenate the surrounding area. In some embodiments, a population of cells is delivered within the GI tract that includes at least about 50% stem cells (at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% stem cells). The term "stem cell" is used herein to refer to a cell that is capable of differentiating into a two or more different cell types. As used herein, the term "a stem cell" may refer to one or more stem cells.

In some embodiments, the stem cells can be hematopoietic stem cells (HSC) capable of differentiating into different types of blood cells, including myeloid and lymphoid lineages of blood cells. HSC can be obtained from bone marrow, cord blood, or peripheral blood, and are commonly used for bone marrow transfusions in combination with chemotherapy to restart the immune system. HSC are $CD34^+$ cells. Cell-surface markers can be identified by any suitable conventional technique, including, for example, positive selection using monoclonal antibodies against cell-surface markers.

The HSC used in the methods described herein can be, with respect to the subject, autologous or allogenic. HSC have a high immunogenicity. As a result, if autologous HSC are not used, the HLA receptors of the donor and receiver must be matched. HSC can be harvested by mobilizing stem cells from the subject (autologous) or an HLA-matched donor (allogeneic)

using granulocyte colony stimulating factor (GCSF) to promote the creation of HSC and their migration into the bloodstream. $CD34^+$ cells can be collected from the peripheral blood or BM of the subject or donor, and then the cells can be cryopreserved until infusion or can be placed in a medium such as an alginate hydrogel. When stored for five days in an alginate-hydrogel in ambient temperature in an air-tight environment, stem cells showed a survival rate of 74-80%. For IBD, HSC therapy is preceded by chemotherapy, which removes the majority of the T-cells causing the inflammation, followed by administration of the HSC in the ingestible device.

In some embodiments, the stem cells used in the methods described herein are capable of differentiating into two or more different cell types other than blood cells. In some embodiments, the stem cells are capable of differentiating into cells of each of the three embryonic germ layers (i.e., endoderm, ectoderm, and mesoderm). As used herein, "capable of differentiating" means that a given cell, or its progeny, can proceed to a differentiated phenotype under the appropriate culture conditions. The capacity of the cells to differentiate into at least two cell types can be assayed by methods known in the art.

Non-limiting examples of stem cells include embryonic stem cells or adult stem cells such as mesenchymal stem cells (MSC) (also can be referred to as mesenchymal stromal cells) or other multipotent stem cells; endothelial progenitor cells; stem cells from a particular tissue or organ such as intestinal stem cells, adipose stem cells, or testes stem cells; or induced pluripotent stem cells (iPSC). In some embodiments, stem cells from a particular tissue also can be classified as MSC.

In some embodiments, the stem cells are MSC, which can differentiate into bone, muscle, cartilage, or adipose type cells. MSC can down-regulate inflammation and have a strong immunoregulatory potential. MSC can be obtained from various tissues, including from, for example, bone marrow, placenta, amniotic fluid, Wharton's jelly, amniotic membrane, chorionic villi, umbilical cord, umbilical cord blood, adipose tissue, dental pulp, synovial membrane, or peripheral blood. Depending on the source of MSC and the stemness (i.e., multipotency), the MSC can express a variety of different markers, including, for example, one or more of CD105, CD73, CD90, CD13, CD29, CD44, CD10, Stro-1, CD271, SSEA-4, CD146, CD49f, CD349, GD2, 3G5, SSEA-3, SISD2, Stro-4, MSCA-1, CD56, CD200, PODXl, Sox11, or TM4SF1 (e.g., 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more , 9 or more, or 10 or more of such markers), and lack expression of one or more of CD45, CD34, CD14, CD19, and HLA-DR (e.g., lack expression of two or more, three or more, four or more, or five or more such markers). In some embodiments, MSC can express CD105, CD73, and CD90. In some

embodiments, MSC can express CD105, CD73, CD90, CD13, CD29, CD44, and CD10. In some embodiments, MSC can express CD105, CD73, and CD90 and one or more stemness markers such as Stro-1, CD271, SSEA-4, CD146, CD49f, CD349, GD2, 3G5, SSEA-3. SISD2, Stro-4, MSCA-1, CD56, CD200, PODXl, Sox11, or TM4SF1. In some embodiments, MSC can express CD105, CD73, CD90, CD13, CD29, CD44, and CD10 and one or more stemness markers such as Stro-1, CD271, SSEA-4, CD146, CD49f, CD349, GD2, 3G5, SSEA-3. SISD2, Stro-4, MSCA-1, CD56, CD200, PODXl, Sox11, or TM4SF1. See, e.g., Lv, *et al.*, *Stem Cells*, 2014, 32:1408-1419.

The MSC used in the methods described herein can be, with respect to the subject, autologous or allogenic. MSC have a low immunogenicity due to a dearth of HLA receptors on their surface. As a result, allogenic MSC therapy is a far more viable option for patients. Furthermore, MSC are able to downregulate the immune system, which can modulate the inflammation caused in many autoimmune disorders.

In some embodiments, MSC are commercially available. See, e.g. Prochymal® from Osiris Therapeutics.

In some embodiments, MSC can be harvested from bone marrow by *ex vivo* culture of the adherent cell fraction of bone marrow aspirates. The solid surface to which the MSC adhere can be a plastic material such as a polystyrene plate, optionally coated with poly-D-Lysine, laminin, or other reagent.

In some embodiments, the stem cells can be PF-05285401 cells (Multistem® cells), which are human stem cells obtained from adult bone marrow or other nonembryonic tissue sources. Multistem® cells are commercially available from Athersys Inc.

In some embodiments, MSC can be harvested from adipose tissue such as brown or white adipose tissue from subcutaneous, omental/visceral, mammary, gonadal, or other adipose tissue site. For example, the MSC can be harvested from subcutaneous white adipose tissue such as that isolated from liposuction. The cells can be harvested by mincing the adipose tissue and washing to remove blood. If the adipose tissue is obtained from a liposuction procedure, mincing is not required. The adipose tissue can be incubated with an enzyme such as Type I collagenase, and the stromal vascular fraction (SVF), which contains a variety of cell types including MSC, can be recovered and the MSC selected from the mixed cell population by adherence to a solid surface such as plastic cell culture surface optionally coated as described above. Yield of MSC from adipose tissue is up to 300-fold higher than the yield of MSC from bone marrow. See, e.g., Fellows, *et al., Front Genet.* 2016, 7:213; Lechanteur, *et al., J Transl Med.* 2016; 14: 145. In

some embodiments, the stem cells can be autologous adipose derived stem cells such as Cx401cells.

In some embodiments, the MSC can be expanded in cultured, e.g., passaged at least two times, at least three times, at least four times, at least five times, at least six times, at least seven times, at least eight times, at least nine times, or at least ten times. In some embodiments, cells can be passaged more than three times to improve the homogeneity of the cell phenotype in the cell population. In some embodiments, the cells can be expanded in culture until needed, so long as the homogeneity of the cell phenotype is improved and differential capacity is maintained. See Martin, *et al.*, *Cytotherapy*, 2016, 18(5): 613-620 for methods of culturing MSCs.

The MSC can be cryopreserved until formulated for the ingestible device or can be placed in a medium such as an alginate hydrogel. The viability of MSC cells stored in alginate hydrogels after 50 days at ambient temperature is 80%.

In some embodiments, the MSC can be coated with antibodies, e.g., anti-vascular cell adhesion molecule-1 (e.g., VCAM-1) antibodies or anti-addressin antibodies, to improve, for example, their ability to target and hone into inflamed lesions in the GI tract. See, e.g., Ko, *et al.*, *Mol Ther.* 2010, 18(7): 1365–1372.

In some embodiments, human iPSCs can be generated from adult somatic cells (e.g., fibroblasts, keratinocytes, dental pulp cells, cord blood, or peripheral blood mononuclear cells) or MSC. iPSCs can be generated using retroviral or non-retroviral methods. See, for example, Loh, *et al.*, *Blood* 2009, 113:5476–5479, Okita, *et al.*, *Nat Methods.* 2011, 8(5):409-12, or Okita, *et al.*, *Stem Cells*, 2013, 31(3): 458–466. In some embodiments, p53 suppression and nontransforming L-Myc can be used to generate human induced pluripotent stem cells (iPSCs) with episomal plasmid vectors encoding OCT3/4, SOX2, KLF4, and LIN28. In some embodiments, adult somatic cells can be transduced with retroviruses encoding four pluripotency factors (SOX2, KLF4, c-MYC, and OCT4). Fully reprogrammed iPSCs have similar properties to embryonic stem cells (ESCs). Patient's cells can be used to derive iPSCs, which can then be induced to undergo differentiation into various types of somatic cells, all with the same genetic information as the patient. See, Azizeh-Mitra, *et al.*, *Stem Cells Int.* 2016; 6180487. In other embodiments, allogenic cells are used to derive iPSCs.

In some embodiments, the stem cells can be intestinal stem cells (ISC), which can differentiate into intestinal cell subtypes such as globet cells, Paneth cells, and enterocytes. ISC are located at the crypt base within the intestine and can be positive for one or more markers such as Musashi-1 (Msi-1), Ascl2, Bmi-1, Doublecortin and Ca^{2+}/calmodulin-dependent kinase-like 1

(DCAMKL1), and Leucin-rich repeat-containing G-protein-coupled receptor 5 (Lgr5). See, e.g., Mohamed, *et al.*, *Cytotechnology*, 2015 67(2): 177–189. In addition, ISC or crypts can be used to produce intestinal organoids using a biodegradable scaffold (e.g., poly-glycolic acid), growth factors such as epidermal growth factor (EGF), R-spondin, Jagged-1 peptide, or Noggin, and extracellular matrix. In some embodiments, mesenchymal cells are included in the culture to support the growth. The intestinal organoid can include a central lumen lined by a villus-like epithelium. See, e.g., US20160287670A1 and WO2015183920A2. Pre-clinical studies have demonstrated the intestinal organoid efficacy in differentiating into all GI cell lineages and regrowing parts of the intestine, muscle layer included. See, Agopian, *et al.*, *J Gastrointest Surg.*, 2009, 13(5):971-82; Kuratnik and Giardina, *Biochem Pharmacol.*, 2013, 85:1721–1726; and Belchior *et al.*, *Semin Pediatr Surg.*, 2014, 23:141–149.

In some embodiments, the stem cells can be allogeneic adipose-derived stem cells (ASC) such as ALLO-ASC cells or expanded ASC (eASC) (e.g., Cx601 cells). See, for example, Panes *et al.*, Lancet; 2016, 388: 1281-90; and U.S. Patent Publication No. 20120020930. Cx601 cells are commercially available from TiGenix. Cx601 cells have been used for treating complex perianal fistulas in Crohn's disease patients. For example, Cx601 cells can be used for treating complex perianal fistulas with non-active/mildly active luminal Crohn's disease. ALLO-ASC cells are commercially available from Anterogen Co., Ltd., and have been used for treating Crohn's disease.

Methods for isolating and culturing ASCs to provide eASCs, and compositions comprising such cell populations are known in the art. See, e.g., U.S. Patent Application No. 20170258851A1. See also Martin, *et al.*, *Cytotherapy*, 2016, 18(5): 613-620 for methods of culturing MSCs. Typically, methods for preparing compositions comprising cell populations can include the following steps:

(i) isolating ASCs from the stromal fraction of adipose tissue and selection by adherence to a suitable surface, e.g., plastic; and

(ii) expanding ASCs to provide cell populations comprising eASCs. Optionally, the cell populations may be subjected to one or more of the following: cryopreserving during and/or subsequent to the expansion step (ii); assessing the phenotype of the cell populations during and/or subsequent to the expansion step (ii); and isolating the cell populations subsequent to the expansion step (ii) and resuspended in a pharmaceutically acceptable carrier and/or diluents.

ASCs can be obtained by any methods standard in the art. Typically the cells are obtained by disassociating the cells from the source tissue (e.g., lipoaspirate or adipose tissue), typically

by treating the tissue with a digestive enzyme such as collagenase. The digested tissue matter is then typically filtered through a filter of between about 20 microns to 1 mm. The cells are then isolated (typically by centrifugation) and cultured on an adherent surface (typically tissue culture plates or flasks). Such methods are known in the art. See, e.g., the methods as disclosed in U.S. Pat. No. 6,777,231. According to this methodology, lipoaspirates are obtained from adipose tissue and the cells derived therefrom. In the course of this methodology, the cells may be washed to remove contaminating debris and red blood cells, preferably with PBS. The cells are digested with collagenase (e.g., at 37°C for 30 minutes, 0.075% collagenase; Type I, Invitrogen, Carlsbad, Calif.) in PBS. To eliminate remaining red blood cells, the digested sample can be washed (e.g., with 10% fetal bovine serum), treated with 160 mmol/L NH_4Cl, and finally suspended in DMEM complete medium (DMEM containing 10% FBS, 2 mmol/L glutamine and 1% penicillin/streptomycin). The cells can be filtered through a 40-µm nylon mesh.

The cells can be cultured in a suitable tissue culture vessel, comprising a surface suitable for the adherence of ASCs, e.g., plastic. Non-adherent cells can be removed, e.g., by washing in a suitable buffer, to provide an isolated population of adherent stromal cells (e.g., ASC). Cells isolated in this way can be seeded (preferably 2-3 x 10^4 cells/cm^2) onto tissue culture flasks and expanded at 37°C and 5% CO_2, changing the culture medium every 3-4 days. Cells are preferably detached from the adherent surface (e.g., using trypsin) and passed ("passaged") to a new culture flask (1,000 cells/cm^2) when cultures reach around 90% of confluence.

Cell isolation is preferably carried out under sterile or GMP conditions.

In one embodiment, a method comprises: (a) collecting adipose tissue from a subject; (b) obtaining a cell suspension by enzymatic digestion; (c) sedimenting the cell suspension and resuspending the cells in a culture medium; (d) culturing of the cells for at least about 10 days; and (g) expanding the cells for at least two culture passages. The adipose tissue-derived stromal stem cells are alloegenic, i.e. not isolated from the adipose tissue of the subject into which the final adipose tissue-derived stromal stem cell-containing composition is to be introduced.

In certain embodiments, the cells are cultured for at least about 15, at least about 20 days, at least about 25 days, or at least about 30 days. Typically the expansion of cells in culture improves the homogeneity of the cell phenotype in the cell population, such that a substantially pure or homogenous population is obtained.

In certain embodiments, the cells are expanded in culture for at least three culture passages or "passaged at least three times." In other embodiments, the cells are passaged at least four times, at least five times, at least six times, at least seven times, at least eight times, at least

nine times, or at least ten times. It is preferable that cells are passaged more than three times to improve the homogeneity of the cell phenotype in the cell population. Indeed, the cells may be expanded in culture indefinitely so long as the homogeneity of the cell phenotype is improved and differential capacity is maintained.

In certain embodiments, the cells are multiplied in culture for at least three population doublings. In certain embodiments, the cells are expanded in culture for at least four, five, six, seven, eight, nine, ten, 15 or 20 population doublings. In certain embodiments, the cells are expanded in culture for less than seven, eight, nine, ten, 15 or 20 population doublings. In certain embodiments, the cells are expanded in culture for between about 5 and 10 population doublings. In certain embodiments, the cells are expanded in culture for between about 10 and 15 population doublings.

In certain embodiments, the cells are expanded in culture for between about 15 and 20 population doublings, for example about 16 population doublings.

Cells may be cultured by any technique known in the art for culturing stem cells. A discussion of various culture techniques, as well as their scale-up, may be found in Freshney, R. I., Culture of Animal Cells: A Manual of Basic Technique, 4th Edition, Wiley-Liss 2000. Cells may be expanded using culture flasks or bioreactors suitable for large-scale expansion. Bioreactors suitable for the large-scale expansion of mesenchymal stromal cells are commercially available and may include both 2D (i.e. substantially planar) and 3D expansion bioreactors. Examples of such bioreactors include, but are not limited to, a plug flow bioreactor, a perfusion bioreactor, a continuous stirred tank bioreactor, a stationary-bed bioreactor. In certain embodiments, the cells are cultured by monolayer culture.

In some embodiments, microcarriers can be used to expand stem cells such as MSC (e.g., allogeneic adipose-derived mesenchymal stem cells). For example, collagen-coated Cytodex-3 (GE Healthcare) and gelatin-coated CultispherS (Percell) microcarriers can be used for human MSC production in serum-containing media or Corning Synthemax II-coated microcarriers in spinner flasks can be used for human MSC under xeno-free, defined conditions (e.g., Mesencult XF (STEMCELL Technologies) medium or stemgro hMSC (Corning) medium). See, Hervy, *et al.*, 2014, *PLoS ONE* 9(3): e92120.

Any medium capable of supporting stromal cells in tissue culture may be used. Media formulations that will support the growth of fibroblasts include, but are not limited to, Dulbecco's Modified Eagle's Medium (DMEM), alpha modified Minimal Essential Medium (alpha.MEM), and Roswell Park Memorial Institute Media 1640 (RPMI Media 1640) and the like. Typically, 0

to 20% Fetal Bovine Serum (FBS) or 1-20% horse serum will be added to the above media in order to support the growth of stromal cells and/or chondrocytes. However, a defined medium could be used if the necessary growth factors, cytokines, and hormones in FBS for stromal cells and chondrocytes are identified and provided at appropriate concentrations in the growth medium. Media useful in the methods of the invention may contain one or more compounds of interest, including, but not limited to antibiotics mitogenic or differentiative compounds for stromal cells. The cells will be grown at temperatures between 31.degree. C. to 37.degree. C. in a humidified incubator. The carbon dioxide content will be maintained between 2% to 10% and the oxygen content between 1% and 22%. Cells may remain in this environment for periods of up to 4 weeks.

In some embodiments, the stem cells can be QBECO SSI cells (Qu Biologics, compassionate use Phase 2 in ulcerative colitis).

In some embodiments, the stem cells can be human placental derived stem cells such as PDA-001 cells from Celgene. PDA-001 cells are a culture-expanded, plastic adherent, undifferentiated *in vitro* cell population that express the nominal phenotype CD34-, CD10+, CD105+ and CD200+. PDA-001 cells constitutively express moderate levels of HLA Class I and undetectable levels of HLA Class II, and they do not express the co-stimulatory molecules CD80 and CD86. PDA-001 is genetically stable, displaying a normal diploid chromosome count, normal karyotype and exhibit normal senescence after prolonged in vitro culture. See, e.g., U.S. Patent No. 8,916,146.

For live biotherapeutics containing stem cells, the cells can be formulated to include one or more additional compounds such as a growth factor, a plurality of different growth factors, an anti-inflammatory agent, an immunosuppressive agent, a biological agent, an antibiotic, an anti-diarrheal agent, an adhesive, or other compounds that affect cell differentiation and/or proliferation. For example, the live biotherapeutic can be formulated to include one or more of the following: granulocyte colony-stimulating factor (G-CSF); an anti-inflammatory agent such as an agent comprising mesalamine; an immunosuppressive agent such as 6-mercaptopurine or azathioprine; a biological agent such as infliximab (Remicade®); an antibiotic, an antidiarrheal agent such as diphenoxylate, loperamide, and codeine; or an adhesive such as fibrin.

Pharmaceutically acceptable carriers and diluents include saline, aqueous buffer solutions, solvents and/or dispersion media. The use of such carriers and diluents is well known in the art. The solution is typically sterile and fluid to the extent that easy syringability exists. Typically, the solution is stable under the conditions of manufacture and storage and preserved against the

contaminating action of microorganisms such as bacteria and fungi through the use of, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. Solutions that are adipose tissue-derived stromal stem cell compositions of the invention can be prepared by incorporating adipose tissue-derived stromal stem cells as described herein in a pharmaceutically acceptable carrier or diluent and, as required, other ingredients enumerated above, followed by filtered sterilization.

Some examples of materials and solutions which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

In certain embodiments, the adipose tissue-derived stromal stem cell-containing compositions further comprise an adhesive. In certain embodiments, the adhesive is a fibrin-based adhesive, such as a fibrin gel or fibrin glue or fibrin-based polymer or adhesive, or other tissue adhesive or surgical glue, such as, for example cyanoacrylate, collagen, thrombin, and polyethylene glycol. Other materials that may be used include but are not limited to calcium alginate, agarose, types I, II, IV or other collagen isoform, poly-lactic/poly-glycolic acid, hyaluronate derivatives or other materials (Perka C. et al. (2000) J. Biomed. Mater. Res. 49:305-311; Sechriest V F. et al. (2000) J. Biomed. Mater. Res. 49:534-541; Chu C R et al. (1995) J. Biomed. Mater. Res. 29:1147-1154; Hendrickson D A et al. (1994) Orthop. Res. 12:485-497). In other embodiments, the adhesive is a liquid bandage, wherein adipose tissue-derived stromal stem cell-containing compositions of the method are mixed with the liquid bandage material. A "liquid bandage" is a solution comprising a compound, e.g. a polymeric material, which is applied to a wound with a spray or a brush, followed by removing the solvent by vaporization to provide a protective film on the wound.

The ingestible device can deliver from about 1 x 10^6, 2 x 10^6, 3 x 10^6, 4 x 10^6, 5 x 10^6, 6 x 10^6, 7 x 10^6, 8 x 10^6, 9 x 10^6, 10 x 10^6, 20 x 10^6, 30 x 10^6, 40 x 10^6, 50 x 10^6, 60 x 10^6, 70 x 10^6, 80 x 10^6, 90 x 10^6, 100 x 10^6, 110 x 10^6, 120 x 10^6, 130 x 10^6, 200 x 10^6, 300 x 10^6, 400 x 10^6 or more cells to the GI tract. For example, in some embodiments, about 1 million to about 20 million stem cells (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 million stem cells) per 100 μL of a solution (e.g., a culture media, including phosphate buffered saline, or a pharmaceutically acceptable carrier or diluent, in which cells remain viable) are delivered to the GI tract. In some embodiments, about 1 million to about 20 million stem cells (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 million stem cells) per 1 mL of a solution (e.g., a culture media or a pharmaceutically acceptable carrier or diluent, in which cells remain viable) are delivered to the GI tract. Total number of cells delivered to the GI tract depends on the total volume of solution. For example, in some embodiments, 10 million cells can be delivered to the GI tract using 100 μL of a solution containing 10 million cells/100 μL. For example, in some embodiments, 120 million cells can be delivered to the GI tract using 24 mL of a solution containing 5 million cells/mL. In some embodiments, the solution containing the stem cells can be stored at 15-25°C for 24-72 hours, preferably 48 hours, before use. In some embodiments, the solution can include one or more additional components such as a growth factor, a plurality of different growth factors, an anti-inflammatory agent, an immunosuppressive agent, a biological agent, an antibiotic, an anti-diarrheal agent, an adhesive, or other compounds that affect cell differentiation and/or proliferation. Depending on the volume of the cells, multiple doses may be required to deliver the desired number of cells.

In some embodiments, the ingestible device includes G-CSF, which can stimulate the bone marrow to produce granulocytes and stem cells and release them into the bloodstream. Nartograstim, myelopoietins, circularly permuted G-CSF sequences, and SB247464 are among the known mimetics of G-CSF.

In some embodiments, medium conditioned by the stem cells can be delivered to the GI tract of the subject. For example, conditioned medium that includes one or more biomolecules secreted or excreted by the stem cells can be delivered to the GI tract of a subject. In some embodiments, the conditioned medium comprises medium in which the stem cells (e.g., MSC or intestinal stem cells) have grown for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 or more days. In some embodiments, the conditioned medium comprises medium in which the stem cells (e.g., MSC or intestinal stem cells) have grown to at least 30%, 40%, 50%, 60%, 70%, 80%, or

90% confluence, or up to 100% confluence. In some embodiments, the conditioned medium comprises medium in which the stem cells have been differentiated into an adult cell type.

In some embodiments, the ingestible device includes an enzyme such as glutathione S-transferase. For example, the ingestible device can include P28GST, a 28 kDa helminth protein from *Schistosoma* with potent immunogenic and antioxidant properties. P28GST prevents intestinal inflammation in experimental colitis through a Th2-type response with mucosal eosinophils and can be recombinantly produced (e.g., in *S. cerevisiae*). See, for example, U.S. Patent No. 9,593,313, Driss *et al.*, *Mucosal Immunology*, 2016 9, 322–335; and Capron *et al.*, *Gastroenterology*, 146(5):S-638. The ingestible device can deliver from about 0.5 mg/ml to 5 mg/ml, 0.5 mg/ml to 2 mg/ml, from 0.3 mg/ml to 1 mg/ml, from 220 µg/ml to 280 µg/ml, or approximately equal to 250 µg/ml of P28GST to the GI tract of the subject.

In some embodiments, the ingestible device includes an ErbB4 receptor tyrosine kinase agonist. An exemplary ERbB4 agonist is Neuregulin-4, which is a selective ligand for ErbB4. Neuregulin-4 is present in GI tissue as well as breast milk. Neuregulin-4 levels are decreased in IBD patients while ErbB4 is present at elevated levels in the inflamed colonic mucosa of IBD patients. Neuregulin-4 exhibits protective and restorative effects on mucosal intestinal tissue. See, Harari, *et al.*, *Oncogene*, 1999, 18:2681-2689; Schumaker, *et al.*, *Cell Death Dis.*, 2017, 8(2): e2622; Bernard, *et al.*, *J. Biol. Chem.*, 2012, 287: 39850–39858; McElroy, *et al.*, *Am. J. Pathol.*, 2014, 184: 2768–2778; PCT/US2012/050970 and PCT/US2016/054027. The ingestible device can deliver from about 0.1 µg/kg to 3 mg/kg of an ErbB4 receptor tyrosine kinase agonist such as Neuregulin-4 to the GI tract of the subject.

In some embodiments, therapeutics that may be used for wound healing (e.g., one or more fistulas or lesions). The therapeutic agent may may comprise nanofiber dressings that can be applied to the site of disease, wherein the dressings comprise fibrous fibronectin nanofibers or soy-based nanofibers that have estrogen-like molecules as described by Chantre, *et al.*, "Production-scale fibronectin nanofibers promote wound closure and tissue repair in a dermal mouse model." *Biomaterials*, (2018) 166:96-108; and Ahn, Seungkuk, *et al.* "Soy Protein/Cellulose Nanofiber Scaffolds Mimicking Skin Extracellular Matrix for Enhanced Wound Healing." Advanced healthcare materials (2018). Dressing may be applied to the GI tract of a subject using a devive that releases the dressing at or near the site of disease.

In some embodiments, the methods described herein can include deep scraping of at least one fistula tract and/or filling of at least one fistula tract with a material such as a fibrin-based polymer or adhesive, such as a fibrin glue or gel. The material can include a therapeutically

effective dose of any of the therapeutic agents, including stem cells, described herein (e.g., at least about 10 x 10^6 adipose tissue-derived stromal stem cells). In some embodiments, the dose of at least about 10 x 10^6 adipose tissue-derived stromal stem cells is already encompassed (e.g., coated, embedded, or encapsulated) within the material, e.g., such that the material comprises the adipose tissue-derived stem cell containing-composition.

The methods described above may further comprise administering a therapeutic agent to the subject being treated, e.g., systemically or locally at a site of suturing. In certain embodiments, the adipose tissue-derived stromal stem cells are formulated in an adipose tissue-derived stromal stem cell-containing composition which contains a therapeutic agent, as described above. In other embodiments, the therapeutic agent is administered separately, e.g. simultaneously with the methods, before the method is performed, or after the method is performed. In some embodiments, the therapeutic agent is administered to the subject before, during and after the methods are performed on the subject. Exemplary therapeutic agents are described above. In preferred embodiments, therapeutic agents for the treatment of Crohn's disease are administered to the subject.

In some embodiments, supportive treatment may be required. For example, immunosuppressants may be administered before, during and/or after treatment to prevent graft versus host disease (GVHD), according to methods known in the art. Prior to administration, the cells may also be modified to suppress an immune reaction from the subject to the cells or vice-versa, according to methods known in the art.

The dosage of any therapeutic agent will vary depending on the symptoms, age and body weight of the patient, the nature and severity of the disorder to be treated or prevented, the route of administration, and the form of the agent. Any of the subject formulations may be administered in a single dose or in divided doses. Dosages for the therapeutic agents may be readily determined by techniques known to those of skill in the art or as taught herein. Also, mixtures of more than one therapeutic agent may be administered, or multiple therapeutic agents administered in separate compositions.

The precise time of administration and amount of any particular agent that will yield the most effective treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), route of administration, and the like. The guidelines presented herein may be used to optimize the treatment, e.g., determining the optimum time and/or amount of

administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

While the subject is being treated, the health of the patient may be monitored by measuring one or more of the relevant indices at predetermined times during a 24-hour period. Treatment, including supplement, amounts, times of administration and formulation, may be optimized according to the results of such monitoring. The patient may be periodically reevaluated to determine the extent of improvement by measuring the same parameters, the first such reevaluation typically occurring at the end of four weeks from the onset of therapy, and subsequent reevaluations occurring every four to eight weeks during therapy and then every three months thereafter. Therapy may continue for several months or even years, with a minimum of one month being a typical length of therapy for humans. Adjustments to the amount(s) of agent administered and possibly to the time of administration may be made based on these reevaluations.

Endoscopes, Ingestible Devices, and Reservoirs

As discussed herein, in some embodiments, a method of treating a disease of the gastrointestinal tract comprises administering to the subject a pharmaceutical formulation wherein the pharmaceutical formulation is delivered proximate to one or more sites of disease by one of various methods. For example, the pharmaceutical formulation may be delivered via a medical device such as an endoscope, ingestible device, or reservoir; the pharmaceutical formulation may be a solid dosage form, a liquid dosage form, a suppository or an enema for rectal administration with different types of release such as sustained or delayed release.

In one embodiment, the pharmaceutical formulation is delivered proximate to one or more sites of disease by an endoscope, ingestible device, or reservoir containing the pharmaceutical formulation.

The GI tract can be imaged using endoscopes, or more recently, by ingestible devices that are swallowed. Direct visualization of the GI mucosa is useful to detect subtle mucosal alterations, as in inflammatory bowel diseases, as well as any flat or sessile lesions.

As discussed herein, in some embodiments, the method of treating a disease of the gastrointestinal tract comprises administering to the subject a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation is delivered proximate to one or more sites of disease by one of various methods. For example, the pharmaceutical formulation may be delivered via a medical device such as an endoscope, ingestible device, or reservoir; the

pharmaceutical formulation may be a solid dosage form, a liquid dosage form, a suppository or an enema for rectal administration with different types of release such as sustained or delayed release.

In one embodiment, the pharmaceutical formulation is delivered proximate to one or more sites of disease by an endoscope, ingestible device, or reservoir containing the pharmaceutical formulation.

The technology behind standard colonoscopy consists of a long, semi-rigid insertion tube with a steerable tip (stiff if compared to the colon), which is pushed by the physician from the outside. However, invasiveness, patient discomfort, fear of pain, and –more often than not– the need for conscious sedation limit the take-up of screening colonoscopy. Diagnosis and treatment in the GI tract are dominated by the use of flexible endoscopes. A few large companies, namely Olympus Medical Systems Co. (Tokyo, Japan), Pentax Medical Co. (Montvale, NJ, USA), Fujinon, Inc. (Wayne, NJ, USA) and Karl Storz GmbH & Co. KG (Tuttlingen, Germany), cover the majority of the market in flexible GI endoscopy.

Endoscopes may comprise a catheter. As an example, the catheter may be a spray catheter. As an example, a spray catheter may be used to deliver dyes for diagnostic purposes. As an example, a spray catheter may be used to deliver a therapeutic agent at the site of disease in the GI tract. For example, the Olympus PW-205V is a ready-to-use spray catheter that enables efficient spraying for maximal differentiation of tissue structures during endoscopy, but may also be used to deliver drugs diseased tissue.

In a review of robotic endoscopic capsules, Journal of Micro-Bio Robotics 11.1-4 (2016): 1-18, Ciuti et al. state that progress in micro-electromechanical systems (MEMS) technologies have led to the development of new endoscopic capsules with enhanced diagnostic capabilities, in addition to traditional visualization of mucosa (embedding, e.g. pressure, pH, blood detection and temperature sensors).

Endoscopic capsules, however, do not have the capability of accurately locating a site autonomously. They require doctor oversight over a period of hours in order to manually determine the location. Autonomous ingestible devices are advantageous in that regard.

Ingestible devices are also advantageous over spray catheters in that they are less invasive, thereby allowing for regular dosing more frequently than spray catheters. Another advantage of ingestible devices is the greater ease with which they can access, relative to a catheter, certain sections of the GI tract such as the ascending colon, the cecum, and all portions of the small intestine.

Methods and Mechanisms for Localization

In addition to, or as an alternative, to directly visualizing the GI tract, one or more different mechanisms can be used to determine the location of an ingestible device within the GI tract. Various implementations may be used for localization of ingestible devices within the GI tract.

For example, certain implementations can include one or more electromagnetic sensor coils, magnetic fields, electromagnetic waves, electric potential values, ultrasound positioning systems, gamma scintigraphy techniques or other radio-tracker technology have been described by others. Alternatively, imaging can be used to localize, for example, using anatomical landmarks or more complex algorithms for 3D reconstruction based on multiple images. Other technologies rely on radio frequency, which relies on sensors placed externally on the body to receive the strength of signals emitted by the capsule. Ingestible devices may also be localized based on reflected light in the medium surrounding the device; pH; temperature; time following ingestion; and/or acoustic signals.

The disclosure provides an ingestible device, as well as related systems and methods that provide for determining the position of the ingestible device within the GI tract of a subject with very high accuracy. In some embodiments, the ingestible device can autonomously determine its position within the GI tract of the subject.

Typically, the ingestible device includes one or more processing devices, and one more machine readable hardware storage devices. In some embodiments, the one or more machine readable hardware storage devices store instructions that are executable by the one or more processing devices to determine the location of the ingestible device in a portion of a GI tract of the subject. In certain embodiments, the one or more machine readable hardware storage devices store instructions that are executable by the one or more processing devices to transmit data to an external device (e.g., a base station external to the subject, such as a base station carried on an article worn by the subject) capable of implementing the data to determine the location of the device within the GI tract of the subject.

In some embodiments, the location of the ingestible device within the GI tract of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. In some embodiments, the location of the ingestible device within the GI tract of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. In such embodiments,

the portion of the GI tract of the subject can include, for example, the esophagus, the stomach, duodenum, the jejunum, and/or the terminal ileum, cecum and colon. An exemplary and non-limiting embodiment is provided below in Example 14.

In certain embodiments, the location of the ingestible device within the esophagus of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. An exemplary and non-limiting embodiment is provided below in Example 14.

In some embodiments, the location of the ingestible device within the stomach of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. An exemplary and non-limiting embodiment is provided below in Example 14.

In certain embodiments, the location of the ingestible device within the duodenum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. An exemplary and non-limiting embodiment is provided below in Example 14.

In some embodiments, the location of the ingestible device within the jejunum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. An exemplary and non-limiting embodiment is provided below in Example 14.

In certain embodiments, the location of the ingestible device within the terminal ileum, cecum and colon of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In some embodiments, the location of the ingestible device within the cecum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%. An exemplary and non-limiting embodiment is provided below in Example 14. In such embodiments, the portion of the portion of the GI tract of the subject can include, for example, the esophagus, the stomach, duodenum, the jejunum, and/or the terminal ileum, cecum and colon.

In certain embodiments, the location of the ingestible device within the esophagus of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In some embodiments, the location of the ingestible device within the stomach of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In certain embodiments, the location of the ingestible device within the duodenum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In some embodiments, the location of the ingestible device within the jejunum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In certain embodiments, the location of the ingestible device within the terminal ileum, cecum and colon of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

In some embodiments, the location of the ingestible device within the cecum of the subject can be determined to an accuracy of at least 85%, e.g., at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, 100%.

As used herein, the term "reflectance" refers to a value derived from light emitted by the device, reflected back to the device, and received by a detector in or on the device. For example, in some embodiments this refers to light emitted by the device, wherein a portion of the light is reflected by a surface external to the device, and the light is received by a detector located in or on the device.

As used herein, the term "illumination" refers to any electromagnetic emission. In some embodiments, an illumination may be within the range of Infrared Light (IR), the visible spectrum and ultraviolet light (UV), and an illumination may have a majority of its power centered at a particular wavelength in the range of 100nm to 1000nm. In some embodiments, it may be advantageous to use an illumination with a majority of its power limited to one of the infrared (750nm-1000nm), red (600nm-750nm), green (495nm-600nm), blue (400nm-495nm), or ultraviolet (100nm-400nm) spectrums. In some embodiments a plurality of illuminations with different wavelengths may be used. For illustrative purposes, the embodiments described herein may refer to the use of green or blue spectrums of light. However, it is understood that these embodiments may use any suitable light having a wavelength that is substantially or approximately within the green or blue spectra defined above, and the localization systems and methods described herein may use any suitable spectra of light.

Referring now to FIG. 1, shown therein is a view of an example embodiment of an ingestible device 100, which may be used to identify a location within a gastrointestinal (GI) tract. In some embodiments, ingestible device 100 may be configured to autonomously determine whether it is located in the stomach, a particular portion of the small intestine such as a duodenum, jejunum, or ileum, or the large intestine by utilizing sensors operating with different wavelengths of light. Additionally, ingestible device 100 may be configured to autonomously determine whether it is located within certain portions of the small intestine or large intestine, such as the duodenum, the jejunum, the cecum, or the colon.

Ingestible device 100 may have a housing 102 shaped similar to a pill or capsule. The housing 102 of ingestible device 100 may have a first end portion 104, and a second end portion 106. The first end portion 104 may include a first wall portion 108, and second end portion 106 may include a second wall portion 110. In some embodiments, first end portion 104 and second end portion 106 of ingestible device 100 may be manufactured separately, and may be affixed together by a connecting portion 112.

In some embodiments, ingestible device 100 may include an optically transparent window 114. Optically transparent window 114 may be transparent to various types of illumination in the visible spectrum, infrared spectrum, or ultraviolet light spectrum, and ingestible device 100 may have various sensors and illuminators located within the housing 102, and behind the transparent window 114. This may allow ingestible device 100 to be configured to transmit illumination at different wavelengths through transparent window 114 to an environment external to housing 102 of ingestible device 100, and to detect a reflectance from a portion of the illumination that is reflected back through transparent window 114 from the environment external to housing 102. Ingestible device 100 may then use the detected level of reflectance in order to determine a location of ingestible device 100 within a GI tract. In some embodiments, optically transparent window 114 may be of any shape and size, and may wrap around the circumference of ingestible device 100. In this case, ingestible device 100 may have multiple sets of sensors and illuminators positioned at different locations azimuthally behind window 114.

In some embodiments, ingestible device 100 may optionally include an opening 116 in the second wall portion 110. In some embodiments, the second wall portion 110 may be configured to rotate around the longitudinal axis of ingestible device 100 (e.g., by means of a suitable motor or other actuator housed within ingestible device 100). This may allow ingestible device 100 to obtain a fluid sample from the GI tract, or release a substance into the GI tract, through opening 116.

FIG. 2 shows an exploded view of ingestible device 100. In some embodiments, ingestible device 100 may optionally include a rotation assembly 118. Optional rotation assembly 118 may include a motor 118-1 driven by a microcontroller (e.g., a microcontroller coupled to printed circuit board 120), a rotation position sensing ring 118-2, and a storage sub-unit 118-3 configured to fit snugly within the second end portion 104. In some embodiments, rotation assembly 118 may cause second end portion 104, and opening 116, to rotate relative to the storage sub-unit 118-3. In some embodiments, there may be cavities on the side of storage sub-unit 118-3 that function as storage chambers. When the opening 116 is aligned with a cavity on the side of the storage sub-unit 118-3, the cavity on the side of the storage sub-unit 118-3 may be exposed to the environment external to the housing 102 of ingestible device 100. In some embodiments, the storage sub-unit 118-3 may be loaded with a medicament or other substance prior to the ingestible device 100 being administered to a subject. In this case, the medicament or other substance may be released from the ingestible device 100 by aligning opening 116 with the cavity within storage sub-unit 118-3. In some embodiments, the storage sub-unit 118-3 may be configured to hold a fluid sample obtained from the GI tract. For example, ingestible device 100 may be configured to align opening 116 with the cavity within storage sub-unit 118-3, thus allowing a fluid sample from the GI tract to enter the cavity within storage sub-unit 118-3. Afterwards, ingestible device 100 may be configured to seal the fluid sample within storage sub-unit 118-3 by further rotating the second end portion 106 relative to storage sub-unit 118-3. In some embodiments, storage sub-unit 118-3 may also contain a hydrophilic sponge, which may enable ingestible device 100 to better draw certain types of fluid samples into ingestible device 100. In some embodiments, ingestible device 100 may be configured to either obtain a sample from within the GI tract, or to release a substance into the GI tract, in response to determining that ingestible device 100 has reached a predetermined location within the GI tract. For example, ingestible device 100 may be configured to obtain a fluid sample from the GI tract in response to determining that the ingestible device has entered the jejunum portion of the small intestine (e.g., as determined by process 900 discussed in relation to FIG. 9). Other ingestible devices capable of obtaining samples or releasing substances are discussed in commonly-assigned PCT Application No. PCT/CA2013/000133 filed February 15, 2013, commonly-assigned U.S. Provisional Application No. 62/385,553, and commonly-assigned U.S. Provisional Application No. 62/376,688, which each are hereby incorporated by reference herein in their entirety. It is understood that any suitable method of obtaining samples or releasing substances may be incorporated into some of the embodiments of the ingestible devices disclosed herein, and that

the systems and methods for determining a location of an ingestible device may be incorporated into any suitable type of ingestible device.

Ingestible device 100 may include a printed circuit board (PCB) 120, and a battery 128 configured to power PCB 120. PCB 120 may include a programmable microcontroller, and control and memory circuitry for holding and executing firmware or software for coordinating the operation of ingestible device 100, and the various components of ingestible device 100. For example, PCB 120 may include memory circuitry for storing data, such as data sets of measurements collected by sensing sub-unit 126, or instructions to be executed by control circuitry to implement a localization process, such as, for example, one or more of the processes, discussed herein, including those discussed below in connection with one or more of the associated flow charts. PCB 120 may include a detector 122 and an illuminator 124, which together form sensing sub-unit 126. In some embodiments, control circuitry within PCB 120 may include processing units, communication circuitry, or any other suitable type of circuitry for operating ingestible device 100. For illustrative purposes, only a single detector 122 and a single illuminator 124 forming a single sensing sub-unit 126 are shown. However, it is understood that in some embodiments there may be multiple sensing sub-units, each with a separate illuminator and detector, within ingestible device 100. For example, there may be several sensing sub-units spaced azimuthally around the circumference of the PCB 120, which may enable ingestible device 100 to transmit illumination and detect reflectances or ambient light in all directions around the circumference of the device. In some embodiments, sensing sub-unit 126 may be configured to generate an illumination using illuminator 124, which is directed through the window 114 in a radial direction away from ingestible device 100. This illumination may reflect off of the environment external to ingestible device 100, and the reflected light coming back into ingestible device 100 through window 114 may be detected as a reflectance by detector 122.

In some embodiments, window 114 may be of any suitable shape and size. For example, window 114 may extend around a full circumference of ingestible device 100. In some embodiments there may be a plurality of sensing sub-units (e.g., similar to sensing sub-unit 126) located at different positions behind the window. For example, three sensing sub-units may be positioned behind the window at the same longitudinal location, but spaced 120 degrees apart azimuthally. This may enable ingestible device 100 to transmit illuminations in all directions radially around ingestible device 100, and to measure each of the corresponding reflectances.

In some embodiments, illuminator 124 may be capable of producing illumination at a variety of different wavelengths in the ultraviolet, infrared, or visible spectrum. For example,

illuminator 124 may be implemented by using Red-Green-Blue Light-Emitting diode packages (RGB-LED). These types of RGB-LED packages are able to transmit red, blue, or green illumination, or combinations of red, blue, or green illumination. Similarly, detector 122 may be configured to sense reflected light of the same wavelengths as the illumination produced by illuminator 124. For example, if illuminator 124 is configured to produce red, blue, or green illumination, detector 122 may be configured to detect different reflectances produced by red, blue, or green illumination (e.g., through the use of an appropriately configured photodiode). These detected reflectances may be stored by ingestible device 100 (e.g., within memory circuitry of PCB 120), and may then be used by ingestible device 100 in determining a location of ingestible device 100 within the GI tract (e.g., through the use of process 500 (FIG. 5), process 600 (FIG. 6), or process 900 (FIG. 9)).

It is understood that ingestible device 100 is intended to be illustrative, and not limiting. It will be understood that modifications to the general shape and structure of the various devices and mechanisms described in relation to FIG. 1 and FIG. 2 may be made without significantly changing the functions and operations of the devices and mechanisms. For example, ingestible device 100 may have a housing formed from a single piece of molded plastic, rather than being divided into a first end portion 104 and a second end portion 106. As an alternate example, the location of window 114 within ingestible device 100 may be moved to some other location, such as the center of ingestible device 100, or to one of the ends of ingestible device 100. Moreover, the systems and methods discussed in relation to FIGS. 1-10 may be implemented on any suitable type of ingestible device, provided that the ingestible device is capable of detecting reflectances or levels of illumination in some capacity. For example, in some embodiments ingestible device 100 may be modified to replace detector 122 with an image sensor, and the ingestible device may be configured to measure relative levels of red, blue, or green light by decomposing a recorded image into its individual spectral components. Other examples of ingestible devices with localization capabilities, which may be utilized in order to implement the systems and methods discussed in relation to FIG. 1-11, are discussed in co-owned PCT Application No. PCT/US2015/052500 filed on September 25, 2015, which is hereby incorporated by reference herein in its entirety. Furthermore, it should be noted that the features and limitations described in any one embodiment may be applied to any other embodiment herein, and the descriptions and examples relating to one embodiment may be combined with any other embodiment in a suitable manner.

FIG. 3 is a diagram of an ingestible device during an example transit through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure. Ingestible device 300 may include any portion of any other ingestible device discussed in this disclosure (e.g., ingestible device 100 (FIG. 1)), and may be any suitable type of ingestible device with localization capabilities. For example, ingestible device 300 may be one embodiment of ingestible device 100 without the optional opening 116 (FIG. 1) or optional rotation assembly 118 (FIG. 2)). In some embodiments, ingestible device 300 may be ingested by a subject, and as ingestible device 300 traverses the GI tract, ingestible device 300 may be configured to determine its location within the GI tract. For example, the movement of ingestible device 300 and the amount of light detected by ingestible device 300 (e.g., via detector 122 (FIG. 2)) may vary substantially depending on the location of ingestible device 300 within the GI tract, and ingestible device 300 may be configured to use this information to determine a location of ingestible device 300 within the GI tract. For instance, ingestible device 300 may detect ambient light from the surrounding environment, or reflectances based on illumination generated by ingestible device 300 (e.g., generated by illuminator 124 (FIG. 1)), and use this information to determine a location of ingestible device 300 through processes, such as described herein. The current location of ingestible device 300, and the time that ingestible device 300 detected each transition between the various portions of the GI tract, may then be stored by ingestible device 300 (e.g., in memory circuitry of PCB 120 (FIG. 2)), and may be used for any suitable purpose.

Shortly after ingestible device 300 is ingested, ingestible device will traverse the esophagus 302, which may connect the subject's mouth to a stomach 306. In some embodiments, ingestible device 300 may be configured to determine that it has entered the esophagus portion GI tract by measuring the amount and type of light (e.g., via detector 122 (FIG. 2)) in the environment surrounding the ingestible device 300. For instance, ingestible device 300 may detect higher levels of light in the visible spectrum (e.g., via detector 122 (FIG. 2)) while outside the subject's body, as compared to the levels of light detected while within the GI tract. In some embodiments, ingestible device 300 may have previously stored data (e.g., on memory circuitry of PCB 120 (FIG. 2)) indicating a typical level of light detected when outside of the body, and the ingestible device 300 may be configured to determine that entry to the body has occurred when a detected level of light (e.g., detected via detector 122 (FIG. 2)) has been reduced beyond a threshold level (e.g., at least a 20-30% reduction) for a sufficient period of time (e.g., 5.0 seconds).

In some embodiments, ingestible device 300 may be configured to detect a transition from esophagus 302 to stomach 306 by passing through sphincter 304. In some embodiments, ingestible device 300 may be configured to determine whether it has entered stomach 306 based at least in part on a plurality of parameters, such as but not limited to the use of light or temperature measurements (e.g., via detector 122 (FIG. 2) or via a thermometer within ingestible device 300), pH measurements (e.g., via a pH meter within ingestible device 300), time measurements (e.g., as detected through the use of clock circuitry included within PCB 120 (FIG. 2)), or any other suitable information. For instance, ingestible device 300 may be configured to determine that ingestible device 300 has entered stomach 306 after detecting that a measured temperature of ingestible device 300 exceeds 31 degrees Celsius. Additionally, or alternately, ingestible device 300 may be configured to automatically determine it has entered stomach 306 after one minute (or another pre-set time duration parameter, 80 seconds, 90 seconds, etc.) has elapsed from the time that ingestible device 300 was ingested, or one minute (or another pre-set time duration parameter, 80 seconds, 90 seconds, etc.) from the time that ingestible device 300 detected that it has entered the GI tract.

Stomach 306 is a relatively large, open, and cavernous organ, and therefore ingestible device 300 may have a relatively large range of motion. By comparison, the motion of ingestible device 300 is relatively restricted within the tube-like structure of the duodenum 310, the jejunum 314, and the ileum (not shown), all of which collectively form the small intestine. Additionally, the interior of stomach 306 has distinct optical properties from duodenum 310 and jejunum 314, which may enable ingestible device 300 to detect a transition from stomach 306 to duodenum 310 through the appropriate use of measured reflectances (e.g., through the use of reflectances measured by detector 122 (FIG. 2)), as used in conjunction with process 600 (FIG. 6)).

In some embodiments, ingestible device 300 may be configured to detect a pyloric transition from stomach 306 to duodenum 310 through the pylorus 308. For instance, in some embodiments, ingestible device 300 may be configured to periodically generate illumination in the green and blue wavelengths (e.g., via illuminator 124 (FIG. 2)), and measure the resulting reflectances (e.g., via detector 122 (FIG. 2)). Ingestible device 300 may be configured to then use a ratio of the detected green reflectance to the detected blue reflectance to determine whether ingestible device 300 is located within the stomach 306, or duodenum 310 (e.g., via process 600 (FIG. 6)). In turn, this may enable ingestible device 300 to detect a pyloric transition from stomach 306 to duodenum 310, an example of which is discussed in relation to FIG. 6.

Similarly, in some embodiments, ingestible device 300 may be configured to detect a reverse pyloric transition from duodenum 310 to stomach 306. Ingestible device 300 will typically transition naturally from stomach 306 to duodenum 310, and onward to jejunum 314 and the remainder of the GI tract. However, similar to other ingested substances, ingestible device 300 may occasionally transition from duodenum 310 back to stomach 306 as a result of motion of the subject, or due to the natural behavior of the organs with the GI tract. To accommodate this possibility, ingestible device 300 may be configured to continue to periodically generate illumination in the green and blue wavelengths (e.g., via illuminator 124 (FIG. 2)), and measure the resulting reflectances (e.g., via detector 122 (FIG. 2)) to detect whether or not ingestible device 300 has returned to stomach 306. An exemplary detection process is described in additional detail in relation to FIG. 6.

After entering duodenum 310, ingestible device 300 may be configured to detect a transition to the jejunum 314 through the duodenojejunal flexure 312. For example, ingestible device 300 may be configured to use reflectances to detect peristaltic waves within the jejunum 314, caused by the contraction of the smooth muscle tissue lining the walls of the jejunum 314. In particular, ingestible device 300 may be configured to begin periodically transmitting illumination (and measuring the resulting reflectances (e.g., via detector 122 and illuminator 124 of sensing sub-unit 126 (FIG. 2)) at a sufficiently high frequency in order to detect muscle contractions within the jejunum 314. Ingestible device 300 may then determine that it has entered the jejunum 314 in response to having detected either a first muscle contraction, or a predetermined number of muscle contractions (e.g., after having detected three muscle contractions in sequence). The interaction of ingestible device 300 with the walls of jejunum 314 is also discussed in relation to FIG. 4, and an example of this detection process is described in additional detail in relation to FIG. 9.

FIG. 4 is a diagram of an ingestible device during an example transit through a jejunum, in accordance with some embodiments of the disclosure. Diagrams 410, 420, 430, and 440 depict ingestible device 400 as it traverses through a jejunum (e.g., jejunum 314), and how ingestible device 400 interacts with peristaltic waves formed by walls 406A and 406B (collectively, walls 406) of the jejunum. In some implementations, ingestible device 400 may include any portion of any other ingestible device discussed in this disclosure (e.g., ingestible device 100 (FIG. 1) or ingestible device 300 (FIG. 3)), and may be any suitable type of ingestible device with localization capabilities. For example, ingestible device 400 may be substantially similar to the ingestible device 300 (FIG. 3) or ingestible device 100 (FIG. 1), with window 404

being the same as window 114 (FIG. 1), and sensing sub-unit 402 being the same as sensing sub-unit 126 (FIG. 2).

Diagram 410 depicts ingestible device 400 within the jejunum, when the walls 406 of the jejunum are relaxed. In some embodiments, the confined tube-like structure of the jejunum naturally causes ingestible device 400 to be oriented longitudinally along the length of the jejunum, with window 404 facing walls 406. In this orientation, ingestible device 400 may use sensing sub-unit 402 to generate illumination (e.g., via illuminator 124 (FIG. 2)) oriented towards walls 406, and to detect the resulting reflectances (e.g., via detector 122 (FIG. 2)) from the portion of the illumination reflected off of walls 406 and back through window 404. In some embodiments, ingestible device 400 may be configured to use sensing sub-unit 402 to generate illumination and measure the resulting reflectance with sufficient frequency to detect peristaltic waves within the jejunum. For instance, in a healthy human subject, peristaltic waves may occur at a rate of approximately 0.1 Hz to 0.2 Hz. Therefore, the ingestible device 400 may be configured to generate illumination and measure the resulting reflectance at least once every 2.5 seconds (i.e., the minimum rate necessary to detect a 0.2 Hz signal), and preferably at a higher rate, such as once every 0.5 seconds, which may improve the overall reliability of the detection process due to more data points being available. It is understood that the ingestible device 400 need not gather measurements at precise intervals, and in some embodiments the ingestible device 400 may be adapted to analyze data gathered at more irregular intervals, provided that there are still a sufficient number of appropriately spaced data points to detect 0.1 Hz to 0.2 Hz signals.

Diagram 420 depicts ingestible device 400 within the jejunum, when the walls 406 of the jejunum begin to contract and form a peristaltic wave. Diagram 420 depicts contracting portion 408A of wall 406A and contracting portion 408B of wall 406B (collectively, contracting portion 408 of wall 406) that form a peristaltic wave within the jejunum. The peristaltic wave proceeds along the length of the jejunum as different portions of wall 406 contract and relax, causing it to appear as if contracting portions 408 of wall 406 proceed along the length of the jejunum (i.e., as depicted by contracting portions 408 proceeding from left to right in diagrams 410-430). While in this position, ingestible device 400 may detect a similar level of reflectance (e.g., through the use of illuminator 124 and detector 122 of sensing sub-unit 126 (FIG. 2)) as detected when there is no peristaltic wave occurring (e.g., as detected when ingestible device 400 is in the position indicated in diagram 410).

Diagram 430 depicts ingestible device 400 within the jejunum, when the walls 406 of the jejunum continue to contract, squeezing around ingestible device 400. As the peristaltic wave proceeds along the length of the jejunum, contracting portions 408 of wall 406 may squeeze tightly around ingestible device 400, bringing the inner surface of wall 406 into contact with window 404. While in this position, ingestible device 400 may detect a change in a reflectance detected as a result of illumination produced by sensing sub-unit 402. The absolute value of the change in the measured reflectance may depend on several factors, such as the optical properties of the window 404, the spectral components of the illumination, and the optical properties of the walls 406. However, ingestible device 400 may be configured to store a data set with the reflectance values over time, and search for periodic changes in the data set consistent with the frequency of the peristaltic waves (e.g., by analyzing the data set in the frequency domain, and searching for peaks between 0.1 Hz to 0.2 Hz). This may enable ingestible device 400 to detect muscle contractions due to peristaltic waves without foreknowledge of the exact changes in reflectance signal amplitude that may occur as a result of detecting the muscle contractions of the peristaltic wave. An example procedure for detecting muscle contractions is discussed further in relation to FIG. 9, and an example of a reflectance data set gathered while ingestible device 400 is located within the jejunum is discussed in relation to FIG. 10.

Diagram 440 depicts ingestible device 400 within the jejunum, when the peristaltic wave has moved past ingestible device 400. Diagram 440 depicts contracting portions 408 that form the peristaltic wave within the jejunum having moved past the end of ingestible device 400. The peristaltic wave proceeds along the length of the jejunum as different portions of wall 406 contract and relax, causing it to appear as if contracting portions 408 of wall 406 proceed along the length of the jejunum (i.e., as depicted by contracting portions 408 proceeding from left to right in diagrams 410-430). While in this position, ingestible device 400 may detect a similar level of reflectance (e.g., through the use of illuminator 124 and detector 122 of sensing sub-unit 126 (FIG. 2)) as detected when there is no peristaltic wave occurring (e.g., as detected when ingestible device 400 is in the position indicated in diagram 410, or diagram 420).

Depending on the species of the subject, peristaltic waves may occur with relatively predictable regularity. After the peristaltic wave has passed over ingestible device 400 (e.g., as depicted in diagram 440), the walls 406 of the jejunum may relax again (e.g., as depicted in diagram 410), until the next peristaltic wave begins to form. In some embodiments, ingestible device 400 may be configured to continue to gather reflectance value data while it is within the GI tract, and may store a data set with the reflectance values over time. This may allow

ingestible device 400 to detect each of the muscle contractions as the peristaltic wave passes over ingestible device 400 (e.g., as depicted in diagram 430), and may enable ingestible device 400 to both count the number of muscle contractions that occur, and to determine that a current location of the ingestible device 400 is within the jejunum. For example, ingestible device 400 may be configured to monitor for possible muscle contractions while is inside either the stomach or the duodenum, and may determine that ingestible device 400 has moved to the jejunum in response to detecting a muscle contraction consistent with a peristaltic wave.

FIG. 5 is a flowchart illustrating some aspects of a localization process used by the ingestible device. Although FIG. 5 may be described in connection with the ingestible device 100 for illustrative purposes, this is not intended to be limiting, and either portions or the entirety of the localization procedure 500 described in FIG. 5 may be applied to any device discussed in this application (e.g., the ingestible devices 100, 300, and 400), and any of the ingestible devices may be used to perform one or more parts of the process described in FIG. 5. Furthermore, the features of FIG. 5 may be combined with any other systems, methods or processes described in this application. For example, portions of the process in FIG. 5 may be integrated into or combined with the pyloric transition detection procedure described by FIG. 6, or the jejunum detection process described by FIG. 9.

At 502, the ingestible device (e.g., ingestible device 100, 300, or 400) gathers measurements (e.g., through detector 122 (FIG. 2)) of ambient light. For example, ingestible device 100 may be configured to periodically measure (e.g., through detector 122 (FIG. 2)) the level of ambient light in the environment surrounding ingestible device 100. In some embodiments, the type of ambient light being measured may depend on the configuration of detector 122 within ingestible device 100. For example, if detector 122 is configured to measure red, green, and blue wavelengths of light, ingestible device 100 may be configured to measure the ambient amount of red, green, and blue light from the surrounding environment. In some embodiments, the amount of ambient light measured by ingestible device 100 will be larger in the area external to the body (e.g., a well-lit room where ingestible device 100 is being administered to a subject) and in the oral cavity of the subject, as compared to the ambient level of light measured by ingestible device 100 when inside of an esophagus, stomach, or other portion of the GI tract (e.g., esophagus 302, stomach 306, duodenum 310, or jejunum 314 (FIG. 3)).

At 504, the ingestible device (e.g., ingestible device 100, 300, or 400) determines (e.g., via control circuitry within PCB 120 (FIG. 2)) whether the ingestible device has detected entry

into the GI tract. For example, ingestible device 100 may be configured to determine when the most recent measurement of ambient light (e.g., the measurement gathered at 502) indicates that the ingestible device has entered the GI tract. For instance, the first time that ingestible device 100 gatherers a measurement of ambient light at 502, ingestible device 100 may store that measurement (e.g., via storage circuitry within PCB 120 (FIG. 2)) as a typical level of ambient light external to the body. Ingestible device 100 may be configured to then compare the most recent measurement of ambient light to the typical level of ambient light external to the body (e.g., via control circuitry within PCB 120 (FIG. 2)), and determine that ingestible device 100 has entered the GI tract when the most recent measurement of ambient light is substantially smaller than the typical level of ambient light external to the body. For example, ingestible device 100 may be configured to detect that it has entered the GI tract in response to determining that the most recent measurement of ambient light is less than or equal to 20% of the typical level of ambient light external to the body. If ingestible device 100 determines that it has detected entry into the GI tract (e.g., that ingestible device 100 has entered at least the esophagus 302 (FIG. 3)), process 500 proceeds to 506. Alternately, if ingestible device 100 determines that it has not detected entry into the GI tract (e.g., as a result of the most recent measurement being similar to the typical level of ambient light external to the body), process 500 proceeds back to 502 where the ingestible device 100 gathers further measurements. For instance, ingestible device 100 may be configured to wait a predetermined amount of time (e.g., five seconds, ten seconds, etc.), and then gather another measurement of the level of ambient light from the environment surrounding ingestible device 100.

At 506, the ingestible device (e.g., ingestible device 100, 300, or 400) waits for a transition from the esophagus to the stomach (e.g., from esophagus 302 to stomach 306 (FIG. 3)). For example, ingestible device 100 may be configured to determine that it has entered the stomach (e.g., stomach 306 (FIG. 3)) after waiting a predetermined period of time after having entered the GI tract. For instance, a typical esophageal transit time in a human patient may be on the order of 15-30 seconds. In this case, after having detected that ingestible device 100 has entered the GI tract at 504 (i.e., after detecting that ingestible device 100 has reached at least esophagus 302 (FIG. 3)), ingestible device 100 may be configured to wait one minute, or a similar amount of time longer than the typical esophageal transmit time (e.g., ninety-seconds), before automatically determining that ingestible device 100 has entered at least the stomach (e.g., stomach 306 (FIG. 3)).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may also determine it has entered the stomach based on measurements of pH or temperature. For example, ingestible device 100 may be configured to determine that it has entered the stomach if a temperature of ingestible device has increased to at least 31 degrees Celsius (i.e., consistent with the temperature inside the stomach), or if a measured pH of the environment surrounding ingestible device 100 is sufficiently acidic (i.e., consistent with the acidic nature of gastric juices that may be found inside the stomach).

At 508, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating the ingestible device has entered the stomach (e.g., stomach 306 (FIG. 3)). For example, after having waited a sufficient amount of time at 506, ingestible device 100 may store data (e.g., within storage circuitry of PCB 120 (FIG. 2)) indicative of ingestible device 100 having entered at least the stomach. Once ingestible device 100 reaches at least the stomach, process 500 proceeds to 510 where ingestible device 100 may be configured to gather data to detect entry into the duodenum (e.g., duodenum 310 (FIG. 3)).

In some embodiments, process 500 may also simultaneously proceed from 508 to 520, where ingestible device 100 may be configured to gather data in order to detect muscle contractions and detect entry into the jejunum (e.g., jejunum 314 (FIG. 3)). In some embodiments, ingestible device 100 may be configured to simultaneously monitor for entry into the duodenum at 516-518, as well as detect for entry into the jejunum at 520-524. This may allow ingestible device 100 to determine when it has entered the jejunum (e.g., as a result of detecting muscle contractions), even when it fails to first detect entry into the duodenum (e.g., as a result of very quick transit times of the ingestible device through the duodenum).

At 510, the ingestible device (e.g., ingestible device 100, 300, or 400) gathers measurements of green and blue reflectance levels (e.g., through the use of illuminator 124 and detector 122 of sensing sub-unit 126 (FIG. 2)) while in the stomach (e.g., stomach 306 (FIG. 3)). For example, ingestible device 100 may be configured to periodically gather measurements of green and blue reflectance levels while in the stomach. For instance, ingestible device 100 may be configured to transmit a green illumination and a blue illumination (e.g., via illuminator 124 (FIG. 2)) every five to fifteen seconds, and measure the resulting reflectance (e.g., via detector 122 (FIG. 2)). Every time that ingestible device 100 gathers a new set of measurements, the measurements may be added to a stored data set (e.g., stored within memory circuitry of PCB 120 (FIG. 2)). The ingestible device 100 may then use this data set to determine whether or not

ingestible device 100 is still within a stomach (e.g., stomach 306 (FIG. 3)), or a duodenum (e.g., duodenum 310 (FIG. 3)).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may be configured to detect a first reflectance based on generating an illumination of a first wavelength in approximately the green spectrum of light (between 495–600 nm), and detecting a second reflectance based on generating an illumination of the second wavelength in approximately the blue spectrum of light (between 400–495 nm). In some embodiments, the ingestible device may ensure that the illumination in the green spectrum and the illumination in the blue spectrum have wavelengths separated by at least 50 nm. This may enable ingestible device 100 to sufficiently distinguish between the two wavelengths when detecting the reflectances (e.g., via detector 122 (FIG. 2)). It is understood that the separation of 50 nm is intended to be illustrative, and not limiting, and depending on the accuracy of the detectors within ingestible device 100, smaller separations may be possible to be used.

At 512, the ingestible device (e.g., ingestible device 100, 300, or 400) determines (e.g., using control circuitry within PCB 120 (FIG. 2)) whether the ingestible device has detected a transition from the stomach (e.g., stomach 306 (FIG. 3)) to a duodenum (e.g., duodenum 310 (FIG. 3)) based on a ratio of green and blue (G/B) reflectance levels. For example, ingestible device 100 may obtain (e.g., from memory circuitry of PCB 120 (FIG. 2)) a data set containing historical data for the respective ratio of the green reflectance to the blue reflectance as measured at a respective time. Generally speaking, a duodenum (e.g., duodenum 310 (FIG. 3)) of a human subject reflects a higher ratio of green light to blue light, as compared to the ratio of green light to blue light that is reflected by a stomach (e.g., stomach 306 (FIG. 3)). Based on this, ingestible device 100 may be configured to take a first set of ratios from the data set, representing the result of recent measurements, and compare them to a second set of ratios from the data set, representing the results of past measurements. When the ingestible device 100 determines that the mean value of the first set of ratios is substantially larger than the mean value of the second set of ratios (i.e., that the ratio of reflected green light to reflected blue light has increased), the ingestible device 100 may determine that it has entered the duodenum (e.g., duodenum 310 (FIG. 3)) from the stomach (e.g., stomach 306 (FIG. 3)). If the ingestible device 100 detects a transition from the stomach (e.g., stomach 306 (FIG. 3)) to a duodenum (e.g., duodenum 310 (FIG. 3)), process 500 proceeds to 514, where ingestible device 100 stores data indicating that the ingestible device 100 has entered the duodenum (e.g., duodenum 310 (FIG. 3)). Alternatively, if the ingestible device determines that the ingestible device has not transitioned from the stomach

(e.g., stomach 306 (FIG. 3)) to the duodenum (e.g., duodenum 310 (FIG. 3)), process 500 proceeds back to 510 to gather more measurements of green and blue reflectance levels while still in the stomach (e.g., stomach 306 (FIG. 3)). An example procedure for using measurements of green and blue reflectances to monitor for transitions between the stomach and the duodenum is discussed in greater detail in relation to FIG. 6.

In some embodiments, the first time that ingestible device 100 detects a transition from the stomach (e.g., stomach 306 (FIG. 3)) to the duodenum (e.g., duodenum 310 (FIG. 3)), ingestible device 100 may be configured to take a mean of the second set of data, (e.g., the set of data previously recorded while in stomach 306 (FIG. 3)) and store this as a typical ratio of green light to blue light detected within the stomach (e.g., stomach 306 (FIG. 3)) (e.g., within memory circuitry of PCB 120 (FIG. 2)). This stored information may later be used by ingestible device 100 to determine when ingestible device 100 re-enters the stomach (e.g., stomach 306 (FIG. 3)) from the duodenum (e.g., duodenum 310 (FIG. 3)) as a result of a reverse pyloric transition.

At 514, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating that the ingestible device has entered the duodenum (e.g., duodenum 310 (FIG. 3)). For example, ingestible device 100 may store a flag within local memory (e.g., memory circuitry of PCB 120) indicating that the ingestible device 100 is currently in the duodenum. In some embodiments, the ingestible device 100 may also store a timestamp indicating the time when ingestible device 100 entered the duodenum. Once ingestible device 100 reaches the duodenum, process 500 proceeds to 520 where ingestible device 100 may be configured to gather data in order to detect muscle contractions and detect entry into the jejunum (e.g., jejunum 314 (FIG. 3)). Process 500 also proceeds from 514 to 516, where ingestible device 100 may be configured to gather data additional data in order to detect re-entry into the stomach (e.g., stomach 306 (FIG. 3)) from the duodenum (e.g., duodenum 310 (FIG. 3)).

At 516, the ingestible device (e.g., ingestible device 100, 300, or 400) gathers measurements (e.g., via sensing sub-unit 126 (FIG. 2)) of green and blue reflectance levels while in the duodenum (e.g., duodenum 310 (FIG. 3)). For example, ingestible device 100 may be configured to periodically gather measurements (e.g., via sensing sub-unit 126 (FIG. 2)) of green and blue reflectance levels while in the duodenum, similar to the measurements made at 510 while in the stomach. For instance, ingestible device 100 may be configured to transmit a green illumination and a blue illumination (e.g., via illuminator 124 (FIG. 2)) every five to fifteen seconds, and measure the resulting reflectance (e.g., via detector 122 (FIG. 2)). Every time that ingestible device 100 gathers a new set of measurements, the measurements may be added to a

stored data set (e.g., stored within memory circuitry of PCB 120 (FIG. 2)). The ingestible device 100 may then use this data set to determine whether or not ingestible device 100 is still within the duodenum (e.g., duodenum 310 (FIG. 3)), or if the ingestible device 100 has transitioned back into the stomach (e.g., stomach 306 (FIG. 3)).

At 518, the ingestible device (e.g., ingestible device 100, 300, or 400) determines a transition from the duodenum (e.g., duodenum 310 (FIG. 3)) to the stomach (e.g., stomach 306 (FIG. 3)) based on a ratio of the measured green reflectance levels to the measured blue reflectance levels. In some embodiments, ingestible device 100 may compare the ratio of the measured green reflectance levels to the measured blue reflectance levels recently gathered by ingestible device 100 (e.g., measurements gathered at 516), and determine whether or not the ratio of the measured green reflectance levels to the measured blue reflectance levels is similar to the average ratio of the measured green reflectance levels to the measured blue reflectance levels seen in the stomach (e.g., stomach 306 (FIG. 3)). For instance, ingestible device 100 may retrieve data (e.g., from memory circuitry of PCB 120 (FIG. 2)) indicative of the average ratio of the measured green reflectance levels to the measured blue reflectance levels seen in the stomach, and determine that ingestible device 100 has transitioned back to the stomach if the recently measured ratio of the measured green reflectance levels to the measured blue reflectance levels is sufficiently similar to the average level in the stomach (e.g., within 20% of the average ratio of the measured green reflectance levels to the measured blue reflectance levels seen in the stomach, or within any other suitable threshold level). If the ingestible device detects a transition from the duodenum (e.g., duodenum 310 (FIG. 3)) to the stomach (e.g., stomach 306 (FIG. 3)), process 500 proceeds to 508 to store data indicating the ingestible device has entered the stomach (e.g., stomach 306 (FIG. 3)), and continues to monitor for further transitions. Alternatively, if the ingestible device does not detect a transition from the duodenum (e.g., duodenum 310 (FIG. 3)) to the stomach (e.g., stomach 306 (FIG. 3)), process 500 proceeds to 516 to gather additional measurements of green and blue reflectance levels while in the duodenum (e.g., duodenum 310 (FIG. 3)), which may be used to continuously monitor for possible transitions back into the stomach. An example procedure for using measurements of green and blue reflectances to monitor for transitions between the stomach and the duodenum is discussed in greater detail in relation to FIG. 6.

At 520, the ingestible device (e.g., ingestible device 100, 300, or 400) gathers periodic measurements of the reflectance levels (e.g., via sensing sub-unit 126 (FIG. 2)) while in the duodenum (e.g., duodenum 310 (FIG. 3)). In some embodiments, the ingestible device (e.g.,

ingestible device 100, 300, or 400) may gather similar periodic measurements while in the stomach as well. In some embodiments, these periodic measurements may enable ingestible device 100 to detect muscle contractions (e.g., muscle contractions due to a peristaltic wave as discussed in relation to FIG. 4), which may be indicative of entry into a jejunum (e.g., jejunum 314 (FIG. 3)). Ingestible device 100 may be configured to gather periodic measurements using any suitable wavelength of illumination (e.g., by generating illumination using illuminator 124, and detecting the resulting reflectance using detector 122 (FIG. 2)), or combinations of wavelengths of illumination. For example, in some embodiments, ingestible device 100 may be configured to generate red, green, and blue illumination, store separate data sets indicative of red, green, and blue illumination, and analyze each of the data sets separately to search for frequency components in the recorded data indicative of detected muscle contractions. In some embodiments, the measurements gathered by ingestible device 100 at 520 may be sufficiently fast as to detect peristaltic waves in a subject. For instance, in a healthy human subject, peristaltic waves may occur at a rate of approximately 0.1 Hz to 0.2 Hz. Therefore, the ingestible device 400 may be configured to generate illumination and measure the resulting reflectance at least once every 2.5 seconds (i.e., the minimum rate necessary to detect a 0.2 Hz signal), and preferably at a higher rate, such as once every 0.5 seconds or faster, and store values indicative of the resulting reflectances in a data set (e.g., within memory circuitry of PCB 120 (FIG. 2)). After gathering additional data (e.g., after gathering one new data point, or a predetermined number of new data points), process 500 proceeds to 522, where ingestible device 100 determines whether or not a muscle contraction has been detected.

At 522, the ingestible device (e.g., ingestible device 100, 300, or 400) determines (e.g., via control circuitry within PCB 120 (FIG .2)) whether the ingestible device detects a muscle contraction based on the measurements of reflectance levels (e.g., as gathered by sensing sub-unit 126 (FIG. 2)). For example, ingestible device 100 may obtain a fixed amount of data stored as a result of measurements made at 520 (e.g., retrieve the past minute of data from memory circuitry within PCB 120 (FIG. 2)). Ingestible device 100 may then convert the obtained data into the frequency domain, and search for peaks in a frequency range that would be consistent with peristaltic waves. For example, in a healthy human subject, peristaltic waves may occur at a rate of approximately 0.1 Hz to 0.2 Hz, and an ingestible device 100 may be configured to search for peaks in the frequency domain representation of the data between 0.1 Hz and 0.2 Hz above a threshold value. If the ingestible device 100 detects a contraction based on the reflectance levels (e.g., based on detecting peaks in the frequency domain representation of the data between 0.1

Hz and 0.2 Hz), process 500 proceeds to 524 to store data indicating that the device has entered the jejunum. Alternatively, if the ingestible device 100 does not detect a muscle contraction, process 500 proceeds to 520 to gather periodic measurements of the reflectance levels while in the duodenum (e.g., duodenum 310 (FIG. 3)). In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may store data (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating that a muscle contraction was detected, and process 500 will not proceed from 522 to 524 until a sufficient number of muscle contractions have been detected.

At 524, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating that the device has entered the jejunum (e.g., jejunum 314 (FIG. 3)). For example, in response to detecting that muscle contraction has occurred at 522, ingestible device 100 may determine that it has entered the jejunum 314, and is no longer inside of the duodenum (e.g., duodenum 310 (FIG. 3)) or the stomach (e.g., stomach 306 (FIG. 3)). In some embodiments, the ingestible device 100 may continue to measure muscle contractions while in the jejunum, and may store data indicative of the frequency, number, or strength of the muscle contractions over time (e.g., within memory circuitry of PCB 120 (FIG. 2)). In some embodiments, the ingestible device 100 may also be configured to monitor for one or more transitions. Such transitions can include a transition from the jejunum to the ileum, an ileoceacal transition from the ileum to the cecum, a transition from the cecum to the colon, or detect exit from the body (e.g., by measuring reflectances, temperature, or levels of ambient light).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may also determine that it has entered the jejunum (e.g., jejunum 314 (FIG. 3)) after a pre-determined amount of time has passed after having detected entry into the duodenum (e.g., duodenum 310 (FIG. 3)). For example, barring a reverse pyloric transition from the duodenum (e.g., duodenum 310 (FIG. 3)) back to the stomach (e.g., stomach 306 (FIG. 3)), the typical transit time for an ingestible device to reach the jejunum from the duodenum in a healthy human subject is less than three minutes. In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may therefore be configured to automatically determine that it has entered the jejunum after spending at least three minutes within the duodenum. This determination may be made separately from the determination made based on measured muscle contractions (e.g., the determination made at 522), and in some embodiments, ingestible device 100 may determine that it has entered the jejunum in response to either detecting muscle contractions, or after three

minutes has elapsed from having entered the duodenum (e.g., as determined by storing data at 514 indicative of the time that ingestible device entered the duodenum).

For illustrative purposes, 512-518 of process 500 describe the ingestible device (e.g., ingestible device 100, 300, or 400) measuring green reflectances and blue reflectances, calculating a ratio of the two reflectances, and using this information to determine when the ingestible device has transitioned between the duodenum and stomach. However, in some embodiments, other wavelengths of light may be used other than green and blue, provided that the wavelengths of light chosen have different reflective properties within the stomach and the duodenum (e.g., as a result of different reflection coefficients of the stomach tissue and the tissue of the duodenum).

It will be understood that the steps and descriptions of the flowcharts of this disclosure, including FIG. 5, are merely illustrative. Any of the steps and descriptions of the flowcharts, including FIG. 5, may be modified, omitted, rearranged, and performed in alternate orders or in parallel, two or more of the steps may be combined, or any additional steps may be added, without departing from the scope of the present disclosure. For example, the ingestible device 100 may calculate the mean and the standard deviation of multiple data sets in parallel in order to speed up the overall computation time. As another example, ingestible device 100 may gather data periodic measurements and detect possible muscle contractions (e.g., at 520-522) while simultaneously gathering green and blue reflectance levels to determine transitions to and from the stomach and duodenum (e.g., at 510-518). Furthermore, it should be noted that the steps and descriptions of FIG. 5 may be combined with any other system, device, or method described in this application, including processes 600 (FIG. 6) and 900 (FIG. 9), and any of the ingestible devices or systems discussed in this application (e.g., ingestible devices 100, 300, or 400) could be used to perform one or more of the steps in FIG. 5.

FIG. 6 is a flowchart illustrating some aspects of a process for detecting transitions from a stomach to a duodenum and from a duodenum back to a stomach, which may be used when determining a location of an ingestible device as it transits through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure. In some embodiments, process 600 may begin when an ingestible device first detects that it has entered the stomach, and will continue as long as the ingestible device determines that it is within the stomach or the duodenum. In some embodiments, process 600 may only be terminated when an ingestible device determines that it has entered the jejunum, or otherwise progressed past the duodenum and the stomach. Although FIG. 6 may be described in connection with the ingestible device 100 for illustrative purposes,

this is not intended to be limiting, and either portions or the entirety of the duodenum detection process 600 described in FIG. 6 may be applied to any device discussed in this application (e.g., the ingestible devices 100, 300, or 400), and any of the ingestible devices may be used to perform one or more parts of the process described in FIG. 6. Furthermore, the features of FIG. 6 may be combined with any other systems, methods or processes described in this application. For example, portions of the process described by the process in FIG. 6 may be integrated into process 500 discussed in relation to FIG. 5.

At 602, the ingestible device (e.g., ingestible device 100, 300, or 400) retrieves a data set (e.g., from memory circuitry within PCB 120 (FIG. 2)) with ratios of the measured green reflectance levels to the measured blue reflectance levels over time. For example, ingestible device 100 may retrieve a data set from PCB 120 containing recently recorded ratios of the measured green reflectance levels to the measured blue reflectance levels (e.g., as recorded at 510 or 516 of process 500 (FIG. 5)). In some embodiments, the retrieved data set may include the ratios of the measured green reflectance levels to the measured blue reflectance levels over time. Example plots of data sets of ratios of the measured green reflectance levels to the measured blue reflectance levels are discussed further in relation to FIG. 7 and FIG. 8.

At 604, the ingestible device (e.g., ingestible device 100, 300, or 400) includes a new measurement (e.g., as made with sensing sub-unit 126 (FIG. 2)) of a ratio of the measured green reflectance level to the measured blue reflectance level in the data set. For example, ingestible device 100 may be configured to occasionally record new data by transmitting green and blue illumination (e.g., via illuminator 124 (FIG. 2)), detecting the amount of reflectance received due to the green and blue illumination (e.g., via detector 122 (FIG. 2)), and storing data indicative of the amount of the received reflectance (e.g., in memory circuitry of PCB 120 (FIG. 2)). The ingestible device 100 may be configured to record new data every five to fifteen seconds, or at any other convenient interval of time. For illustrative purposes, ingestible device 100 is described as storing and retrieving the ratio of the measured green reflectance levels to the measured blue reflectance levels (e.g., if the amount of detected green reflectance was identical to the amount of detected blue reflectance at a given time, the ratio of the green and blue reflectances would be “1.0” at that given time); however, it is understood that the green reflectance data and the blue reflectance data may be stored separately within the memory of ingestible device 100 (e.g., stored as two separate data sets within memory circuitry of PCB 120 (FIG. 2)).

At 606, the ingestible device (e.g., ingestible device 100, 300, or 400) retrieves a first subset of recent data by applying a first sliding window filter to the data set. For example, ingestible device 100 may use a sliding window filter to obtain a predetermined amount of the most recent data within the data set, which may include any new values of the ratio of the measured green reflectance level to the measured blue reflectance level obtained at 604. For instance, the ingestible device may be configured to select between ten and forty data points from the data set, or ingestible device 100 may be configured to select a predetermined range of data values between fifteen seconds of data and five minutes of data. In some embodiments, other ranges of data may be selected, depending on how frequently measurements are recorded, and the particular application at hand. For instance, any suitable amount of data may be selected in the sliding window, provided that it is sufficient to detect statistically significant differences between the data selected in a second sliding window (e.g., the second subset of data selected at 614).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may also be configured to remove outliers from the data set, or to smooth out unwanted noise in the data set. For example, ingestible device 100 may select the first subset of data, or any other subset of data, by first obtaining a raw set of values by applying a window filter to the data set (e.g., selecting a particular range of data to be included). Ingestible device 100 may then be configured to identify outliers in the raw set of values; for instance, by identifying data points that are over three standard deviations away from the mean value of the raw set of values, or any other suitable threshold. Ingestible device 100 may then determine the subset of data by removing outliers from the raw set of values. This may enable ingestible device 100 to avoid spurious information when determining whether or not it is located within the stomach or the duodenum.

At 608, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether the most recently detected location was the duodenum (e.g., duodenum 310 (FIG. 3)). In some embodiments, ingestible device 100 may store a data flag (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating the most recent portion of the GI tract that the ingestible device 100 detected itself to be within. For instance, every time ingestible device 100 detects entry to the stomach (e.g., detects entry into stomach 306 (FIG. 3) as a result of the decision made at 610), a flag is stored in memory indicating the ingestible device 100 is in the stomach (e.g., as part of storing data at 612). If ingestible device 100 subsequently detects entry into the duodenum (e.g., detects entry into duodenum 310 (FIG. 3) as a result of a decision made at 624), another different flag is stored in memory indicating that the ingestible device 100 is in the duodenum (e.g., as part

of storing data at 624). In this case, ingestible device 100 may retrieve the most recently stored flag at 608, and determine whether or not the flag indicates that the ingestible device 100 was most recently within the duodenum. If ingestible device 100 detects that it was most recently in the duodenum, process 600 proceeds to 610 where the ingestible device compares the recent measurements of the ratios of the measured green reflectance levels to the measured blue reflectance levels (e.g., measurements that include the recent measurement made at 606) to the typical ratios measured within the stomach, and uses this information to determine whether a reverse pyloric transition from the duodenum back to the stomach has occurred. Alternately, if ingestible device 100 detects that it was not most recently in the duodenum (e.g., because it was in the stomach instead), process 600 proceeds to 614 where the ingestible device compares the recent measurements of the ratios of the measured green reflectance levels to the measured blue reflectance levels (e.g., measurements that include the recent measurement made at 606) to past measurements, and uses this information to determine whether a pyloric transition from the stomach to the duodenum has occurred.

Process 600 proceeds from 608 to 610 when the ingestible device determined that it was most recently in the duodenum. At 610, the ingestible device (e.g., ingestible device 100, 300, or 400) determines (e.g., via control circuitry within PCB 120 (FIG. 2)) whether the current G/B signal is similar to a recorded average G/B signal in the stomach. For example, ingestible device 100 may be configured to have previously stored data (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicative of the average ratio of the measured green reflectance levels to the measured blue reflectance levels measured in the stomach. Ingestible device 100 may then retrieve this stored data indicative of the average ratio of the measured green reflectance levels to the measured blue reflectance levels in the stomach, and compare this against the recent measurements in order to determine whether or not ingestible device 100 has returned back to the stomach from the duodenum. For instance, ingestible device 100 may determine if the mean value of the first subset of recent data (i.e., the average value of the recently measured ratios of the measured green reflectance levels to the measured blue reflectance levels) is less than the average ratio of the measured green reflectance levels to the measured blue reflectance levels within the stomach, or less that the average ratio measured within the stomach plus a predetermined number times the standard deviation of the ratios measured within the stomach. For instance, if the average ratio of the measured green reflectance levels to the measured blue reflectance levels in the stomach was “1,” with a standard deviation of “0.2,” ingestible device 100 may determine whether or not the mean value of the first subset of data is less than “1.0 +

k*0.2," where "k" is a number between zero and five. It is understood that, in some embodiments, the ingestible device 100 may be configured to use a different threshold level to determine whether or not the mean value of the first subset of recent data is sufficiently similar to the average ratio of the measured green reflectance levels to the measured blue reflectance levels within the stomach. In response to determining that the recent ratio of the measured green reflectance levels to the measured blue reflectance levels is similar to the average ratio of measured green and blue reflectance levels seen in the stomach, process 600 proceeds to 612 where ingestible device 100 stores data indicating that it has re-entered the stomach from the duodenum. Alternately, in response to determining that the recent ratio of measured green and blue reflectance levels is sufficiently different from the average ratio of measured green and blue reflectance levels seen in the stomach, ingestible device 100 proceeds directly to 604, and continues to obtain new data on an ongoing basis.

At 612, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating a reverse pyloric transition from the duodenum to the stomach was detected. For example, ingestible device 100 may store a data flag (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating that the ingestible device 100 most recently detected itself to be within the stomach portion of the GI tract (e.g., stomach 306 (FIG. 3)). In some embodiments, ingestible device 100 may also store data (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating a time that ingestible device 100 detected the reverse pyloric transition from the duodenum to the stomach. This information may be used by ingestible device 100 at 608, and as a result process 600 may proceed from 608 to 614, rather than proceeding from 618 to 610. After ingestible device 100 stores the data indicating a reverse pyloric transition from the duodenum to the stomach was detected, process 600 proceeds to 604 where ingestible device 100 continues to gather additional measurements, and continues to monitor for further transitions between the stomach and the duodenum.

Process 600 proceeds from 608 to 614 when the ingestible device determined that it was not most recently in the duodenum (e.g., as a result of having most recently been in the stomach instead). At 614, the ingestible device (e.g., ingestible device 100, 300, or 400) retrieves a second subset of previous data by applying a second sliding window filter to the data set. For example, ingestible device 100 may use a sliding window filter to obtain a predetermined amount of older data from a past time range, which may be separated from recent time range used to select the first subset of data gathered at 606 by a predetermined period of time. In some embodiments, any suitable amount of data may be selected by the first and second window

filters, and the first and second window filters may be separated by any appropriate predetermined amount of time. For example, in some embodiments, the first window filter and the second window filter may each be configured to select a predetermined range of data values from the data set, the predetermined range being between fifteen seconds of data and five minutes of data. In some embodiments, the recent measurements and the past measurements may then be separated by a predetermined period of time that is between one to five times the predetermined range of data values. For instance, ingestible device 100 may select the first subset of data and the second subset of data to each be one minute of data selected from the dataset (i.e., selected to have a predetermined range of one minute), and the first subset of data and the second subset of data are selected from recorded measurements that are at least two minutes apart (i.e., the predetermined period of time is two minutes, which is twice the range used to select the subsets of data using the window filters). As another example, ingestible device 100 may select the first subset of data and the second subset of data to each be five minutes of data selected from the dataset (i.e., selected to have a predetermined range of five minutes), and the first subset of data and the second subset of data are selected from recorded measurements that are at least 10 minutes apart (i.e., the predetermined period of time is two minutes, which is twice the range used to select the subsets of data using the window filters).

In some embodiments, if ingestible device 100 recently transitioned to the stomach from the duodenum (e.g., as determined by checking for recent data stored within ingestible device 100 at 612), ingestible device 100 may select the second subset of data at 614 from a time frame when ingestible device 100 is known to be within the stomach. In some embodiments, ingestible device 100 may alternately select a previously recorded average and standard deviation for ratios of green reflectances and blue reflectances within the stomach (e.g., an average and standard deviation typical of data recorded within the stomach, as previously recorded within memory circuitry of PCB 120 at 620) in place of the second subset of data. In this case, ingestible device 100 may simply use the previously recorded average and previously recorded standard deviation when making a determination at 616, rather than expending resources to calculate the mean and standard deviation of the second subset.

At 616, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether the difference between the mean of the second subset and the mean of the first subset is greater than a predetermined multiple of the standard deviation of the first subset. For example, ingestible device 100 may compute a difference between a mean of the first subset of recent data and a mean of a second subset of past data, and determine whether this difference is greater than

three times the standard deviation of the second subset of past data. In some embodiments, it is understood that any convenient threshold level may be used other than three times the standard deviation, such as any value between one and five times the standard deviation. Also, in some embodiments, the ingestible device may instead set the threshold level based on the standard deviation of the second subset instead of the first subset. In response to determining that the difference between the mean of the first subset and the mean of the second subset is greater than a predetermined multiple of the standard deviation of the second subset, process 600 proceeds to 618. Otherwise, process 600 proceeds back to 604, where the ingestible device 604 continues to gather new data to be used in monitoring for transitions between the stomach (e.g., stomach 306 (FIG. 3)) and the duodenum (e.g., duodenum 310 (FIG. 3)).

At 618, the ingestible device (e.g., ingestible device 100, 300, or 400) determines (e.g., via control circuitry within PCB 120 (FIG. 2)) whether the determination made at 616 is the first time that the difference between the mean of the first subset of recent data and the mean of the second subset of past data is calculated to be greater than the standard deviation of the second subset. If the ingestible device determines that this is the first time that the difference between the mean of the first subset and the mean of the second subset is calculated to be greater than the standard deviation of the second subset, process 600 proceeds to 620 to store the mean of the second subset of past data as an average G/B signal in the stomach. Alternatively, if the ingestible device determines that the immediately preceding determination made at 616 is not the first time that the difference between the mean of the first subset of recent data and the mean of the second subset of past data is calculated to be greater than the standard deviation of the second subset, process 600 proceeds directly to 622.

At 620, the ingestible device (e.g., ingestible device 100, 300, or 400) stores the mean of the second subset as an average G/B signal in the stomach. For example, ingestible device 100 may be configured to store the mean of the second subset of past data (e.g., store within memory circuitry of PCB 120 (FIG. 2)) as the average ratio of the measured green reflectance levels to the measured blue reflectance levels measured in the stomach. In some embodiments, ingestible device 100 may also store the standard deviation of the second subset of past data as a typical standard deviation of the ratios of the measured green reflectance levels to the measured blue reflectance levels detected within the stomach. This stored information may be used by the ingestible device later on (e.g., at 610) to compare against future data, which may enable the ingestible device to detect reverse pyloric transitions from the duodenum (e.g., duodenum 310 (FIG. 3)) back to the stomach (e.g., stomach 306 (FIG. 3)), and may generally be used in place of

other experimental data gathered from the stomach (e.g., in place of the second subset of data at 616). After storing the mean of the second subset as an average G/B signal in the stomach, process 600 proceeds to 622.

At 622, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether a difference of the mean of the first subset of recent data to the mean of the second subset of past data is greater than a predetermined threshold, "M". In some embodiments, the predetermined threshold, "M," will be sufficiently large to ensure that the mean of the first subset is substantially larger than the mean of the second subset, and may enable ingestible device 100 to ensure that it detected an actual transition to the duodenum. This may be particularly advantageous when the determination made at 616 is potentially unreliable due to the standard deviation of the second subset of past data being abnormally small. For example, a typical value of the predetermined threshold "M," may be on the order of 0.1 to 0.5. If ingestible device 100 determines that the difference of the mean of the first subset of recent data to the second subset of past data is greater than a predetermined threshold, process 600 proceeds to 624 to store data indicating that a pyloric transition from the stomach to the duodenum (e.g., from stomach 306 to duodenum 310 (FIG. 3)) was detected. Alternatively, if the ingestible device determines that the ratio of the mean of the first subset to the second subset is less than or equal to the predetermined threshold, "M" (i.e., determines that a transition to the duodenum has not occurred), process 600 proceeds directly to 604 where ingestible device 100 continues to make new measurements and monitor for possible transitions between the stomach and the duodenum.

In some embodiments, instead of using a difference of the mean of the first subset of recent data to the mean of the second subset of past data, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether the ratio of the mean of the first subset of recent data to the mean of the second subset of past data is greater than a predetermined threshold, "M". In some embodiments, the predetermined threshold, "M," will be sufficiently large to ensure that the mean of the first subset is substantially larger than the mean of the second subset, and may enable ingestible device 100 to ensure that it detected an actual transition to the duodenum. This may be particularly advantageous when the determination made at 616 is potentially unreliable due to the standard deviation of the second subset of past data being abnormally small. For example, a typical value of the predetermined threshold "M," may be on the order of 1.2 to 2.0. It is understood any convenient type of threshold or calculation may be used to determine whether or not the first subset of data and the second subset of data are both statistically distinct

from one another, and also substantially different from one another in terms of overall average value.

At 624, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating a pyloric transition from the stomach to the duodenum was detected. For example, ingestible device 100 may store a data flag (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating that the ingestible device 100 most recently detected itself to be within the duodenum portion of the GI tract (e.g., duodenum 310 (FIG. 3)). In some embodiments, ingestible device 100 may also store data (e.g., within memory circuitry of PCB 120 (FIG. 2)) indicating a time that ingestible device 100 detected the pyloric transition from the stomach to the duodenum. This information may be used by ingestible device 100 at 608, and as a result process 600 may proceed from 608 to 610, rather than proceeding from 618 to 614. After ingestible device 100 stores the data indicating a pyloric transition from the stomach to the duodenum was detected, process 600 proceeds to 604 where ingestible device 100 continues to gather additional measurements, and continues to monitor for further transitions between the stomach and the duodenum.

It will be understood that the steps and descriptions of the flowcharts of this disclosure, including FIG. 6, are merely illustrative. Any of the steps and descriptions of the flowcharts, including FIG. 6, may be modified, omitted, rearranged, and performed in alternate orders or in parallel, two or more of the steps may be combined, or any additional steps may be added, without departing from the scope of the present disclosure. For example, the ingestible device 100 may calculate the mean and the standard deviation of multiple data sets in parallel in order to speed up the overall computation time. Furthermore, it should be noted that the steps and descriptions of FIG. 6 may be combined with any other system, device, or method described in this application, and any of the ingestible devices or systems discussed in this application could be used to perform one or more of the steps in FIG. 6. For example, portions of process 600 may be incorporated into 508-516 of process 500 (FIG. 5), and may be part of a more general process for determining a location of the ingestible device. As another example, the ratio of detected blue and green light (e.g., as measured and added to the data set at 604) may continue even outside of the stomach or duodenum, and similar information may be recorded by the ingestible device throughout its transit in the GI tract. Example plots of data sets of ratios of measured green and blue reflectance levels, which may be gathered throughout the GI tract, are discussed further in relation to FIG. 7 and FIG. 8 below.

FIG. 7 is a plot illustrating data collected during an example operation of an ingestible device (e.g., ingestible device 100, 300, or 400), which may be used when determining a location of an ingestible device as it transits through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure.

Although FIG. 7 may be described in connection with ingestible device 100 for illustrative purposes, this is not intended to be limiting, and plot 700 and data set 702 may be typical of data gathered by any device discussed in this application. Plot 700 depicts the ratios of the measured green reflectance levels to the measured blue reflectance levels over time. For example, ingestible device 100 may have computed the value for each point in the data set 702 by transmitting green and blue illumination at a given time (e.g., via illuminator 124 (FIG. 2)), measuring the resulting green and blue reflectances (e.g., via detector 122 (FIG. 2)), calculating the ratio of the resulting reflectances, and storing the ratio in the data set along with a timestamp indicating the time that the reflectances were gathered.

At 704, shortly after ingestible device 100 begins operation, ingestible device 100 determines that it has reached at least the stomach (e.g., as a result of making a determination similar to the determination discussed in relation to 506 in process 500 (FIG. 5)). Ingestible device 100 continues to gather additional measurements of green and blue reflectance levels, and at 706 ingestible device 100 determines that a pyloric transition has occurred from the stomach to the duodenum (e.g., as a result of making a determination similar to the determinations discussed in relation to 616-624 of process 600 (FIG. 6)). Notably, the values in data set 702 around 706 jump up precipitously, which is indicative of the higher ratios of measured green reflectance levels to measured blue reflectance levels typical of the duodenum.

The remainder of the data set 702 depicts the ratios of the measured green reflectance levels to the measured blue reflectance levels throughout the remainder of the GI tract. At 708, ingestible device 100 has reached the jejunum (e.g., as determined through measurements of muscle contractions, as discussed in relation to FIG. 9), and by 710, ingestible device 100 has reached the cecum. It is understood that, in some embodiments, the overall character and appearance of data set 702 changes within the small intestine (i.e., the duodenum, jejunum, and ileum) versus the cecum. Within the jejunum and ileum, there may typically be a wide variation in the ratios of the measured green reflectance levels to the measured blue reflectance levels, resulting in relatively noisy data with a high standard deviation. By comparison, within the cecum ingestible device 100 may measure a relatively stable ratio of the measured green reflectance levels to the measured blue reflectance levels. In some embodiments, ingestible

device 100 may be configured to determine transitions from the small intestine to the cecum based on these differences. For example, ingestible device 100 may compare recent windows of data to past windows of data, and detect a transition to the cecum in response to determining that the standard deviation of the ratios in the recent window of data is substantially less than the standard deviation of the ratios in the past window of data.

FIG. 8 is another plot illustrating data collected during an example operation of an ingestible device, which may be used when determining a location of an ingestible device as it transits through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure. Similar to FIG. 7, FIG. 8 may be described in connection with the ingestible device 100 for illustrative purposes. However, this is not intended to be limiting, and plot 800 and data set 802 may be typical of data gathered by any device discussed in this application.

At 804, shortly after ingestible device 100 begins operation, ingestible device 100 determines that it has reached at least the stomach (e.g., as a result of making a determination similar to the determination discussed in relation to 506 in process 500 (FIG. 5)). Ingestible device 100 continues to gather additional measurements of green and blue reflectance levels (e.g., via sensing sub-unit 126 (FIG. 2)), and at 806 ingestible device 100 determines that a pyloric transition has occurred from the stomach to the duodenum (e.g., as a result of making a determination similar to the determinations discussed in relation to 616-624 of process 600 (FIG. 6)). Notably, the values in data set 802 around 806 jump up precipitously, which is indicative of the higher ratios of measured green reflectance levels to measured blue reflectance levels typical of the duodenum, before falling shortly thereafter. As a result of the reduced values in data set 802, ingestible device 100 determines that a reverse pyloric transition has occurred from the duodenum back to the stomach at 808 (e.g., as a result of making a determination similar to the determinations discussed in relation to 610-612 of process 600 (FIG. 6)). At 810, as a result of the values in data set 802 increasing again, ingestible device 100 determines that another pyloric transition has occurred from the stomach to the duodenum, and shortly thereafter ingestible device 100 proceeds onwards to the jejunum, ileum, and cecum.

The remainder of the data set 802 depicts the ratios of the measured green reflectance levels to the measured blue reflectance levels throughout the remainder of the GI tract. Notably, at 812, ingestible device reaches the transition point between the ileum and the cecum. As discussed above in relation to FIG. 7, the transition to the cecum is marked by a reduced standard deviation in the ratios of measured green reflectances and measured blue reflectances over time, and ingestible device 100 may be configured to detect a transition to the cecum based on

determining that the standard deviation of a recent set of measurements is substantially smaller than the standard deviation of past measurements taken from the jejunum or ileum.

FIG. 9 is a flowchart of illustrative steps for detecting a transition from a duodenum to a jejunum, which may be used when determining a location of an ingestible device as it transits through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure. Although FIG. 9 may be described in connection with the ingestible device 100 for illustrative purposes, this is not intended to be limiting, and either portions or the entirety of process 900 described in FIG. 9 may be applied to any device discussed in this application (e.g., the ingestible devices 100, 300, and 400), and any of these ingestible devices may be used to perform one or more parts of the process described in FIG. 9. Furthermore, the features of FIG. 9 may be combined with any other systems, methods or processes described in this application. For example, portions of the process described by the process in FIG. 9 may be integrated into the localization process described by FIG. 5 (e.g., as part of 520-524 of process 500 (FIG. 5)). In some embodiments, an ingestible device 100 may perform process 900 while in the duodenum, or in response to detecting entry to the duodenum. In other embodiments, an ingestible device 100 may perform process 900 while in the stomach, or in response to detecting entry into the GI tract. It is also understood that process 900 may be performed in parallel with any other process described in this disclosure (e.g., process 600 (FIG. 6)), which may enable ingestible device 100 to detect entry into various portions of the GI tract, without necessarily detecting entry into a preceding portion of the GI tract.

For illustrative purposes, FIG. 9 may be discussed in terms of ingestible device 100 generating and making determinations based on a single set of reflectance levels generated at a single wavelength by a single sensing sub-unit (e.g., sensing sub-unit 126 (FIG. 2)). However, it is understood that ingestible device 100 may generate multiple wavelengths of illumination from multiple different sensing sub-units positioned around the circumference of ingestible device (e.g., multiple sensing sub-units positioned at different locations behind window 114 of ingestible device 100 (FIG. 1), and each of the resulting reflectances may be stored as a separate data set. Moreover, each of these sets of reflectance levels may be used to detect muscle contractions by running multiple versions of process 900, each one of which processes data for a different set of reflectances corresponding to data sets obtained from measurements of different wavelengths or measurements made by different sensing sub-units.

At 902, the ingestible device (e.g., ingestible device 100, 300, or 400) retrieves a set of reflectance levels. For example, ingestible device 100 may retrieve a data set of previously

recorded reflectance levels from memory (e.g., from memory circuitry of PCB 120 (FIG. 2)). Each of the reflectance levels may correspond to reflectances previously detected by ingestible device 100 (e.g., via detector 122 (FIG. 2)) from illumination generated by ingestible device 100 (e.g., via illuminator 124 (FIG. 2)), and may represent a value indicative of an amount of light detected in a given reflectance. However, it is understood that any suitable frequency of light may be used, such as light in the infrared, visible, or ultraviolet spectrums. In some embodiments, the reflectance levels may correspond to reflectances previously detected by ingestible device 100 at periodic intervals.

At 904, the ingestible device (e.g., ingestible device 100, 300, or 400) includes new measurements of reflectance levels in the data set. For example, ingestible device 100 may be configured to detect a new reflectance (e.g., transmit illumination and detect the resulting reflectance using sensing sub-unit 126 (FIG. 2)) at regular intervals, or with sufficient speed as to detect peristaltic waves. For example, ingestible device 100 may be configured to generate illumination and measure the resulting reflectance once every three seconds (i.e., the minimum rate necessary to detect a 0.17 Hz signal), and preferably at a higher rate, as fast at 0.1 second or even faster. It is understood that the periodic interval between measurements may be adapted as needed based on the species of the subject, and the expected frequency of the peristaltic waves to be measured. Every time ingestible device 100 makes a new reflectance level measurement at 904, the new data is included to the data set (e.g., a data set stored within memory circuitry of PCB 120 (FIG. 2)).

At 906, the ingestible device (e.g., ingestible device 100, 300, or 400) obtains a first subset of recent data by applying a sliding window filter to the data set. For example, ingestible device 100 may retrieve a one-minute worth of data from the data set. If the data set includes values for reflectances measured every second, this would be approximately 60 data points worth of data. Any suitable type of window size may be used, provided that the size of the window is sufficiently large to detect peristaltic waves (e.g., fluctuations on the order of 0.1 Hz to 0.2 Hz for healthy human subjects). In some embodiments, ingestible device 100 may also clean the data, for example, by removing outliers from the first subset of data obtained through the use of the sliding window filter.

At 908, the ingestible device (e.g., ingestible device 100, 300, or 400) obtains a second subset of recent data by interpolating the first subset of recent data. For example, ingestible device 100 may interpolate the first subset of data in order to generate a second subset of data with a sufficient number of data points (e.g., data points spaced every 0.5 seconds or greater). In

some embodiments, this may enable ingestible device 100 to also replace any outlier data points that may have been removed as part of applying the window filter at 906.

At 910, the ingestible device (e.g., ingestible device 100, 300, or 400) calculates a normalized frequency spectrum from the second subset of data. For example, ingestible device 100 may be configured to perform a fast Fourier transform to convert the second subset of data from a time domain representation into a frequency domain representation. It is understood that depending on the application being used, and the nature of the subset of data, any number of suitable procedures (e.g., Fourier transform procedures) may be used to determine a frequency spectrum for the second subset of data. For example, the sampling frequency and size of the second subset of data may be known in advance, and ingestible device 100 may be configured to have pre-stored values of a normalized discreet Fourier transform (DFT) matrix, or the rows of the DFT matrix corresponding to the 0.1 Hz to 0.2 Hz frequency components of interest, within memory (e.g., memory circuitry of PCB 120 (FIG. 2)). In this case, the ingestible device may use matrix multiplication between the DFT matrix and the data set to generate an appropriate frequency spectrum. An example data set and corresponding frequency spectrum that may be obtained by the ingestible device is discussed in greater detail in relation to FIG. 10.

At 912, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether at least a portion of the normalized frequency spectrum is between 0.1 Hz and 0.2 Hz above a threshold value of 0.5 Hz. Peristaltic waves in a healthy human subject occur at a rate between 0.1 Hz and 0.2 Hz, and an ingestible device experiencing peristaltic waves (e.g., ingestible device 400 detecting contractions in walls 406 of the jejunum (FIG. 4)) may detect sinusoidal variations in the amplitude of detected reflectances levels that follow a similar 0.1 Hz to 0.2 Hz frequency. If the ingestible device determines that a portion of the normalized frequency spectrum between 0.1 Hz and 0.2 Hz is above a threshold value of 0.5, this measurement may be consistent with peristaltic waves in a healthy human subject, and process 900 proceeds to 914 where ingestible device 100 stores data indicating a muscle contraction was detected. Alternatively, if the ingestible device determines that no portion of the normalized frequency spectrum between 0.1 Hz and 0.2 Hz above a threshold value of 0.5, process 900 proceeds directly to 904 to make new measurements and to continue to monitor for new muscle contractions. It is understood that a threshold value other than 0.5 may be used, and that the exact threshold may depend on the sampling frequency and type of frequency spectrum used by ingestible device 100.

At 914, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating a muscle contraction was detected. For example, ingestible device 100 may store data

in memory (e.g., memory circuitry of PCB 120 (FIG. 2)) indicating that a muscle contraction was detected, and indicating the time that the muscle contraction was detected. In some embodiments, ingestible device 100 may also monitor the total number of muscle contractions detected, or the number of muscle contractions detected in a given time frame. In some embodiments, detecting a particular number of muscle contractions may be consistent with ingestible device 100 being within the jejunum (e.g., jejunum 314 (FIG. 3)) of a healthy human subject. After detecting a muscle contraction, process 900 proceeds to 916.

At 916, the ingestible device (e.g., ingestible device 100, 300, or 400) determines whether a total number of muscle contractions exceeds a predetermined threshold number. For example, ingestible device 100 may retrieve the total number of muscle contractions detected from memory (e.g., from memory circuitry of PCB 120 (FIG. 2)), and compare the total number to a threshold value. In some embodiments, the threshold value may be one, or any number larger than one. The larger the threshold value, the more muscle contractions need to be detected before ingestible device 100 stores data indicating that it has entered the jejunum. In practice, setting the threshold value as three or higher may prevent the ingestible device from detecting false positives (e.g., due to natural movement of the GI tract organs, or due to movement of the subject). If the total number of contractions exceeds the predetermined threshold number, process 900 proceeds to 918 to store data indicating detection of a transition from the duodenum to the jejunum. Alternatively, if the total number of contractions does not exceed a predetermined threshold number, process 900 proceeds to 904 to include new measurements of reflectance levels in the data set. An example plot of the muscle contractions detected over time is discussed in greater detail in relation to FIG. 11.

At 918, the ingestible device (e.g., ingestible device 100, 300, or 400) stores data indicating detection of a transition from the duodenum to the jejunum. For example, ingestible device 100 may store data in memory (e.g., from memory circuitry of PCB 120 (FIG. 2)) indicating that the jejunum has been reached. In some embodiments, if ingestible device 100 is configured to perform all or part of process 900 while in the stomach, ingestible device 100 may store data at 918 indicating detection of a transition from the stomach directly to the jejunum (e.g., as a result of transitioning too quickly through the duodenum for the pyloric transition to be detected using process 600 (FIG. 6)).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may be configured to obtain a fluid sample from the environment external to a housing of the ingestible device in response to identifying a change in the location of the ingestible device. For

example, ingestible device 100 may be configured to obtain a fluid sample from the environment external to the housing of ingestible device 100 (e.g., through the use of optional opening 116 and optional rotating assembly 118 (FIG. 2)) in response to determining that the ingestible device is located within the jejunum (e.g., jejunum 314 (FIG. 3)). In some embodiments, ingestible device 100 may also be equipped with appropriate diagnostics to detect certain medical conditions based on the retrieved fluid sample, such as small intestinal bacterial overgrowth (SIBO).

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may be configured to deliver a dispensable substance that is pre-stored within the ingestible device from the ingestible device into the gastrointestinal tract in response to identifying the change in the location of the ingestible device. For example, ingestible device 100 may have a dispensable substance pre-stored within the ingestible device 100 (e.g., within a storage chamber or cavity on optional storage sub-unit 118-3 (FIG. 2)), and ingestible device 100 may be configured to dispense the substance into the gastrointestinal tract (e.g., through the use of optional opening 116 and optional rotating assembly 118 (FIG. 2)) when the ingestible device 100 detects that the ingestible device 100 is located within the jejunum (e.g., jejunum 314 (FIG. 3)). In some embodiments, this may enable ingestible device 100 to deliver substances (e.g., therapeutics and medicaments) at targeted locations within the GI tract.

In some embodiments, the ingestible device (e.g., ingestible device 100, 300, or 400) may be configured to perform an action based on the total number of detected muscle contractions. For example, ingestible device 100 may be configured to retrieve data indicative of the total number of muscle contractions (e.g., from memory circuitry of PCB 120 (FIG. 2)), and compare that to an expected number of muscle contractions in a healthy individual. In response, the ingestible device may either dispense a substance into the gastrointestinal tract (e.g., through the use of optional opening 116 and optional rotating assembly 118 (FIG. 2)), or may obtain a fluid sample from the environment external to the housing of ingestible device 100 (e.g., through the use of optional opening 116 and optional rotating assembly 118 (FIG. 2)). For instance, ingestible device 100 may be configured to obtain a sample in response to determining that a number of detected muscle contractions is abnormal, and differs greatly from the expected number. As another example, ingestible device 100 may be configured to deliver a substance into the GI tract (such as a medicament), in response to determining that the detected muscle contractions are consistent with a functioning GI tract in a healthy individual.

It will be understood that the steps and descriptions of the flowcharts of this disclosure, including FIG. 9, are merely illustrative. Any of the steps and descriptions of the flowcharts, including FIG. 9, may be modified, omitted, rearranged, performed in alternate orders or in parallel, two or more of the steps may be combined, or any additional steps may be added, without departing from the scope of the present disclosure. For example, the ingestible device 100 may calculate the mean and the standard deviation of multiple data sets in parallel (e.g., multiple data sets, each one corresponding to a different wavelength of reflectance or different sensing sub-unit used to detect the reflectance) in order to speed up the overall computation time. Furthermore, it should be noted that the steps and descriptions of FIG. 9 may be combined with any other system, device, or method described in this application, and any of the ingestible devices or systems discussed in this application could be used to perform one or more of the steps in FIG. 9.

FIG. 10 is a plot illustrating data collected during an example operation of an ingestible device, which may be used when detecting a transition from a duodenum to a jejunum, in accordance with some embodiments of the disclosure. Diagram 1000 depicts a time domain plot 1002 of a data set of reflectance levels measured by an ingestible device (e.g., the second subset of data discussed in relation to 908 of FIG. 9). In some embodiments, ingestible device 100 may be configured to gather data points at semi-regular intervals approximately 0.5 seconds apart. By comparison, diagram 1050 depicts a frequency domain plot 1004 of the same data set of reflectance levels measured by an ingestible device (e.g., as a result of ingestible device 100 calculating a frequency spectrum at 910 of FIG. 9). In some embodiments, ingestible device 100 may be configured to calculate the frequency spectrum through any convenient means.

In diagram 1050, the range of frequencies 1006 between 0.1 Hz and 0.2 Hz may be the range of frequencies that ingestible device 100 searches in order to detect muscle contractions. As shown in diagram 1050, there is a strong peak in the frequency domain plot 1004 around 0.14 Hz, which is consistent with the frequency of peristaltic motion in a healthy human individual. In this case, an ingestible device 100 analyzing frequency domain plot 1004 may be configured to determine that the data is consistent with a detected muscle contraction (e.g., using a process similar to 912 of process 900 (FIG. 9)), and may store data (e.g., in memory circuitry of PCB 120 (FIG. 2)) indicating that a muscle contraction has been detected. Because the muscle contraction was detected from the one-minute window of data ending at 118 minutes, ingestible device 100 may also store data indicating that the muscle contraction was detected at the 118-minute mark

(i.e., which may indicate that the ingestible device 100 was turned on and ingested by the subject 118 minutes ago).

FIG. 11 is a plot illustrating muscle contractions detected by an ingestible device over time, which may be used when determining a location of an ingestible device as it transits through a gastrointestinal (GI) tract, in accordance with some embodiments of the disclosure. In some embodiments, ingestible device 100 may be configured to detect muscle contractions, and store data indicative of when each muscle contraction is detected (e.g., as part of 914 of process 900 (FIG. 9)). Plot 1100 depicts the detected muscle contractions 1106 over time, with each muscle contraction being represented by a vertical line reaching from "0" to "1" on the y-axis.

At 1102, around the 10-minute mark, ingestible device 100 first enters the duodenum (e.g., as determined by ingestible device 100 performing process 600 (FIG. 6)). Shortly thereafter, at 1108, ingestible device 100 begins to detect several muscle contractions 1106 in quick succession, which may be indicative of the strong peristaltic waves that form in the jejunum (e.g., jejunum 314 (FIG. 3)). Later, around 1110, ingestible device 100 continues to detect intermittent muscle contractions, which may be consistent with an ingestible device 100 within the ileum. Finally, at 1104, ingestible device 100 transitions out of the small intestine, and into the cecum. Notably, ingestible device 100 detects more frequent muscle contractions in the jejunum portion of the small intestine as compared to the ileum portion of the small intestine, and ingestible device 100 does not measure any muscle contractions after having exited the small intestine. In some embodiments, ingestible device 100 may incorporate this information into a localization process. For example, ingestible device 100 may be configured to detect a transition from a jejunum to an ileum in response to determining that a frequency of detected muscle contractions (e.g., the number of muscle contractions measured in a given 10-minute window) has fallen below a threshold number. As another example, ingestible device 100 may be configured to detect a transition from an ileum to a cecum in response to determining that no muscle contractions have been detected for a threshold period of time. It is understood that these examples are intended to be illustrative, and not limiting, and that measurements of muscle contractions may be combined with any of the other processes, systems, or methods discussed in this disclosure.

FIG. 12 is a flowchart 1200 for certain embodiments for determining a transition of the device from the jejunum to the ileum. It is to be noted that, in general, the jejunum is redder and more vascular than the ileum. Moreover, generally, in comparison to the ileum, the jejunum has a thicker intestine wall with more messentary fat. These differences between the jejunum and the

ileum are expected to result in differences in optical responses in the jejunum relative to the ileum. Optionally, one or more optical signals may be used to investigate the differences in optical responses. For example, the process can include monitoring a change in optical response in reflected red light, blue light, green light, ratio of red light to green light, ratio of red light to blue light, and/or ratio of green light to blue light. In some embodiments, reflected red light is detected in the process.

Flowchart 1200 represents a single sliding window process. In step 1210, the jejenum reference signal is determined based on optical reflection. Typically, this signal is as the average signal (e.g., reflected red light) over a period of time since the device was determined to enter the jejenum. The period of time can be, for example, from five minutes to 40 minutes (e.g., from 10 minutes to 30 minutes, from 15 minutes to 25 minutes). In step 1220, the detected signal (e.g., reflected red light) just after the period of time used in step 1210 is normalized to the reference signal determined in step 1210. In step 1230, the signal (e.g., reflected red light) is detected. In step 1240, the mean signal detected based on the single sliding window is compared to a signal threshold. The signal threshold in step 1240 is generally a fraction of the reference signal of the jejenum reference signal determined in step 1210. For example, the signal threshold can be from 60% to 90% (e.g., from 70% to 80%) of the jejenum reference signal. If the mean signal exceeds the signal threshold, then the process determines that the device has entered the ileum at step 1250. If the mean signal does not exceed the signal threshold, then the process returns to step 1230.

FIG. 13 is a flowchart 1200 for certain embodiments for determining a transition of the device from the jejunum to the ileum using a two sliding window process. In step 1310, the jejenum reference signal is determined based on optical reflection. Typically, this signal is as the average signal (e.g., reflected red light) over a period of time since the device was determined to enter the jejenum. The period of time can be, for example, from five minutes to 40 minutes (e.g., from 10 minutes to 30 minutes, from 15 minutes to 25 minutes). In step 1320, the detected signal (e.g., reflected red light) just after the period of time used in step 1310 is normalized to the reference signal determined in step 1310. In step 1330, the signal (e.g., reflected red light) is detected. In step 1340, the mean difference in the signal detected based on the two sliding windows is compared to a signal threshold. The signal threshold in step 1340 is based on whether the mean difference in the detected signal exceeds a multiple (e.g., from 1.5 times to five times, from two times to four times) of the detected signal of the first window. If signal

threshold is exceeded, then the process determines that the device has entered the ileum at step 1350. If the signal threshold is not exceeded, then the process returns to step 1330.

FIG. 14 is a flowchart 1400 for a process for certain embodiments for determining a transition of the device from the ileum to the cecum. In general, the process involves detecting changes in the reflected optical signal (e.g., red light, blue light, green light, ratio of red light to green light, ratio of red light to blue light, and/or ratio of green light to blue light). In some embodiments, the process includes detecting changes in the ratio of reflected red light to reflected green light, and also detecting changes in the ratio of reflected green light to reflected blue light. Generally, in the process 1400, the sliding window analysis (first and second windows) discussed with respect to process 600 is continued.

Step 1410 includes setting a first threshold in a detected signal, e.g., ratio of detected red light to detected green light, and setting a second threshold for the coefficient of variation for a detected signal, e.g., the coefficient of variation for the ratio of detected green light to detected blue light. The first threshold can be set to a fraction (e.g., from 0.5 to 0.9, from 0.6 to 0.8) of the average signal (e.g., ratio of detected red light to detected green light) in the first window, or a fraction (e.g., from 0.4 to 0.8, from 0.5 to 0.7) of the mean difference between the detected signal (e.g., ratio of detected red light to detected green light) in the two windows. The second threshold can be set to 0.1 (e.g., 0.05, 0.02).

Step 1420 includes detecting the signals in the first and second windows that are to be used for comparing to the first and second thresholds.

Step 1430 includes comparing the detected signals to the first and second thresholds. If the corresponding value is not below the first threshold or the corresponding value is not below the second threshold, then it is determined that the device has not left the ileum and entered the cecum, and the process returns to step 1420. If the corresponding value is below the first threshold and the corresponding value is below the second threshold, then it is determined that the device has left the ileum and entered the cecum, and the proceeds to step 1440.

Step 1450 includes determining whether it is the first time that that the device was determined to leave the ileum and enter the cecum. If it is the first time that the device was determined to leave the ileum and enter the cecum, then the process proceeds to step 1460. If it is not the first time that the device has left the ileum and entered the cecum, then the process proceeds to step 1470.

Step 1460 includes setting a reference signal. In this step the optical signal (e.g., ratio of detected red light to detected green light) as a reference signal.

Step 1470 includes determining whether the device may have left the cecum and returned to the ileum. The device is determined to have left the cecum and returned to the ileum if the corresponding detected signal (e.g., ratio of detected red light to detected green light) is statistically comparable to the reference signal (determined in step 1460) and the coefficient of variation for the corresponding detected signal (e.g., ratio of detected green light to detected blue light) exceeds the second threshold. If it is determined that the device may have left the cecum and returned to the ileum, the process proceeds to step 1480.

Step 1480 includes continuing to detect the relevant optical signals for a period of time (e.g., at least one minute, from five minutes to 15 minutes).

Step 1490 includes determining whether the signals determined in step 1480 indicate (using the methodology discussed in step 1470) that the device re-entered the ileum. If the signals indicate that the device re-entered the ileum, the process proceeds to step 1420. If the signals indicate that the device is in the cecum, the process proceeds to step 1492.

Step 1492 includes continuing to monitor the relevant optical signals for a period of time (e.g., at least 30 minutes, at least one hour, at least two hours).

Step 1494 includes determining whether the signals determined in step 1492 indicate (using the methodology discussed in step 1470) that the device re-entered the ileum. If the signals indicate that the device re-entered the ileum, the process proceeds to step 1420. If the signals indicate that the device is in the cecum, the process proceeds to step 1496.

At step 1496, the process determines that the device is in the cecum.

FIG. 15 is a flowchart 1500 for a process for certain embodiments for determining a transition of the device from the cecum to the colon. In general, the process involves detecting changes in the reflected optical signal (e.g., red light, blue light, green light, ratio of red light to green light, ratio of red light to blue light, and/or ratio of green light to blue light). In some embodiments, the process includes detecting changes in the ratio of reflected red light to reflected green light, and also detecting changes in the ratio of reflected blue light. Generally, in the process 1500, the sliding window analysis (first and second windows) discussed with respect to process 1400 is continued.

In step 1510, optical signals (e.g., the ratio of reflected red signal to reflected green signal, and reflected blue signal) are collected for a period of time (e.g., at least one minute, at least five minutes, at least 10 minutes) while the device is in the cecum (e.g., during step 1480). The average values for the recorded optical signals (e.g., the ratio of reflected red signal to reflected green signal, and reflected blue signal) establish the cecum reference signals.

In step 1520, the optical signals are detected after it has been determined that the device entered the cecum (e.g., at step 1440). The optical signals are normalized to the cecum reference signals.

Step 1530 involves determining whether the device has entered the colon. This includes determining whether any of three different criteria are satisfied. The first criterion is satisfied if the mean difference in the ratio of a detected optical signal (e.g., ratio of detected red signal to the detected green) is a multiple greater than one (e.g., 2X, 3X, 4X) the standard deviation of the corresponding signal (e.g., ratio of detected red signal to the detected green) in the second window. The second criterion is satisfied if the mean of a detected optical signal (e.g., a ratio of detected red light to detected green light) exceeds a given value (e.g., exceeds one). The third criterion is satisfied if the coefficient of variation of an optical signal (e.g., detected blue light) in the first window exceeds a given value (e.g., exceeds 0.2). If any of the three criteria are satisfied, then the process proceeds to step 1540. Otherwise, none of the three criteria are satisfied, the process returns to step 1520.

For illustrative purposes the disclosure focuses primarily on a number of different example embodiments of an ingestible device, and example embodiments of methods for determining a location of an ingestible device within a GI tract. However, the possible ingestible devices that may be constructed are not limited to these embodiments, and variations in the shape and design may be made without significantly changing the functions and operations of the device. Similarly, the possible procedures for determining a location of the ingestible device within the GI tract are not limited to the specific procedures and embodiments discussed (e.g., process 500 (FIG. 5), process 600 (FIG. 6), process 900 (FIG. 9), process 1200 (FIG. 12), process 1300 (FIG. 13), process 1400 (FIG. 14) and process 1500 (FIG. 15)). Also, the applications of the ingestible devices described herein are not limited merely to gathering data, sampling and testing portions of the gastrointestinal tract, or delivering medicament. For example, in some embodiments the ingestible device may be adapted to include a number of chemical, electrical, or optical diagnostics for diagnosing a number of diseases. Similarly, a number of different sensors for measuring bodily phenomenon or other physiological qualities may be included on the ingestible device. For example, the ingestible device may be adapted to measure elevated levels of certain chemical compounds or impurities in the gastrointestinal tract, or the combination of localization, sampling, and appropriate diagnostic and assay techniques incorporated into a sampling chamber may be particularly well suited to determine the presence of small intestinal bacterial overgrowth (SIBO).

At least some of the elements of the various embodiments of the ingestible device described herein that are implemented via software (e.g., software executed by control circuitry within PCB 120 (FIG. 2)) may be written in a high-level procedural language such as object oriented programming, a scripting language or both. Accordingly, the program code may be written in C, C^{++} or any other suitable programming language and may comprise modules or classes, as is known to those skilled in object oriented programming. Alternatively, or in addition, at least some of the elements of the embodiments of the ingestible device described herein that are implemented via software may be written in assembly language, machine language or firmware as needed. In either case, the language may be a compiled or an interpreted language.

At least some of the program code used to implement the ingestible device can be stored on a storage media or on a computer readable medium that is readable by a general or special purpose programmable computing device having a processor, an operating system and the associated hardware and software that is necessary to implement the functionality of at least one of the embodiments described herein. The program code, when read by the computing device, configures the computing device to operate in a new, specific and predefined manner in order to perform at least one of the methods described herein.

Furthermore, at least some of the programs associated with the systems, devices, and methods of the example embodiments described herein are capable of being distributed in a computer program product comprising a computer readable medium that bears computer usable instructions for one or more processors. The medium may be provided in various forms, including non-transitory forms such as, but not limited to, one or more diskettes, compact disks, tapes, chips, and magnetic and electronic storage. In some embodiments, the medium may be transitory in nature such as, but not limited to, wire-line transmissions, satellite transmissions, internet transmissions (e.g. downloads), media, digital and analog signals, and the like. The computer useable instructions may also be in various formats, including compiled and non-compiled code.

The techniques described above can be implemented using software for execution on a computer. For instance, the software forms procedures in one or more computer programs that execute on one or more programmed or programmable computer systems (which may be of various architectures such as distributed, client/server, or grid) each including at least one processor, at least one data storage system (including volatile and non-volatile memory and/or storage elements), at least one input device or port, and at least one output device or port.

The software may be provided on a storage medium, such as a CD-ROM, readable by a general or special purpose programmable computer or delivered (encoded in a propagated signal) over a communication medium of a network to the computer where it is executed. All of the functions may be performed on a special purpose computer, or using special-purpose hardware, such as coprocessors. The software may be implemented in a distributed manner in which different parts of the computation specified by the software are performed by different computers. Each such computer program is preferably stored on or downloaded to a storage media or device (e.g., solid state memory or media, or magnetic or optical media) readable by a general or special purpose programmable computer, for configuring and operating the computer when the storage media or device is read by the computer system to perform the procedures described herein. The inventive system may also be considered to be implemented as a computer-readable storage medium, configured with a computer program, where the storage medium so configured causes a computer system to operate in a specific and predefined manner to perform the functions described herein.

Methods and Mechanisms of Delivery

FIG. 16 provides an example mock-up diagram illustrating aspects of a structure of an ingestible device 1600 for delivering a dispensable substance, such as a formulation of a therapeutic agent described herein, according to some embodiments described herein. In some embodiments, the ingestible device 1600 may generally be in the shape of a capsule, a pill or any swallowable form that may be orally consumed by an individual. In this way, the ingestible device 1600 may be ingested by a patient and may be prescribed by healthcare practitioners and patients.

The ingestible device 1600 includes a housing 1601 that may take a shape similar to a capsule, a pill, and/or the like, which may include two ends 1602a-b. The housing 1601 may be designed to withstand the chemical and mechanical environment of the GI tract (e.g., effects of muscle contractile forces and concentrated hydrochloric acid in the stomach). A broad range of materials that may be used for the housing 1601. Examples of these materials include, but are not limited to, thermoplastics, fluoropolymers, elastomers, stainless steel and glass complying with ISO 10993 and USP Class VI specifications for biocompatibility; and any other suitable materials and combinations thereof.

In some embodiment, the wall of the housing 1601 may have a thickness of 0.5mm-1mm, which is sufficient to sustain an internal explosion (e.g., caused by hydrogen ignition or over pressure inside the housing).

The housing 1601 may or may not have a pH-sensitive enteric coating to detect or otherwise be sensitive to a pH level of the environment external to the ingestible device. As discussed elsewhere in the application in more detail, the ingestible device 1600 may additionally or alternatively include one more sensors, e.g., temperature sensor, optical sense.

The housing 1601 may be formed by coupling two enclosure portions together. The ingestible device 1600 may include an electronic component within the housing 1600. The electronic component may be placed proximally to an end 1602b of the housing, and includes a printed circuit board (PCB), a battery, an optical sensing unit, and/or the like.

The ingestible device 1600 further includes a gas generating cell 1603 that is configured to generate gas and thus cause an internal pressure within the housing 1601. In some embodiments, the gas generating cell may include or be connected to a separate channel or valve of the ingestible device such that gas may be release through the channel or valve to create a motion to alter the position of the ingestible device within the GI tract. Such gas release can also be used to position the ingestible device relative to the intestinal lining. In another embodiment, gas may be released through the separate channel or valve to alter the surface orientation of the intestinal tissue prior to delivery of the dispensable substance.

A traveling plunger 1604 may be placed on top of the gas generating cell 1603 within the housing 1601. The traveling plunger 1604 is a membrane that separates the gas generating cell 1603 and a storage reservoir that stores the dispensable substance 1605. In some embodiments, the traveling plunger 1604 may be a movable piston. In some embodiments, the traveling plunger 1604 may instead be a flexible membrane such as but not limited to a diaphragm. In some embodiments, the traveling plunger 1604, which may have the form of a flexible diaphragm, may be placed along an axial direction of the housing 1601, instead of being placed on top of the gas generating cell 1603. The traveling plunger or the membrane 1604 may move (when the membrane 1604 is a piston) or deform (when the membrane 1604 is a diaphragm) towards a direction of the end 1602a of the housing, when the gas generating cell 1603 generates gas to create an internal pressure that pushes the membrane 1604. In this way, the membrane or traveling plunger 1604 may push the dispensable substance 1605 out of the housing via a dispensing outlet 1607.

The housing 1601 may include a storage reservoir storing one or more dispensable substances 1605 adjacent to the traveling plunger 1604. The dispensable substance 1605 may be a therapeutic or medical agent that may take a form of a powder, a compressed powder, a fluid, a semi-liquid gel, or any other dispensable or deliverable form. The delivery of the dispensable substance 1605 may take a form such as but not limited to bolus, semi-bolus, continuous, burst drug delivery, and/or the like. In some embodiments, a single bolus is delivered proximate to the disease location. In some embodiments, more than one bolus is released at one location or more than one location. In some embodiments the release of more than one bolus is triggered according to a pre-programmed algorithm. In some embodiments the release profile is continuous. In some embodiments the release profile is time-based. In some embodiments the release profile is location-based. In some embodiments, the amount delivered is based on the severity and/or extent of the disease in the following manner. In some embodiments, the bolus is delivered in one or more of the following locations: stomach; duodenum; proximal jejunum; ileum; cecum; ascending colon; transverse colon; descending colon.

In some embodiments the dispensable substance is a small molecule therapeutic that is released in the cecum and/or other parts of the large intestine. Small molecules that are administered by typical oral routes are primarily absorbed in the small intestine, with much lower absorption taking place in the large intestine (outside of the rectum). Accordingly, an ingestible device that is capable of releasing a small molecule selectively in the large intestine (e.g., the cecum) with resulting low systemic levels (even when high doses are used) is attractive for subjects with inflammatory bowel disease in the large intestine.

In some embodiments, the storage reservoir may include multiple chambers, and each chamber stores a different dispensable substance. For example, the different dispensable substances can be released at the same time via the dispensing outlet 1607. Alternatively, the multiple chambers may take a form of different layers within the storage reservoir such that the different dispensable substance from each chamber is delivered sequentially in an order. In one example, each of the multiple chambers is controlled by a separate traveling plunger, which may be propelled by gas generation. The electronic component may control the gas generating cell 1603 to generate gas to propel a specific traveling plunger, e.g., via a separate gas generation chamber, etc., to deliver the respective substance. In some embodiments, the content of the multiple chambers may be mixed or combined prior to release, for example, to activate the drug.

The ingestible device 1600 may include a dispensing outlet 1607 at one end 1602a of the housing 1601 to direct the dispensable substance 105 out of the housing. The dispensing outlet

1607 may include an exit valve, a slit or a hole, a jet injection nozzle with a syringe, and/or the like. When the traveling plunger 1604 moves towards the end 1602a of the housing 1601, an internal pressure within the storage reservoir may increase and push the dispensing outlet to be open to let the dispensable substance 1605 be released out of the housing 1601.

In an embodiment, a pressure relief device 1606 may be placed within the housing 1601, e.g., at the end 1602a of the housing 1601.

In some embodiments, the housing 1601 may include small holes (e.g., with a diameter smaller than 2 mm), e.g., on the side of the housing 1601, or at the end 1602a to facilitate loading the dispensable substance into the storage reservoir.

In some embodiments, a feedback control circuit (e.g., a feedback resistor, etc.) may be added to send feedback from the gas generating cell 1603 to the electronic component such that when the internal pressure reaches a threshold level, the electronic component may control the gas generating cell 1603 to turn off gas generation, or to activate other safety mechanism (e.g., feedback-controlled release valve, etc.). For example, an internal pressure sensor may be used to measure the internal pressure within the ingestible device and generate feedback to the feedback control circuit.

FIG. 17 provides an example diagram illustrating aspects of a mechanism for a gas generating cell 1603 configured to generate a gas to dispense a substance, according to some embodiments described herein. As shown in FIG. 17, the gas generating cell 1603 generates a gas 1611 which can propel the dispensable substance 1605 out of the dispensing outlet 1607. A variable resistor 1608 may be connected to a circuit with the gas generating cell 1603 such that the variable resistor 1608 may be used to control an intensity and/or an amount of gas 1611 (e.g., hydrogen) generated by the cell 1603. Specifically, the gas generating cell 1603 may be a battery form factor cell that is capable of generating hydrogen when a resistor is applied. In this way, as the gas generating cell 1603 only needs the use of a resistor only without any active power requirements, the gas generating cell 1603 may be integrated into an ingestible device such as a capsule with limited energy/power available. For example, the gas generating cell 1603 may be compatible with a capsule at a size of 26mm x 13mm or smaller.

In some embodiments, based on the elution rate of gas from the cell, and an internal volume of the ingestible device, it may take time to generate sufficient gas 1611 to deliver the substance 1605, and the time required may be 30 seconds or longer. For example, the time to generate a volume of hydrogen equivalent to 500μL of fluid would be approximately 5 minutes. A longer period of time may be needed based upon non-ideal conditions within the ingestible

device, such as friction, etc. Thus, given that the production of gas (e.g., hydrogen) may take time, gas generation may need to start prior to the ingestible device arriving at the site of delivery to build pressure up within the device. The ingestible device may then need to know when it is approaching the site of delivery. For example, the device may start producing gas on an "entry transition," which is determined by temperature, so as to produce enough gas to be close to the pressure high enough to deliver the dispensable substance. The ingestible device may then only start producing gas again when it arrives at the site of delivery, which will cause the internal pressure within the ingestible device to reach a level required by the dispensing outlet to release the dispensable substance. Also, for regio-specific delivery, the ingestible device may estimate the time it takes to build up enough pressure to deliver the dispensable substance before the ingestible device arrives at a specific location, to activate gas generation.

For example, for systemic delivery, when an internal volume of the ingestible device is around 500μL, a gas generation time of 2 hours, an initial pressure of approximately 300 pound per square inch absolute (psia) may be generated, with higher and lower pressures possible. The generated pressure may drop when air enters the storage reservoir which was previously occupied by the dispensable substance during the dispensing process. For systemic drug delivery, a force with a generated pressure of approximately 100 to 360 pound per square inch (psi) may be required for dermal penetration, e.g., to penetrate the mucosa or epithelial layer. The pressure may also vary depending on the nozzle design at the dispensing outlet, fluid viscosity, and surrounding tissue proximity and properties.

The gas 1611 that may be generated for a continuous delivery of drug (e.g., 1cc H_2 in 4 hours, 16 breaths per minute at 0.5L tidal volume) may equate to 1 cc hydrogen in approximately 2000L of exhaled air, or approximately 0.5 ppm H2, which is below physiologic values of exhaled hydrogen. Reducing this time to 10 minutes equates to approximately 13 ppm hydrogen. Thus, due to the length of intestine that may be covered during this time period, the ingestible device may possess a higher localized value than physiologic.

FIGs. 18 and 19, disclosed in US Provisional Application No. 62/385,553, incorporated by reference herein in its entirety, illustrates an example of an ingestible device for localized delivery of pharmaceutical compositions disclosed herein, in accordance with particular implementations. The ingestible device 1600 includes a piston or drive element 1634 to push for drug delivery, in accordance with particular implementations described herein. The ingestible device 1600 may have one or more batteries 1631 placed at one end 1602a of a housing 1601 to provide power for the ingestible device 1600. A printed circuit board (PCB) 1632 may be placed

adjacent to a battery or other power source 1631, and a gas generating cell 1603 may be mounted on or above the PCB 1632. The gas generating cell 1603 may be sealed from the bottom chamber (e.g., space including 1631 and 1632) of the ingestible device 1600. A movable piston 1634 may be placed adjacent to the gas generating cell 1603. In this way, gas generation from the gas generating cell 1603 may propel a piston 1634 to move towards another end 1602b of the housing 1601 such that the dispensable substance in a reservoir compartment 1635 can be pushed out of the housing through a dispensing outlet 1607, e.g., the movement is shown at 1636, with the piston 1634 at a position after dispensing the substance. The dispensing outlet 1607 may comprise a plug. The reservoir compartment 1635 can store the dispensable substance (e.g., drug substance), or alternatively the reservoir compartment can house a storage reservoir 1661 which comprises the dispensable substance. The reservoir compartment 1635 or storage reservoir 1661 may have a volume of approximately 600μL or even more dispensable substance, which may be dispensed in a single bolus, or gradually over a period of time.

The battery cells 1631 may have a height of 1.65 mm each, and one to three batteries may be used. The height of the piston may be reduced with custom molded part for around 1.5mm to save space. If the gas generating cell 1603 is integrated with the piston 1634, the overall height of the PCB, batteries and gas generating cell in total can be reduced to around 5 mm, thus providing more space for drug storage. For example, for an ingestible device of 7.8 mm in length (e.g., from end 1602a to the other end 1602b), a reservoir compartment 1635 or a storage reservoir 1661 of approximately 600μL may be used for drug delivery. For another example, for an ingestible device of 17.5 mm in length, a reservoir compartment 1635 or a storage reservoir 1661 of approximately 1300μL may be used for drug release.

In some implementations, at the reservoir 1635 or 1661 for storing a therapeutically effective amount of the stem cell forms at least a portion of the device housing 1601. The therapeutically effective amount of the stem cell can be stored in the reservoir 1635 or 1661 at a particular pressure, for example, determined to be higher than a pressure inside the GI tract so that once the reservoir 1635 or 1661 is in fluid communication with the GI tract, the stem cell is automatically released. In certain implementations, the reservoir compartment 1635 includes a plurality of chambers, and each of the plurality of the chambers stores a different dispensable substance or a different storage reservoir 1661.

In certain embodiments, the storage reservoir 1661 is a compressible component or has compressible side walls. In particular embodiments, the compressible component can be composed, at least in part, or coated (e.g., internally) with polyvinyl chloride (PVC), silicone,

DEHP (di-2-ethylhexyl phthalate), Tyvek, polyester film, polyolefin, polyethylene, polyurethane, or other materials that inhibit the stem cell from sticking to the reservoir and provide a sterile reservoir environment for the stem cell. The storage reservoir 1661 can be hermetically sealed. The reservoir compartment 1635 or storage reservoir 1661 can be configured to store stem cell in quantities in the range of 0.01 mL – 2 mL, such as 0.05 mL – 2 mL, such as 0.05 mL – 2 mL, such as 0.6mL – 2 mL. In some embodiments, the storage reservoir 1661 is attachable to the device housing 1601, for example, in the reservoir compartment. Accordingly, the storage reservoir 1635 can be loaded with the stem cell prior to being positioned in and/or coupled to the ingestible device housing 1601. The ingestible device housing 1601 includes one or more openings configured as a loading port to load the dispensable substance into the reservoir compartment. In another embodiment, the ingestible device housing 1601 includes one or more openings configured as a vent.

As noted above, in some embodiments, a storage reservoir (optionally, containing a stem cell, such as a therapeutically effective amount of stem cell) is attachable to an ingestible device. In general, in such embodiments the storage reservoir and ingestible device can be designed in any appropriate fashion so that the storage reservoir can attach to the ingestible device when desired. Examples of designs include a storage reservoir that fits entirely within the ingestible device (e.g., in the ingestible device so that the storage reservoir is sealed within the device at the time the device is ingested by a subject), a storage reservoir that fits partially within the ingestible device, and a storage reservoir that is carried by the housing of the device. In some embodiments, the storage reservoir snap fits with the ingestible device. In certain embodiments, the storage reservoir is friction fit with the ingestible device. In some embodiments, the storage reservoir is held together with the ingestible device via a biasing mechanism, such as one or more springs, one or more latches, one or more hooks, one or more magnets, and/or electromagnetic radiation. In certain embodiments, the storage reservoir can be a piercable member. In some embodiments, the ingestible device has a sleeve into which the storage reservoir securely fits. In some embodiments, the storage reservoir is disposed in/on a slidable track/groove so that it can move onto a piercing needle when delivery of the therapeutic agent is desired. In certain embodiments, the storage reservoir is made of a soft plastic coating, which is contacted with a needle at any orientation to deliver the therapeutic agent when desired. Generally, the storage reservoir can be made of one or more appropriate materials, such as, for example, one or more plastics and/or one or more metals or alloys. Exemplary materials include silicone, polyvinyl chloride, polycarbonate and stainless steel. Optionally, the design may be such that the storage

reservoir carries some or all of the electrical componentry to be used by the ingestible device. Although the foregoing discussion relates to one storage reservoir, it is to be understood that an ingestible device can be designed to carry any desired number (e.g., two, three, four, five) storage reservoirs. Different storage reservoirs can have the same or different designs. In some embodiments, the ingestible device (when fully assembled and packaged) satisfies the regulatory requirements for marketing a medical device in one or more jurisdictions selected from the United States of America, the European Union or any member state thereof, Japan, China, Brazil, Canada, Mexico, Colombia, Argentina, Chile, Peru, Russia, the UK, Switzerland, Norway, Turkey, Israel, any member state of the Gulf Cooperative Council, South Africa, India, Australia, New Zealand, South Korea, Singapore, Thailand, the Philippines, Malaysia, Viet Nam, Indonesia, Taiwan and Hong Kong.

In certain embodiments, the ingestible device housing 1601 includes one or more actuation systems (e.g., gas generating cell 1603) for pumping the stem cell from the reservoir 1635. In some embodiments, the actuation system can include a mechanical, electrical, electromechanical, hydraulic, and/or fluid actuation system. For example, a chemical actuation means may use chemical reaction of mixing one or more reagents to generate a sufficient volume of gas to propel the piston or drive element 1634 for drug release. The actuation system can be integrated into the reservoir compartment 1635 or can be an auxiliary system acting on or outside of the reservoir compartment 1635. For example, the actuation system can include pumping system for pushing/pulling the stem cell out of the reservoir compartment 1635 or the actuation system can be configured to cause the reservoir compartment 1635 to change structurally so that the volume inside of the reservoir compartment 1635 changes, thereby dispensing the stem cell from the reservoir compartment 1635. The actuation system can include an energy storage component such as a battery or a capacitor for powering the actuation system. The actuation system can be actuated via gas pressure or a system storing potential energy, such as energy from an elastic reservoir component being expanded during loading of the reservoir and after being positioned in the ingestible device housing 1601 being subsequently released from the expanded state when the ingestible device housing is at the location for release within the GI tract. In certain embodiments, the reservoir compartment 1635 can include a membrane portion, whereby the stem cell is dispensed from the reservoir compartment 1635 or storage reservoir 1661 via osmotic pressure.

In particular embodiments the storage reservoir 1661 is in a form of a bellow that is configured to be compressed via a pressure from the gas generating cell. The stem cell may be

loaded into the bellow, which may be compressed by gas generation from the gas generating cell or other actuation means to dispense the dispensable substance through the dispensing outlet 1607 and out of the housing 1601. In some embodiments, the ingestible device includes a capillary plate placed between the gas generating cell and the first end of the housing, and a wax seal between the gas generating cell and the reservoir, wherein the wax seal is configured to melt and the dispensable substance is pushed through the capillary plate by a pressure from the gas generating cell. The shape of the bellow may aid in controlled delivery. The reservoir compartment 1635 includes a dispensing outlet, such as a valve or dome slit 1662 extending out of an end of the housing 1601, in accordance with particular implementations. Thus when the bellow is being compressed, the dispensable substance may be propelled out of the bellow through the valve or the dome slit.

In certain embodiments, the reservoir compartment 1635 includes one or more valves (e.g. a valve in the dispensing outlet 1607) that are configured to move or open to fluidly couple the reservoir compartment 1635 to the GI tract. In certain embodiments, a housing wall of the housing 1601 can form a portion of the reservoir compartment 1635. In certain embodiments, the housing walls of the reservoir serve as a gasket. One or more of the one or more valves are positioned in the housing wall of the device housing 1601, in accordance with particular implementations. One or more conduits may extend from the reservoir 1635 to the one or more valves, in certain implementations.

In certain embodiments, a housing wall of the housing 1601 can be formed of a material that is configured to dissolve, for example, in response to contact at the disease site. In certain embodiments, a housing wall of the housing 1601 can be configured to dissolve in response to a chemical reaction or an electrical signal. The one or more valves and/or the signals for causing the housing wall of the housing 1601 to dissolve or dissipate can be controlled by one or more processors or controllers positioned on PCB 1632 in the device housing 1601. The controller is communicably coupled to one or more sensors or detectors configured to determine when the device housing 1601 is proximate to a disease site. The sensors or detectors comprise a plurality of electrodes comprising a coating, in certain implementations. Releasing of the stem cell from the reservoir compartment 1635 is triggered by an electric signal from the electrodes resulting from the interaction of the coating with the one or more sites of disease site. The one or more sensors can include a chemical sensor, an electrical sensor, an optical sensor, an electromagnetic sensor, a light sensor, a gas sensor, and/or a radiofrequency sensor. Methods for detecting volatile organic compounds (VOCs) and other gases from a biological sample include resistive

metal oxide gas sensors/mixed metal oxide gas sensors, electrochemical gas sensors, optical/IR gas sensors, conducting polymer/composite polymer resistive/capacitive gas sensors, quartz crystal microbalance gas sensors, carbon nanotubes, and pellister/calorimetric gas sensors. Examples of ingestible gas sensors are described in US Patent Publication No. US20130289368, which published on October 31, 2013, US Patent Publication No. US20170284956, which published on October 5, 2017, and PCT Patent Publication No. WO2016197181, which published on December 15, 2016. Examples of gases that can be detected in the gastrointestinal tract using a sensor include, but are not limited to, oxygen, hydrogen, and carbon dioxide.

In particular embodiments, the device housing 1601 can include one or more pumps configured to pump the therapeutically effective amount of the stem cell from the reservoir compartment 1635. The pump is communicably coupled to the one or more controllers. The controller is configured to activate the pump in response to detection by the one or more detectors of the disease site and activation of the valves to allow the reservoir 1635 to be in fluid communication with the GI tract. The pump can include a fluid actuated pump, an electrical pump, or a mechanical pump.

In certain embodiments, the device housing 1601 comprises one or more anchor systems for anchoring the device housing 1601 or a portion thereof at a particular location in the GI tract adjacent the disease site. In some embodiments, a storage reservoir comprises an anchor system, and the storage reservoir comprising a releasable substance is anchored to the GI tract. The anchor system can be activated by the controller in response to detection by the one or more detectors of the disease site. In certain implementations, the anchor system includes legs or spikes configured to extend from the housing wall(s) of the device housing 1601. The spikes can be configured to retract and/or can be configured to dissolve over time. An example of an attachable device that becomes fixed to the interior surface of the GI tract is described in PCT Patent Application PCT/US2015/012209, “Gastrointestinal Sensor Implantation System”, filed January 21, 2015, which is hereby incorporated by reference herein in its entirety.

FIG. 20 provides an example structural diagram having a flexible diaphragm 1665 that may deform towards the dispensing outlet 1607 when the gas generating cell 1603 generates gas. The dispensable substance may then be propelled by the deformed diaphragm out of the housing through the dispensing outlet 1607. The dispensing outlet 1607 shown at FIG. 20 is in the form of a ring valve, however, any outlet design can be applied.

In some embodiments, an ingestible device can have an umbrella-shaped exit valve structure as a dispensing outlet of the ingestible device. Optionally, an ingestible device can

have a flexible diaphragm to deform for drug delivery, and/or an integrated piston and gas generating cell such that the gas generating cell is movable with the piston to push for drug delivery.

In certain embodiments, an ingestible device can be anchored within the intestine by extending hooks from the ingestible device after it has entered the region of interest. For example, when the ingestible device determines it has arrived at a location within the GI tract, the hooks can be actuated to extend outside of the ingestible device to catch in the intestinal wall and hold the ingestible device in the respective location. In some embodiments, the hook can pierce into the intestinal wall to hold the ingestible device 100 in place. The hooks can be hollow. A hollow hook can be used to anchor the ingestible device and/or to dispense a substance from the dispensable substance, e.g., into the intestinal wall.

In some embodiments an ingestible device includes an intestinal gripper to grip a portion of the intestinal wall for delivering the dispensable substance. Such a gripper can include two or more arms configured to out of the device and close to grip a portion of the intestinal wall.

An injecting needle can be used with the anchoring arms to inject dispensable substance into the intestinal wall after a portion of the intestinal wall is gripped.

In some embodiments, when the gas generating cell generates gas to propel the piston to move towards the nozzle such that the dispensable substance can be pushed under the pressure to break a burst disc to be injected via the nozzle.

In some embodiments, an ingestible device has a jet delivery mechanism with enhanced usable volume of dispensable substance. For example, the nozzle may be placed at the center of the ingestible device, and gas channels may be placed longitudinally along the wall of the ingestible device to transport gas from the gas generating cell to propel the piston, which is placed at an end of the ingestible device.

In some embodiments, the ingestible device can use osmotic pressure to adhere a suction device of the ingestible device to the intestinal wall. For example, the ingestible device may have an osmotic mechanism that has a chamber storing salt crystals. The chamber can include a mesh placed in proximate to a burst valve at one end of the chamber, and a reverse osmosis (RO) membrane placed in proximate to a valve on the other end of the chamber. A suction device, e.g., two or more suction fingers, is placed outside of the chamber with an open outlet exposed to luminal fluid in the GI tract. When the osmotic mechanism is inactivated, e.g., the valve is closed so that no luminal fluid is drawn into the osmotic chamber. When the osmotic mechanism is activated by opening the valve, luminal fluid enters the ingestible device through an outlet of

the suction device and enters the osmotic chamber through the valve. The salt in the chamber is then dissolved into the fluid. The RO membrane prevents any fluid to flow in the reverse direction, e.g., from inside the chamber to the valve. The fluid continues to flow until all the salt contained in the chamber is dissolved or until intestinal tissue is drawn into the suction device. As luminal fluid keeps flowing into the chamber, the solution of the luminal fluid with dissolved salt in the chamber may reduce osmotic pressure such that the suction force at may also be reduced. In this way, suction of the intestinal tissue may stall before the tissue is in contact with the valve to avoid damage to the intestinal tissue.

An ingestible device employing an osmotic mechanism can also include a suction device as illustrated. The suction device can be two or more suction fingers 347a-b disposed proximate to the outlet. The outlet can be connected to a storage reservoir storing the dispensable substance (e.g., therapeutic agent). The storage reservoir can contact a piston (similar to 104 in FIG. 16), which can be propelled by pressure generated from the osmotic pump to move towards the outlet. The osmotic pump can be similar to the osmotic mechanism described in the preceding paragraph. A breakaway section can be placed in proximate to the other end (opposite to the end where the outlet 107 is disposed) of the ingestible device.

In some embodiments, tumbling suction by an ingestible device is used. Such an ingestible device does not require any electronics or other actuation elements. Such an ingestible device may constantly, intermittently, or periodically tumble when travelling through the intestine. When the ingestible device tumbles to a position that the outlet is in direct contact with the intestinal wall, a suction process similar to that described in the preceding paragraph may occur. Additional structural elements such as fins, flutes or the like may be added to the outer wall of the ingestible device 100 to promote the tumbling motion.

In certain embodiments, the reservoir is an anchorable reservoir, which is a reservoir comprising one or more anchor systems for anchoring the reservoir at a particular location in the GI tract adjacent the disease site. In certain embodiments, the anchor system includes legs or spikes or other securing means such as a piercing element, a gripping element, a magnetic-flux-guiding element, or an adhesive material, configured to extend from the anchorable reservoir of the device housing. The spikes can be configured to retract and/or can be configured to dissolve over time. In some embodiments, the anchorable reservoir is suitable for localizing, positioning and/or anchoring. In some embodiments, the anchorable reservoir is suitable for localizing, and positioning and/or anchoring by an endoscope. In some embodiments, the anchorable reservoir is connected to the endoscope. In some embodiments, the anchorable reservoir is connected to

the endoscope in a manner suitable for oral administration. In some embodiments, the anchorable reservoir is connected to the endoscope in a manner suitable for rectal administration. Accordingly, provided herein in some embodiments is an anchorable reservoir is connected to an endoscope wherein the anchorable reservoir comprises a therapeutically effective amount of the stem cell. In some embodiments the endoscope is fitted with a spray catheter.

Exemplary embodiments of anchorable reservoirs are as follows. In more particular examples of the following exemplary embodiments the reservoir is connected to an endoscope.

In one embodiment, the anchorable reservoir comprises an implant capsule for insertion into a body canal to apply radiation treatment to a selected portion of the body canal. The reservoir includes a body member defining at least one therapeutic treatment material receiving chamber and at least one resilient arm member associated with the body member for removably engaging the body canal when the device is positioned therein.

In one embodiment the anchorable reservoir has multiple suction ports and permits multiple folds of tissue to be captured in the suction ports with a single positioning of the device and attached together by a tissue securement mechanism such as a suture, staple or other form of tissue bonding. The suction ports may be arranged in a variety of configurations on the reservoir to best suit the desired resulting tissue orientation.

In some embodiments an anchorable reservoir comprises a tract stimulator and/or monitor IMD comprising a housing enclosing electrical stimulation and/or monitoring circuitry and a power source and an elongated flexible member extending from the housing to an active fixation mechanism adapted to be fixed into the GI tract wall is disclosed. After fixation is effected, the elongated flexible member bends into a preformed shape that presses the housing against the mucosa so that forces that would tend to dislodge the fixation mechanism are minimized. The IMD is fitted into an esophageal catheter lumen with the fixation mechanism aimed toward the catheter distal end opening whereby the bend in the flexible member is straightened. The catheter body is inserted through the esophagus into the GI tract cavity to direct the catheter distal end to the site of implantation and fix the fixation mechanism to the GI tract wall. The IMD is ejected from the lumen, and the flexible member assumes its bent configuration and lodges the hermetically sealed housing against the mucosa. A first stimulation/sense electrode is preferably an exposed conductive portion of the housing that is aligned with the bend of the flexible member so that it is pressed against the mucosa. A second stimulation/sense electrode is located at the fixation site.

In some embodiments a reservoir for sensing one or more parameters of a patient is anchored to a tissue at a specific site and is released from a device, using a single actuator operated during a single motion. As an example, a delivery device may anchor the capsule to the tissue site and release the reservoir from the delivery device during a single motion of the actuator.

In some embodiments a device is provided comprising: a reservoir configured to contain a fluid, the reservoir having at least one outlet through which the fluid may exit the reservoir; a fluid contained within the reservoir; a primary material contained within the reservoir and having a controllable effective concentration in the fluid; and at least one electromagnetically responsive control element located in the reservoir or in a wall of the reservoir and adapted for modifying the distribution of the primary material between a first active form carried in the fluid and a second form within the reservoir in response to an incident electromagnetic control signal, the effective concentration being the concentration of the first active form in the fluid, whereby fluid exiting the reservoir carries the primary material in the first active form at the effective concentration.

In some embodiments systems and methods are provided for implementing or deploying medical or veterinary devices or reservoirs (a) operable for anchoring at least partly within a digestive tract, (b) small enough to pass through the tract per vias naturales and including a wireless-control component, (c) having one or more protrusions positionable adjacent to a mucous membrane, (d) configured to facilitate redundant modes of anchoring, (e) facilitating a "primary" material supply deployable within a stomach for an extended and/or controllable period, (f) anchored by one or more adaptable extender modules supported by a subject's head or neck, and/or (g) configured to facilitate supporting at least a sensor within a subject's body lumen for up to a day or more.

In certain embodiments, the reservoir is attachable to an ingestible device. In certain embodiments, the ingestible device comprises a housing and the reservoir is attachable to the housing. In certain embodiments, the attachable reservoir is also an anchorable reservoir, such as an anchorable reservoir comprising one or more anchor systems for anchoring the reservoir at a particular location in the GI tract as disclosed hereinabove.

Accordingly, in certain embodiments, provided herein is a stem cell for use in a method of treating a disease of the gastrointestinal tract as disclosed herein, wherein the stem cell is contained in a reservoir suitable for attachment to a device housing, and wherein the method

comprises attaching the reservoir to the device housing to form the ingestible device, prior to orally administering the ingestible device to the subject.

In certain embodiments, provided herein is an attachable reservoir containing a stem cell for use in a method of treating a disease of the gastrointestinal tract, wherein the method comprises attaching the reservoir to a device housing to form an ingestible device and orally administering the ingestible device to a subject, wherein the stem cell is released by device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In certain embodiments, provided herein is an attachable reservoir containing a stem cell, wherein the reservoir is attachable to a device housing to form an ingestible device that is suitable for oral administration to a subject and that is capable of releasing the stem cell at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

In particular implementation the ingestible device includes cameras (e.g., video cameras) that affords inspection of the entire GI tract without discomfort or the need for sedation, thus avoiding many of the potential risks of conventional endoscopy. Video imaging can be used to help determine one or more characteristics of the GI tract, including the location of disease (e.g., presence or location of inflamed tissue and/or lesions associated with inflammatory bowel disease). In some embodiments, the ingestible device 101 may comprise a camera for generating video imaging data of the GI tract which can be used to determine, among other things, the location of the device. Examples of video imaging capsules include Medtronic's PillCam™, Olympus' Endocapsule®, and IntroMedic's MicroCam™. For a review of imaging capsules, see Basar et al. "Ingestible Wireless Capsule Technology: A Review of Development and Future Indication" International Journal of Antennas and Propagation (2012); 1-14). Other imaging technologies implemented with the device 101 can include thermal imaging cameras, and those that employ ultrasound or Doppler principles to generate different images (see Chinese patent application CN104473611: "Capsule endoscope system having ultrasonic positioning function".

Ingestible devices can be equipped with sources for generating reflected light, including light in the Ultraviolet, Visible, Near-infrared and/or Mid-infrared spectrum, and the corresponding detectors for spectroscopy and hyperspectral imaging. Likewise, autofluorescense may be used to characterize GI tissue (e.g., subsurface vessel information), or low-dose radiation (see Check-Cap™) can be used to obtain 3D reconstructed images.

Device Components

An ingestible device in accordance with particular embodiments of the present invention may comprise a component made of a non-digestible material and contain the stem cell. In some embodiments, the material is plastic.

It is envisaged that the device is single-use. The device is loaded with a drug prior to the time of administration. In some embodiments, it may be preferred that there is provided a medicinal product comprising the device pre-filled with the drug.

Anchoring components

Several systems may actively actuate and control the capsule position and orientation in different sections of the GI tract. Examples include leg-like or anchor-like mechanisms that can be deployed by an ingestible device to resist peristaltic forces in narrowed sections of the GI tract, such as the intestine, and anchor the device to a location. Other systems employ magnetic shields of different shapes that can interact with external magnetic fields to move the device. These mechanisms may be particularly useful in areas outside of the small intestine, like the cecum and large intestine.

An anchoring mechanism may be a mechanical mechanism. For example, a device may be a capsule comprising a plurality of legs configured to steer the capsule. The number of legs in the capsule may be, for example, two, four, six, eight, ten or twelve. The aperture between the legs of the device may be up to about 35 mm; about 30 to about 35 mm; about 35 to about 75 mm; or about 70 to about 75 mm. The contact area of each leg may be varied to reduce impact on the tissue. One or more motors in the capsule may each actuate a set of legs independently from the other. The motors may be battery-powered motors.

An anchoring mechanism may be a non-mechanical mechanism. For example, a device may be a capsule comprising a permanent magnet located inside the capsule. The capsule may be anchored at the desired location of the GI tract by an external magnetic field.

An anchoring mechanism may comprise a non-mechanical mechanism and a mechanical mechanism. For example, a device may be a capsule comprising one or more legs, one or more of which are coated with an adhesive material.

Locomotion components

Ingestible devices can be active or passive, depending on whether they have controlled or non-controlled locomotion. Passive (non-controlled) locomotion is more commonly used among

ingestible devices given the challenges of implementing a locomotion module. Active (controlled) locomotion is more common in endoscopic ingestible capsules. For example, a capsule may comprise a miniaturized locomotion system (internal locomotion). Internal locomotion mechanisms may employ independent miniaturized propellers actuated by DC brushed motors, or the use of water jets. As an example, a mechanism may comprise flagellar or flap-based swimming mechanisms. As an example, a mechanism may comprise cyclic compression/extension shape-memory alloy (SMA) spring actuators and anchoring systems based on directional micro-needles. As an example, a mechanism may comprise six SMA actuated units, each provided with two SMA actuators for enabling bidirectional motion. As an example, a mechanism may comprise a motor adapted to electrically stimulating the GI muscles to generate a temporary restriction in the bowel.

As an example, a capsule may comprise a magnet and motion of the capsule is caused by an external magnetic field. For example, a locomotion system may comprise an ingestible capsule and an external magnetic field source. For example, the system may comprise an ingestible capsule and magnetic guidance equipment such as, for example, magnetic resonance imaging and computer tomography, coupled to a dedicated control interface.

In some embodiments drug release mechanisms may also be triggered by an external condition, such as temperature, pH, movement, acoustics, or combinations thereof.

Use of an endoscope or an ingestible device in biopsy and surgery

Sampling

Ingestible devices may comprise a mechanism adapted to permit the collection of tissue samples. In some examples, this is achieved using electro-mechanical solutions to collect and store the sample inside an ingestible device. As an example, a biopsy mechanism may include a rotational tissue cutting razor fixed to a torsional spring or the use of microgrippers to fold and collect small biopsies. As an example, Over-the-scope clips (OTSC®) may be used to perform endoscopic surgery and/or biopsy. As an example of the methods disclosed herein, the method may comprise releasing a stem cell and collecting a sample inside the device. As an example, the method may comprise releasing a stem cell and collecting a sample inside the device in a single procedure.

FIG. 21 illustrates an example ingestible device 2100 with multiple openings in the housing. The ingestible device 2100 has an outer housing with a first end 2102A, a second end 2102B, and a wall 2104 extending longitudinally from the first end 2102A to the second end

2102B. Ingestible device 2100 has a first opening 2106 in the housing, which is connected to a second opening 2108 in the housing. The first opening 2106 of the ingestible device 2100 is oriented substantially perpendicular to the second opening 2108, and the connection between the first opening 2106 and the second opening 2108 forms a curved chamber 2110 within the ingestible device 2100.

The overall shape of the ingestible device 2100, or any of the other ingestible devices discussed in this disclosure, may be similar to an elongated pill or capsule.

In some embodiments, a portion of the curved chamber 2110 may be used as a sampling chamber, which may hold samples obtained from the GI tract. In some embodiments the curved chamber 2110 is subdivided into sub-chambers, each of which may be separated by a series of one or more valves or interlocks.

In some embodiments, the first opening 2106, the second opening 2108, or the curved chamber 2110 include one or more of a hydrophilic or hydrophobic material, a sponge, a valve, or an air permeable membrane.

The use of a hydrophilic material or sponge may allow samples to be retained within the curved chamber 2110, and may reduce the amount of pressure needed for fluid to enter through the first opening 2106 and dislodge air or gas in the curved chamber 2110. Examples of hydrophilic materials that may be incorporated into the ingestible device 2100 include hydrophilic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, and the like. Similarly, materials that have undergone various types of treatments, such as plasma treatments, may have suitable hydrophilic properties, and may be incorporated into the investible device 2100. Sponges may be made of any suitable material or combination of materials, such as fibers of cotton, rayon, glass, polyester, polyethylene, polyurethane, and the like. Sponges generally may be made from commercially available materials, such as those produced by Porex®.

As discussed in more detail below, in some embodiments, the sponges may be treated in order to change their absorbency or to help preserve samples.

In some embodiments, the sponges may be cut or abraded to change their absorbency or other physical properties.

Hydrophobic materials located near the second opening 2108 may repel liquids, discouraging liquid samples from entering or exiting the curved chamber 2110 through the second opening 2108. This may serve a similar function as an air permeable membrane. Examples of hydrophobic materials which may be incorporated into the ingestible device 2100

include polycarbonate, acrylics, fluorocarbons, styrenes, certain forms of vinyl, stainless steel, silicone, and the like.

The various materials listed above are provided as examples, and are not limiting. In practice, any type of suitable hydrophilic, hydrophobic, or sample preserving material may be used in the ingestible device 2100.

In some embodiments, an ingestible device includes a moveable valve as a diaphragm valve, which uses a mechanical actuator to move a flexible diaphragm in order to seal or unseal an aperture in a second portion of an inlet region, which may effectively block or unblock the inlet region. However, it will be understood that, in some embodiments, the moveable valve may be a different type of valve. For example, in some embodiments the moveable valve may be replaced by a pumping mechanism. As another example, in some embodiments the moveable valve is replaced with an osmotic valve

A sampling chamber of an ingestible device can have an exit port to allow air or gas to exit the sampling chamber, while preventing at least a portion of the sample obtained by the ingestible device from exiting the sampling chamber. For example, the exit port may include a gas-permeable membrane. An ingestible device can include one-way valve as part of its exit port.

An ingestible device can include an outlet port connected to the volume within housing of the ingestible device. The outlet port may provide a path for the gas to exit the ingestible device and be released into the environment surrounding the ingestible device. This may prevent pressure from building up within the housing of the ingestible device. In some embodiments, an ingestible device does not include an outlet port, and the gas stays inside the volume of the ingestible device. In some embodiments, the outlet port may contain a gas permeable membrane, a one-way valve, a hydrophobic channel, or some other mechanism to avoid unwanted material, (e.g., fluids and solid particulates from within the GI tract), from entering the ingestible device through the outlet port.

In some embodiments, the ingestible device may include a sensor within or proximate to the sampling chamber. For example, this sensor may be used to detect various properties of a sample contained within the sampling chamber, or this sensor may be used to detect the results of an assay technique applied to the sample contained within the sampling chamber.

In some embodiments, a hydrophilic sponge is located within the sampling chamber, and the hydrophilic sponge may be configured to absorb the sample as the sample enters the sampling chamber. In some embodiments, the hydrophilic sponge fills a substantial portion of the

sampling chamber, and holds the sample for an extended period of time. This may be particularly advantageous if the sample is collected from the ingestible device after the ingestible device exits the body. In some embodiments, the hydrophilic sponge is placed on only certain surfaces or fills only certain portions of the sampling chamber. For example, it may be possible to line certain walls (or all walls) of the sampling chamber with a hydrophilic sponge to assist in drawing in the sample, while leaving some (or none) of the walls of the sampling chamber uncovered. Leaving walls uncovered may allow the use of diagnostics or assay techniques that require a relatively un-obscured optical path.

In some embodiments, the ingestible device may include a sealed vacuum chamber connected to the exit port, or connected directly or indirectly to the sampling chamber. In some embodiments a pin valve may be used as a moveable valve (e.g., as moveable valve of ingestible device). In certain embodiments, a rotary valve may be used as a moveable valve (e.g., as moveable valve of ingestible device). In some embodiments, a flexible diaphragm, or diaphragm valve, may be used as a moveable valve (e.g., as moveable valve of ingestible device). In certain embodiments, a mechanism is near the diaphragm or in direct contact with the diaphragm. The spring mechanism may apply pressure to the diaphragm to oppose the pressure applied by the mechanical actuator, which may cause the flexible diaphragm to be moved into an open position when the mechanical actuator is not applying pressure to the flexible diaphragm. Additionally, this may ensure that the diaphragm valve remains open when the mechanical actuator is not applying pressure across the flexible diaphragm. In some embodiments, moving the mechanical actuator from a closed position to an open position causes a volume of the inlet region within the ingestible device to increase. This may cause the pressure within the inlet region to be reduced, generating suction to draw a sample into the inlet region. Similarly, moving the mechanical actuator from an open position to a closed position may cause the volume of the inlet region to be reduced. This may cause the pressure within the inlet region to be increased, pushing the sample out of the inlet region. Depending on the design of the inlet region, the mechanical actuator, and the moveable valve, this may push the sample into the sampling chamber rather than pushing the sample back through the opening in the ingestible device.

FIG. 22 depicts a cross-sectional view of a portion of the interior of ingestible device 3000. As shown in FIG. 22, the interior of ingestible device 3000 includes a valve system 3100 and a sampling system 3200. Valve system 3100 is depicted as having a portion that is flush with the opening 3018 so that valve system 3100 prevents fluid exterior to ingestible device 2000 from entering sampling system 3200. However, as described in more detail below with reference

to FIGs. 22-27, valve system 3100 can change position so that valve system 3100 allows fluid exterior to ingestible device 3000 to enter sampling system 3200.

FIGs. 23 and 27 illustrate valve system 3100 in more detail. As shown in FIG. 23, valve system 3100 includes an actuation mechanism 3110, a trigger 3120, and a gate 3130. In FIGs. 23 and 7, a leg 3132 of gate 3130 is flush against, and parallel with, housing wall 3016 so that gate leg 3132 covers opening 3018 to prevent fluid exterior to ingestible device 3000 (e.g., fluid in the GI tract) from entering the interior of ingestible device 3000. A protrusion 3134 of gate 3130 engages a lip 3122 of trigger 3120. A peg 3124 of trigger 3120 engages a wax pot 3112 of actuation mechanism 3110. Referring to FIG. 27, a biasing mechanism 3140 includes a compression spring 3142 that applies an upward force on gate 3130. Biasing mechanism 3140 also includes a torsion spring 3144 that applies a force on trigger 3120 in the counter-clockwise direction. In FIGs. 23 and 27, the force applied by torsion spring 3144 is counter-acted by the solid wax in pot 3112, and the force applied by compression spring 3142 is counter-acted by lip 3122.

FIGs. 24A and FIG 24B show an embodiment of the manner in which actuation mechanism 3110 actuates movement of trigger 3120. Similar to FIGs. 23 and 27, FIG. 24A shows a configuration in which peg 3124 applies a force against solid wax pot 3112 due to torsion spring 3144, and in which the solid nature of wax pot 3112 resists the force applied by peg 3124. A control unit 3150 is in signal communication with valve system 3100. During use of ingestible device 3000, a control unit 3150 receives a signal, indicating that the position of valve system 3100 should change, e.g., so that ingestible device 3000 can take a sample of a fluid in the GI tract. Control unit 3150 sends a signal that causes a heating system 3114 of actuation system 3100 to heat the wax in pot 3112 so that the wax melts. As shown in FIG. 24B, the melted wax is not able to resist the force applied by peg 3124 so that, under the force of torsion spring 3144, trigger 3120 moves in a counter-clockwise fashion.

FIGs. 25A and 25B illustrate the interaction of trigger 3120 and gate 3130 before and after actuation. As shown in FIG 25A, when wax pot 3112 is solid (corresponding to the configuration shown in FIG. 24A), protrusion 3134 engages lip 3122, which prevents the force of compression spring 3142 from moving gate 3130 upward. As shown in FIG. 25B, when the wax in pot 3112 melts (FIG. 24B), trigger 3120 moves counter-clockwise, and lip 3122 disengages from protrusion 3134. This allows the force of compression spring 3142 to move gate 3130 upward. As seen by comparing FIG. 25A to FIG. 25B, the upward movement of gate 3130 results in an upward movement of an opening 3136 in gate leg 3132.

FIGs. 26A and 26B illustrate the impact of the upward movement of opening 3136 on the ability of ingestible device 3000 to obtain a sample. As shown in FIG. 26A, when the wax in pot 3112 is solid (FIGs. 24A and 25A), opening 3136 in is not aligned with opening 3018 in wall 3016 of ingestible device 3000. Instead, gate leg 3132 covers opening 3018 and blocks fluid from entering the interior of ingestible device 3000. As shown in FIG. 26B, when the wax in pot 3112 is melted and trigger 3120 and gate 3130 have moved (FIGs. 24B and 42B), opening 3136 in gate 3130 is aligned with opening 3018 in wall 3016. In this configuration, fluid that is exterior to ingestible device 3000 (e.g., in the GI tract) can enter the interior of ingestible device 3000 via openings 3018 and 3036.

FIG. 27 illustrates a more detailed view of ingestible device 3000 including valve system 3100 and sampling system 3200.

While the foregoing description is made with regard to a valve system having one open position and one closed position (e.g., a two-stage valve system), the disclosure is not limited in this sense. Rather, the concepts described above with regard to a two stage valve system can be implemented with a valve system have more than two stages (e.g., three stages, four stages, five stages, etc.).

As noted above in addition to a valve system, an ingestible device includes a sampling system. FIG. 28 illustrates a partial cross sectional view of ingestible device 3000 with sampling system 3200 and certain components of valve system 3100. Sampling system 3200 includes a series of sponges configured to absorb fluid from an opening, move the fluid to a location within the housing, and prepare the fluid for testing. Preparation for testing may include filtering the fluid and combining the fluid with a chemical assay. The assay may be configured to dye cells in the filtered sample. The series of sponges includes a wicking sponge 3210, a transfer sponge 3220, a volume sponge 3230, and an assay sponge 3240. Sampling system 3200 also includes a membrane 3270 located between assay sponge 3240 and a vent 3280 for gases to leave sampling system 3200. A cell filter 3250 is located between distal end 3214 of wicking sponge 3210 and a first end 3222 of transfer sponge 3220. Membrane 3270 is configured to allow one or more gases to leave sampling system 3200 via an opening 3280, while maintaining liquid in sampling system 3200.

FIG. 29 is a highly schematic illustration of an ingestible device 4000 that contains multiple different systems that cooperate for obtaining a sample and analyzing a sample, e.g., within the GI tract of a subject. Ingestible device 4000 includes a power system 4100 (e.g., one or more batteries), configured to power an electronics system 4200 (e.g., including a control

system, optionally in signal communication with an external base station), a valve system 4300, a sampling system 4400, and an analytic system 4500. Exemplary analytical systems include assay systems, such as, for example, optical systems containing one or more sources of radiation and/or one more detectors.

Some or all of the sponges of the above-described sampling systems may contain one or more preservatives (see discussion above). Typically, the assay sponge and/or the volume sponge 3230 and/or the transfer sponge contain one or more preservatives. Typically, the preservative(s) are selected based on the analyte of interest, e.g., an analyte (such as a protein biomarker) for a GI disorder.

Communication systems

An ingestible device may be equipped with a communication system adapted to transmit and/or receive data, including imaging and/or localization data. As an example, a communication system may employ radiofrequency transmission. Ingestible devices using radiofrequency communication are attractive because of their efficient transmission through the layers of the skin. This is especially true for low frequency transmission (UHF-433 ISM and lower, including the Medical Device Radio Communication Service band (MDRS) band 402-406MHz). In another embodiment, acoustics are used for communications, including the transmission of data. For example, an ingestible capsule may be able to transmit information by applying one or more base voltages to an electromechanical transducer or piezoelectric (e.g., PZT, PVDF, etc.) device to cause the piezoelectric device to ring at particular frequencies, resulting in an acoustic transmission. A multi-sensor array for receiving the acoustic transmission may include a plurality of acoustic transducers that receive the acoustic transmission from a movable device such as an ingestible capsule as described in US Patent Application No. 11/851214 filed September 6, 2007, incorporated by reference herein in its entirety.

As an example, a communication system may employ human body communication technology. Human body communication technology uses the human body as a conductive medium, which generally requires a large number of sensor electrodes on the skin. As an example, a communication system may integrate a data storage system.

For example, an ingestible device may be configured to communicate with an external base station. As an example, an ingestible device can have a communications unit that communicates with an external base station which itself has a communications unit. FIG. 62 illustrates exemplary implementation of such an ingestible device. As shown in FIG. 62, a

subject ingests an ingestible device as disclosed herein. Certain data about the subject (e.g., based on a collected sample) and/or the location of the ingestible device in the GI tract of the subject is collected or otherwise available and provided to a mobile device, which then forwards the data via the internet and a server/data store to a physician's office computer. The information collected by the ingestible device is communicated to a receiver, such as, for example, a watch or other object worn by the subject. The information is then communicated from the receiver to the mobile device which then forwards the data via the internet and a server/data store to a physician's office computer. The physician is then able to analyze some or all of the data about the subject to provide recommendations, such as, for example, delivery a therapeutic agent. While FIG. 62 shows a particular approach to collecting and transferring data about a subject, the disclosure is not limited. As an example, one or more of the receiver, mobile device, internet, and/or server/data store can be excluded from the data communication channel. For example, a mobile device can be used as the receiver of the device data, e.g., by using a dongle. In such embodiments, the item worn by the subject need not be part of the communication chain. As another example, one or more of the items in the data communication channel can be replaced with an alternative item. For example, rather than be provided to a physician's office computer, data may be provided to a service provider network, such as a hospital network, an HMO network, or the like. In some embodiments, subject data may be collected and/or stored in one location (e.g., a server/data store) while device data may be collected and/or stored in a different location (e.g., a different server/data store).

Environmental Sensors

In some embodiments the device may comprise environmental sensors to measure pH, temperature, transit times, or combinations thereof. Other examples of environmental sensors include, but are not limited to a capacitance sensor, an impedance sensor, a heart rate sensor, acoustic sensor such as a microphone or hydrophone, image sensor, and/or a movement sensor. In one embodiment, the ingestible device comprises a plurality of different environmental sensors for generating different kinds of environmental data.

In order to avoid the problem of capsule retention, a thorough past medical and surgical history should be undertaken. In addition, several other steps have been proposed, including performing investigations such as barium follow-through. In cases where it is suspected that there is a high risk of retention, the patient is given a patency capsule a few days before swallowing an ingestible device. Any dissolvable non-endoscopic capsule may be used to determine the

patency of the GI tract. The patency capsule is usually the same size as the ingestible device and can be made of cellophane. In some embodiments, the patency capsule contains a mixture of barium and lactose, which allows visualization by x-ray. The patency capsule may also include a radiotag or other label, which allows for it to be detected by radio-scanner externally. The patency capsule may comprise wax plugs, which allow for intestinal fluid to enter and dissolve the content, thereby dividing the capsule into small particles.

Accordingly, in some embodiments, the methods herein comprise (a) identifying a subject having a disease of the gastrointestinal tract and (b) evaluating the subject for suitability to treatment. In some embodiments, the methods herein comprise evaluating for suitability to treatment a subject identified as having a disease of the gastrointestinal tract. In some embodiments, evaluating the subject for suitability to treatment comprises determining the patency of the subject's GI tract.

In some embodiments, an ingestible device comprises a tissue anchoring mechanism for anchoring the ingestible device to a subject's tissue. For example, an ingestible device could be administered to a subject and once it reaches the desired location, the tissue attachment mechanism can be activated or deployed such that the ingestible device, or a portion thereof, is anchored to the desired location. In some embodiments, the tissue anchoring mechanism is reversible such that after initial anchoring, the tissue attachment device is retracted, dissolved, detached, inactivated or otherwise rendered incapable of anchoring the ingestible device to the subject's tissue. In some embodiments the attachment mechanism is placed endoscopically.

In some embodiments, a tissue anchoring mechanism comprises an osmotically-driven sucker. In some embodiments, the osmotically-driven sucker comprises a first valve on the near side of the osmotically-driven sucker (e.g., near the subject's tissue) and a second one-way valve that is opened by osmotic pressure on the far side of the osmotically-driven sucker, and an internal osmotic pump system comprising salt crystals and semi-permeable membranes positioned between the two valves. In such embodiments, osmotic pressure is used to adhere the ingestible device to the subject's tissue without generating a vacuum within the ingestible capsule. After the osmotic system is activated by opening the first valve, fluid is drawn in through the sucker and expelled through the second burst valve. Fluid continues to flow until all the salt contained in the sucker is dissolved or until tissue is drawn into the sucker. As liminal fluid is drawn through the osmotic pump system, solutes build up between the tissue and the first valve, reducing osmotic pressure. In some embodiments, the solute buildup stalls the pump before the tissue contacts the valve, preventing tissue damage. In some embodiments, a burst

valve is used on the far side of the osmotically-driven sucker rather than a one-way valve, such that luminal fluid eventually clears the saline chamber and the osmotic flow reverses, actively pushing the subject's tissue out of the sucker. In some embodiments, the ingestible device may be anchored to the interior surface of tissues forming the GI tract of a subject. In one embodiment, the ingestible device comprises a connector for anchoring the device to the interior surface of the GI tract. The connector may be operable to ingestible device to the interior surface of the GI tract using an adhesive, negative pressure and/or fastener.

In some embodiments a device comprises a tract stimulator and/or monitor IMD comprising a housing enclosing electrical stimulation and/or monitoring circuitry and a power source and an elongated flexible member extending from the housing to an active fixation mechanism adapted to be fixed into the GI tract wall is disclosed. After fixation is effected, the elongated flexible member bends into a preformed shape that presses the housing against the mucosa so that forces that would tend to dislodge the fixation mechanism are minimized. The IMD is fitted into an esophageal catheter lumen with the fixation mechanism aimed toward the catheter distal end opening whereby the bend in the flexible member is straightened. The catheter body is inserted through the esophagus into the GI tract cavity to direct the catheter distal end to the site of implantation and fix the fixation mechanism to the GI tract wall. The IMD is ejected from the lumen, and the flexible member assumes its bent configuration and lodges the hermetically sealed housing against the mucosa. A first stimulation/sense electrode is preferably an exposed conductive portion of the housing that is aligned with the bend of the flexible member so that it is pressed against the mucosa. A second stimulation/sense electrode is located at the fixation site.

In some embodiments a device includes a fixation mechanism to anchor the device to tissue within a body lumen, and a mechanism to permit selective de-anchoring of the device from the tissue anchoring site without the need for endoscopic or surgical intervention. An electromagnetic device may be provided to mechanically actuate the de-anchoring mechanism. Alternatively, a fuse link may be electrically blown to de-anchor the device. As a further alternative, a rapidly degradable bonding agent may be exposed to a degradation agent to de-anchor the device from a bonding surface within the body lumen.

In some embodiments a device is as disclosed in patent publication WO2015112575A1, incorporated by reference herein in its entirety. The patent publication is directed to a gastrointestinal sensor implantation system. In some embodiments an orally-administrable capsule comprises a tissue capture device or reservoir removably coupled to the orally-

administrable capsule, where the tissue capture device including a plurality of fasteners for anchoring the tissue capture device to gastrointestinal tissue within a body

In some embodiments, the ingestible device contains an electric energy emitting means, a radio signal transmitting means, a medicament storage means and a remote actuatable medicament releasing means. The capsule signals a remote receiver as it progresses through the alimentary tract in a previously mapped route and upon reaching a specified site is remotely triggered to release a dosage of medicament. Accordingly, in some embodiments, releasing the stem cell is triggered by a remote electromagnetic signal.

In some embodiments, the ingestible device includes a housing introducible into a body cavity and of a material insoluble in the body cavity fluids, but formed with an opening covered by a material which is soluble in body cavity fluids. A diaphragm divides the interior of the housing into a medication chamber including the opening, and a control chamber. An electrolytic cell in the control chamber generates a gas when electrical current is passed therethrough to deliver medication from the medication chamber through the opening into the body cavity at a rate controlled by the electrical current. Accordingly, in some embodiments, releasing the stem cell is triggered by generation in the composition of a gas in an amount sufficient to expel the stem cell.

In some embodiments, the ingestible device includes an oral drug delivery device having a housing with walls of water permeable material and having at least two chambers separated by a displaceable membrane. The first chamber receives drug and has an orifice through which the drug is expelled under pressure. The second chamber contains at least one of two spaced apart electrodes forming part of an electric circuit which is closed by the ingress of an aqueous ionic solution into the second chamber. When current flows through the circuit, gas is generated and acts on the displaceable membrane to compress the first chamber and expel the active ingredient through the orifice for progressive delivery to the gastrointestinal tract.

In some embodiments, the ingestible device includes an ingestible device for delivering a substance to a chosen location in the GI tract of a mammal includes a receiver of electromagnetic radiation for powering an openable part of the device to an opened position for dispensing of the substance. The receiver includes a coiled wire that couples the energy field, the wire having an air or ferrite core. In a further embodiment the invention includes an apparatus for generating the electromagnetic radiation, the apparatus including one or more pairs of field coils supported in a housing. The device optionally includes a latch defined by a heating resistor and a fusible restraint. The device may also include a flexible member that may serve one or both the functions

of activating a transmitter circuit to indicate dispensing of the substance; and restraining of a piston used for expelling the substance.

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In some embodiments, the ingestible device is a swallowable capsule. A sensing module is disposed in the capsule. A bioactive substance dispenser is disposed in the capsule. A memory and logic component is disposed in the capsule and in communication with the sensing module and the dispenser.

In some embodiments, localized administration is implemented via an electronic probe which is introduced into the intestinal tract of a living organism and which operates autonomously therein, adapted to deliver one or more therapy agents. In one embodiment, the method includes loading the probe with one or more therapy agents, and selectively releasing the agents from the probe at a desired location of the intestinal tract in order to provide increased efficacy over traditional oral ingestion or intravenous introduction of the agent(s).

In some embodiments, the ingestible device includes electronic control means for dispensing the drug substantially to the diseased tissue sites of the GI tract, according to a pre-determined drug release profile obtained prior to administration from the specific mammal. Accordingly, in some embodiments, releasing the stem cell is triggered by an electromagnetic signal generated within the device. The releasing may occur according to a pre-determined drug release profile.

In some embodiments, the ingestible device can include at least one guide tube, one or more tissue penetrating members positioned in the guide tube, a delivery member, an actuating mechanism and a release element. The release element degrades upon exposure to various conditions in the intestine so as to release and actuate the actuating mechanism. Embodiments of

the invention are particularly useful for the delivery of drugs which are poorly absorbed, tolerated and/or degraded within the GI tract.

In some embodiments, the ingestible device includes an electronic pill comprising at least one reservoir with a solid powder or granulate medicament or formulation, a discharge opening and an actuator responsive to control circuitry for displacing medicine from the reservoir to the discharge opening. The medicament or formulation comprises a dispersion of one or more active ingredients--e.g., solids in powder or granulate form--in an inert carrier matrix. Optionally, the active ingredients are dispersed using intestinal moisture absorbed into the pill via a semi-permeable wall section.

In some embodiments, the ingestible device includes a sensor comprising a plurality of electrodes having a miniature size and a lower power consumption and a coating exterior to the electrodes, wherein the coating interacts with a target condition thereby producing a change in an electrical property of the electrodes, wherein the change is transduced into an electrical signal by the electrodes. Accordingly, in some embodiments, releasing the stem cell is triggered by an electric signal by the electrodes resulting from the interaction of the coating with the one or more sites of disease. Further provided herein is a system for medication delivery comprising such sensor and a pill.

In some embodiments, the ingestible device includes an electronic pill comprising a plurality of reservoirs, each of the reservoirs comprising a discharge opening covered by a removable cover. The pill comprises at least one actuator responsive to control circuitry for removing the cover from the discharge opening. The actuator can for example be a spring loaded piston breaking a foil cover when dispensing the medicament. Alternatively, the cover can be a rotatable disk or cylinder with an opening which can be brought in line with the discharge opening of a reservoir under the action of the actuator.

In some embodiments, the ingestible device includes an electronically and remotely controlled pill or medicament delivery system. The pill includes a housing; a reservoir for storing a medicament; an electronically controlled release valve or hatch for dispensing one or more medicaments stored in the reservoir while traversing the gastrointestinal tract; control and timing circuitry for opening and closing the valve; and a battery. The control and timing circuitry opens and closes the valve throughout a dispensing time period in accordance with a preset dispensing timing pattern which is programmed within the control and timing circuitry. RF communication circuitry receives control signals for remotely overriding the preset dispensing timing pattern, reprogramming the control and timing circuitry or terminating the dispensing of the medicament

within the body. The pill includes an RFID tag for tracking, identification, inventory and other purposes.

In some embodiments, the ingestible device includes an electronic capsule which has a discrete drive element comprising: a housing, electronics for making the electronic capsule operable, a pumping mechanism for dosing and displacing a substance, a power source for powering the electronic capsule and enabling the electronics and the pumping mechanism to operate, and a locking mechanism; and a discrete payload element comprising: a housing, a reservoir for storing the substance, one or more openings in the housing for releasing the substance from the reservoir and a locking mechanism for engaging the drive element locking mechanism. Engagement of the drive element locking mechanism with the payload element locking mechanism secures the drive element to the payload element, thereby making the electronic capsule operable and specific.

In some embodiments, the ingestible device may be a mucoadhesive device configured for release of an active agent.

In some embodiments, the ingestible device includes an apparatus that includes an ingestible medical treatment device, which is configured to initially assume a contracted state having a volume of less than 4 cm^3. The device includes a gastric anchor, which initially assumes a contracted size, and which is configured to, upon coming in contact with a liquid, expand sufficiently to prevent passage of the anchor through a round opening having a diameter of between 1 cm and 3 cm. The device also includes a duodenal unit, which is configured to pass through the opening, and which is coupled to the gastric anchor such that the duodenal unit is held between 1 cm and 20 cm from the gastric anchor.

In some embodiments, the ingestible device includes a medical robotic system and method of operating such comprises taking intraoperative external image data of a patient anatomy, and using that image data to generate a modeling adjustment for a control system of the medical robotic system (e.g., updating anatomic model and/or refining instrument registration), and/or adjust a procedure control aspect (e.g., regulating substance or therapy delivery, improving targeting, and/or tracking performance).

In one embodiment the ingestible device may also include one or more environmental sensors. Environmental sensor may be used to generate environmental data for the environment external to device in the gastrointestinal (GI) tract of the subject. In some embodiments, environmental data is generated at or near the location within the GI tract of the subject where a drug is delivered. Examples of environmental sensor include, but are not limited to a capacitance

sensor, a temperature sensor, an impedance sensor, a pH sensor, a heart rate sensor, acoustic sensor, image sensor (e.g., a hydrophone), and/or a movement sensor (e.g., an accelerometer). In one embodiment, the ingestible device comprises a plurality of different environmental sensors for generating different kinds of environmental data.

In one embodiment, the image sensor is a video camera suitable for obtaining images *in vivo* of the tissues forming the GI tract of the subject. In one embodiment, the environmental data is used to help determine one or more characteristics of the GI tract, including the location of disease (e.g., presence or location of inflamed tissue and/or lesions associated with inflammatory bowel disease). In some embodiments, the ingestible device may comprise a camera for generating video imaging data of the GI tract which can be used to determine, among other things, the location of the device.

In another embodiment, the ingestible device described herein may be localized using a gamma scintigraphy technique or other radio-tracker technology as employed by Phaeton Research's Enterion™ capsule (See Teng, Renli, and Juan Maya. "Absolute bioavailability and regional absorption of ticagrelor in healthy volunteers." Journal of Drug Assessment 3.1 (2014): 43-50), or monitoring the magnetic field strength of permanent magnet in the ingestible device (see T. D. Than, et al., "A review of localization systems for robotic endoscopic capsules," IEEE Trans. Biomed. Eng., vol. 59, no. 9, pp. 2387–2399, Sep. 2012).

In one embodiment, drug delivery is triggered when it encounters the site of disease in the GI tract.

In one embodiment, the one or more environmental sensors measure pH, temperature, transit times, or combinations thereof.

In some embodiments, releasing the stem cell is dependent on the pH at or in the vicinity of the location. In some embodiments the pH in the jejunum is from 6.1 to 7.2, such as 6.6. In some embodiments the pH in the mid small bowel is from 7.0 to 7.8, such as 7.4. In some embodiments the pH in the ileum is from 7.0 to 8.0, such as 7.5. In some embodiments the pH in the right colon is from 5.7 to 7.0, such as 6.4. In some embodiments the pH in the mid colon is from 5.7 to 7.4, such as 6.6. In some embodiments the pH in the left colon is from 6.3 to 7.7, such as 7.0. In some embodiments, the gastric pH in fasting subjects is from about 1.1 to 2.1, such as from 1.4 to 2.1, such as from 1.1 to 1.6, such as from 1.4 to 1.6. In some embodiments, the gastric pH in fed subjects is from 3.9 to 7.0, such as from 3.9 to 6.7, such as from 3.9 to 6.4, such as from 3.9 to 5.8, such as from 3.9 to 5.5, such as from 3.9 to 5.4, such as from 4.3 to 7.0, such as from 4.3 to 6.7, such as from 4.3 to 6.4, such as from 4.3 to 5.8, such as from 4.3 to 5.5,

such as from 4.3 to 5.4. In some embodiments, the pH in the duodenum is from 5.8 to 6.8, such as from 6.0 to 6.8, such as from 6.1 to 6.8, such as from 6.2 to 6.8, such as from 5.8 to 6.7, such as from 6.0 to 6.7, such as from 6.1 to 6.7, such as from 6.2 to 6.7, such as from 5.8 to 6.6, such as from 6.0 to 6.6, such as from 6.1 to 6.6, such as from 6.2 to 6.6, such as from 5.8 to 6.5, such as from 6.0 to 6.5, such as from 6.1 to 6.5, such as from 6.2 to 6.5.

In some embodiments, releasing the stem cell is not dependent on the pH at or in the vicinity of the location. In some embodiments, releasing the stem cell is triggered by degradation of a release component located in the capsule. In some embodiments, the stem cell is not triggered by degradation of a release component located in the capsule. In some embodiments, wherein releasing the stem cell is not dependent on enzymatic activity at or in the vicinity of the location. In some embodiments, releasing the stem cell is not dependent on bacterial activity at or in the vicinity of the location.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

a reservoir located within the housing and containing the stem cell,

wherein a first end of the reservoir is attached to the first end of the housing;

a mechanism for releasing the stem cell from the reservoir; and;

an exit valve configured to allow the stem cell to be released out of the housing from the reservoir.

In some embodiments, the ingestible device further comprises:

an electronic component located within the housing; and

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas.

In some embodiments, the ingestible device further comprises:

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an exit valve located at the first end of the housing,

wherein the exit valve is configured to allow the dispensable substance to be released out of the first end of the housing from the reservoir; and

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing,

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing,

wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an injection device located at the first end of the housing,

wherein the jet injection device is configured to inject the dispensable substance out of the housing from the reservoir; and

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an optical sensing unit located on a side of the housing, wherein the optical sensing unit is configured to detect a reflectance from an environment external to the housing;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas in response to identifying a location of the ingestible device based on the reflectance;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

a membrane in contact with the gas generating cell and configured to move or deform into the reservoir by a pressure generated by the gas generating cell; and

a dispensing outlet placed at the first end of the housing, wherein the dispensing outlet is configured to deliver the dispensable substance out of the housing from the reservoir.

In one embodiment, drug delivery is triggered when it encounters the site of disease in the GI tract.

In one embodiment, the one or more environmental sensors measure pH, temperature, transit times, or combinations thereof.

In some embodiments, releasing the live biotherapeutic, such as a stem cell, is dependent on the pH at or in the vicinity of the location. In some embodiments the pH in the jejunum is from 6.1 to 7.2, such as 6.6. In some embodiments the pH in the mid small bowel is from 7.0 to 7.8, such as 7.4. In some embodiments the pH in the ileum is from 7.0 to 8.0, such as 7.5. In some embodiments the pH in the right colon is from 5.7 to 7.0, such as 6.4. In some embodiments the pH in the mid colon is from 5.7 to 7.4, such as 6.6. In some embodiments the pH in the left colon is from 6.3 to 7.7, such as 7.0. In some embodiments, the gastric pH in fasting subjects is from about 1.1 to 2.1, such as from 1.4 to 2.1, such as from 1.1 to 1.6, such as from 1.4 to 1.6. In some embodiments, the gastric pH in fed subjects is from 3.9 to 7.0, such as from 3.9 to 6.7, such as from 3.9 to 6.4, such as from 3.9 to 5.8, such as from 3.9 to 5.5, such as from 3.9 to 5.4, such as from 4.3 to 7.0, such as from 4.3 to 6.7, such as from 4.3 to 6.4, such as from 4.3 to 5.8, such as from 4.3 to 5.5, such as from 4.3 to 5.4. In some embodiments, the pH in the duodenum is from 5.8 to 6.8, such as from 6.0 to 6.8, such as from 6.1 to 6.8, such as from 6.2 to 6.8, such as from 5.8 to 6.7, such as from 6.0 to 6.7, such as from 6.1 to 6.7, such as from 6.2 to 6.7, such as from 5.8 to 6.6, such as from 6.0 to 6.6, such as from 6.1 to 6.6, such as from

6.2 to 6.6, such as from 5.8 to 6.5, such as from 6.0 to 6.5, such as from 6.1 to 6.5, such as from 6.2 to 6.5.

In some embodiments, releasing the live biotherapeutic, such as a stem cell, is not dependent on the pH at or in the vicinity of the location. In some embodiments, releasing the live biotherapeutic, such as a stem cell, is triggered by degradation of a release component located in the capsule. In some embodiments, the live biotherapeutic, such as a stem cell, is not triggered by degradation of a release component located in the capsule. In some embodiments, wherein releasing the live biotherapeutic, such as a stem cell, is not dependent on enzymatic activity at or in the vicinity of the location. In some embodiments, releasing the live biotherapeutic, such as a stem cell, is not dependent on bacterial activity at or in the vicinity of the location.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

a reservoir located within the housing and containing the live biotherapeutic, such as a stem cell, wherein a first end of the reservoir is attached to the first end of the housing;

a mechanism for releasing the live biotherapeutic, such as a stem cell, from the reservoir; and;

an exit valve configured to allow the live biotherapeutic, such as a stem cell, to be released out of the housing from the reservoir.

In some embodiments, the ingestible device further comprises:

an electronic component located within the housing; and

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas.

In some embodiments, the ingestible device further comprises:

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an exit valve located at the first end of the housing, wherein the exit valve is configured to allow the dispensable substance to be released out of the first end of the housing from the reservoir; and

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing,

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an injection device located at the first end of the housing, wherein the jet injection device is configured to inject the dispensable substance out of the housing from the reservoir; and

a safety device placed within or attached to the housing, wherein the safety device is configured to relieve an internal pressure within the housing.

In some embodiments, the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an optical sensing unit located on a side of the housing, wherein the optical sensing unit is configured to detect a reflectance from an environment external to the housing;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component, wherein the electronic component is configured to activate the gas generating cell to generate gas in response to identifying a location of the ingestible device based on the reflectance;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

a membrane in contact with the gas generating cell and configured to move or deform into the reservoir by a pressure generated by the gas generating cell; and

a dispensing outlet placed at the first end of the housing, wherein the dispensing outlet is configured to deliver the dispensable substance out of the housing from the reservoir.

In some embodiments, the pharmaceutical composition is an ingestible device as disclosed in US Patent Application Ser. No. 62/385,553, incorporated by reference herein in its entirety.

In some embodiments, the pharmaceutical composition is an ingestible device as disclosed in the following applications, each of which is incorporated by reference herein in its entirety:

USSNs 14/460,893; 15/514,413; 62/376,688; 62/385,344; 62/478,955; 62/434,188; 62/434,320; 62/431,297; 62/434,797; 62/480,187; 62/502,383; and 62/540,873.

In some embodiments, the pharmaceutical composition is an ingestible device comprising a localization mechanism as disclosed in international patent application PCT/US2015/052500, incorporated by reference herein in its entirety.

In some embodiments, the pharmaceutical composition is not a dart-like dosage form.

In some embodiments of any ingestible device disclosed herein comprising a live biotherapeutic such as a stem cell, the live biotherapeutic is present in a therapeutically effective amount.

In case of conflict between the present specification and any subject matter incorporated by reference herein, the present specification, including definitions, will control.

<u>Devices and Methods for Detection of Analytes in GI tract</u>

Detection of certain analytes in the GI tract may be useful in the identification of the nature and severity of the disease, in accurately locating the site(s) of disease, and in assessing patient response to a therapeutic agent. The appropriate therapeutic agent may accordigly be released at the correct locations(s), dosage, or timing for the disease. As discussed further herein, analytes may include biomarkers associated with a disease or associated with patient response and/or therapeutic agents previously administered to treat the disease.In some embodiments, the

disclosure provides an ingestible device for detecting an analyte in a sample, the ingestible device comprising a sampling chamber that is configured to hold a composition comprising: (1) a plurality of donor particles, each of the plurality of donor particles comprising a photosensitizer and having coupled thereto a first antigen-binding agent that binds to the analyte, wherein the photosensitizer, in its excited state, is capable of generating singlet oxygen; and (2) a plurality of acceptor particles, each of the plurality of acceptor particles comprising a chemiluminescent compound and having coupled thereto a second antigen-binding agent that binds to the analyte, wherein the chemiluminescent compound is capable of reacting with singlet oxygen to emit luminescence. In some embodiments, the first and the second analyte-binding agents are antigen-binding agents (e.g., antibodies). In some embodiments, the first and the second antigen-binding agents bind to the same epitope of the analyte (e.g., a protein). In some embodiments, the first and the second antigen-binding agents bind to separate epitopes of the analyte (e.g., a protein) that spatially overlap. In some embodiments, the first and the second antigen-binding agents bind to the separate epitopes of the analyte (e.g., a protein) that do not spatially overlap.

In some embodiments, this disclcosure provides an ingestible device for detecting an analyte in a sample, the ingestible device comprising a sampling chamber that is configured to hold an absorbable material (*e.g.*, an absorbable pad or sponge) having absorbed therein a composition comprising: (1) a plurality of donor particles, each of the plurality of donor particles comprising a photosensitizer and having coupled thereto a first antigen-binding agent that binds to the analyte, wherein the photosensitizer, in its excited state, is capable of generating singlet oxygen; and (2) a plurality of acceptor particles, each of the plurality of acceptor particles comprising a chemiluminescent compound and having coupled thereto a second antigen-binding agent that binds to the analyte, wherein the chemiluminescent compound is capable of reacting with singlet oxygen to emit luminescence. In some embodiments, the first and the second analyte-binding agents are antigen-binding agents (e.g., antibodies). In some embodiments, the first and the second antigen-binding agents bind to the same epitope of the analyte (e.g., a protein). In some embodiments, the first and the second antigen-binding agents bind to separate epitopes of the analyte (e.g., a protein) that spatially overlap. In some embodiments, the first and the second antigen-binding agents bind to the separate epitopes of the analyte (e.g., a protein) that do not spatially overlap.

In certain embodiments, the disclosure provides a kit comprising an ingestible device as described herein. In some embodiments, the kit further comprises instructions, *e.g.*, for detecting or quantifying an analyte in a sample.

In some embodiments, the disclosure provides methods for determining an analyte in a sample. In certain embodiments, this disclosure provides a method of detecting an analyte in a fluid sample of a subject, comprising: (1) providing an ingestible device; (2) transferring the fluid sample of the subject into the sampling chamber of the ingestible device *in vivo*; (3) irradiating the composition held in the sampling chamber of the ingestible device with light to excite the photosensitizer; and (4) measuring total luminescence or rate of change of luminescence emitted from the composition held in the sampling chamber of the ingestible device as a function of time, thereby determining the level of the analyte in the fluid sample. In some embodiments, the method further comprises comparing the level of the analyte in the fluid sample with the level of analyte in a reference sample (e.g., a reference sample obtained from a healthy subject). In some embodiments, the level of the analyte in the sample is used to diagnose and/or monitor a disease or disorder in the subject.

In some embodiments, the disclosure provides a method of detecting an analyte in a fluid sample of a subject, comprising: (1) providing an ingestible device, the device comprising a sampling chamber that is configured to hold an absorbable material (*e.g.*, an absorbable pad or sponge) having absorbed therein a composition, as described herein; (2) transferring the fluid sample of the subject into the sampling chamber of the ingestible device *in vivo*; (3) fully or partially saturating the absorbable material held in the sampling chamber of the ingestible device with the fluid sample; (4) irradiating the absorbable material held in the sampling chamber of the ingestible device with light to excite the photosensitizer; and (5) measuring total luminescence or rate of change of luminescence emitted from the composition held in the sampling chamber of the ingestible device as a function of time, thereby determining the level of the analyte in the fluid sample. In some embodiments, the method further comprises comparing the level of the analyte in the fluid sample with the level of analyte in a reference sample (e.g., a reference sample obtained from a healthy subject). In some embodiments, the level of the analyte in the sample is used to diagnose and/or monitor a disease or disorder in the subject.

In some embodiments, the disclosure provides a method of assessing or monitoring the need to treat a subject suffering from or at risk of overgrowth of bacterial cells in the gastrointestinal (GI) tract, comprising: (1) providing an ingestible device for detecting an analyte; (2) transferring a fluid sample from the GI tract of the subject into the sampling chamber of the ingestible device *in vivo*; (3) irradiating the composition held in the sampling chamber of the ingestible device with light to excite the photosensitizer; (4) measuring total luminescence or rate of change of luminescence emitted from the composition held in the sampling chamber of the

ingestible device as a function of time; (5) correlating the total luminescence or the rate of change of luminescence as a function of time measured in step (4) to the amount of the analyte in the fluid sample; and (6) correlating the amount of the analyte in the fluid sample to the number of viable bacterial cells in the fluid sample.. In some embodiments, a number of viable bacterial cells determined in step (6) greater than a control number of viable bacterial cells, indicates a need for treatment (e.g., with an antibiotic agent described herein). In some embodiments, the control number of viable bacterial cells is 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or more. For example, in some embodiments, a number of viable bacterial cells determined in step (6) greater that about 10^3 CFU/mL indicates a need for treatment. In some embodiments, a number of viable bacterial cells determined in step (6) greater that about 10^4 CFU/mL indicates a need for treatment. In some embodiments, a number of the viable bacterial cells determined in step (6) greater than about 10^5 CFU/mL indicates a need for treatment, *e.g.*, with an antibiotic agent as described herein. In some embodiments, a number of viable bacterial cells determined in step (6) greater that about 10^6 or more CFU/mL indicates a need for treatment.

In some embodiments, the total luminescence or the rate of change of luminescence as a function of time of the sponge is measured over multiple time points for an extended period of time in step (4). For instance, in some embodiments, the total luminescence or rate of change of luminescence as a function of time of the sample is measured continuously for a period of 0-1800 minutes, 0-1600 minutes, 0-1500 minutes, 0-1440 minutes, 0-1320 minutes, 0-1000 minutes, 0-900 minutes, 0-800 minutes, 0-700 minutes, 0-600 minutes, 0-500 minutes, 0-400 minutes, 0-350 minutes, 0-330 minutes, 0-300 minutes, 0-270 minutes, or 0-220 minutes. In some embodiments, the total luminescence or the rate of change of luminescence as a function of time of said sample is measured continuously for a period of 0-330 minutes. In some embodiments, the method is performed *in vivo*. In some embodiments, the method includes communicating the results of the onboard assay(s) to an *ex vivo* receiver. In some embodiments, the total luminescence or the rate of change of luminescence as a function of time of the sponge is measured over multiple time points for an extended period of time in step (5). For instance, in some embodiments, the total luminescence or rate of change of luminescence as a function of time of the sample is measured continuously for a period of 0-1800 minutes, 0-1600 minutes, 0-1500 minutes, 0-1440 minutes, 0-1320 minutes, 0-1000 minutes, 0-900 minutes, 0-800 minutes, 0-700 minutes, 0-600 minutes, 0-500 minutes, 0-400 minutes, 0-350 minutes, 0-330 minutes, 0-300 minutes, 0-270 minutes, or 0-220 minutes. In some embodiments, the total luminescence or the rate of change of luminescence as a function of time of said sample is measured continuously for a period of 0-330

minutes. In some embodiments, the method is performed *in vivo*. In some embodiments, the method includes communicating the results of the onboard assay(s) to an *ex vivo* receiver.

In some embodiments, the disclosure provides a method of assessing or monitoring the need to treat a subject suffering from or at risk of overgrowth of bacterial cells in the gastrointestinal tract, comprising: (1) providing an ingestible device for detecting an analyte, the device comprising a sampling chamber that is configured to hold an absorbable material (*e.g.*, an absorbable pad or sponge) having absorbed therein a composition, as described herein; (2) transferring a fluid sample from the GI tract of the subject into the sampling chamber of the ingestible device *in vivo*; (3) fully or partially saturating the absorbable material held in the sampling chamber of the ingestible device with the fluid sample; (4) irradiating the absorbable material held in the sampling chamber of the ingestible device with light to excite the photosensitizer; (5) measuring total luminescence or rate of change of luminescence emitted from the composition held in the sampling chamber of the ingestible device as a function of time; (6) correlating the total luminescence or the rate of change of luminescence as a function of time measured in step (5) to the amount of the analyte in the fluid sample; and (7) correlating the amount of the analyte in the fluid sample to the number of viable bacterial cells in the fluid sample. In some embodiments, a number of viable bacterial cells determined in step (7) greater than a control number of viable bacterial cells indicates a need for treatment (e.g., with an antibiotic agent described herein). In some embodiments, the control number of viable bacterial cells is 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or more. For example, in some embodiments, a number of viable bacterial cells determined in step (7) greater that about 10^3 CFU/mL indicates a need for treatment. In some embodiments, a number of viable bacterial cells determined in step (7) greater that about 10^4 CFU/mL indicates a need for treatment. In some embodiments, a number of the viable bacterial cells determined in step (7) greater than about 10^5 CFU/mL indicates a need for treatment, *e.g.*, with an antibiotic agent as described herein. In some embodiments, a number of viable bacterial cells determined in step (7) greater that about 10^6 or more CFU/mL indicates a need for treatment.

In some embodiments, the disclosure, provides a method of measuring the presence, absence or amount of one or more analytes from one or more samples in the gastrointestinal tract. In some embodiments the one or more analytes are measured multiple times, for example, at different time points or at different locations. In one embodiment, a single device measures one or more analytes or more time points or locations; thereby creating a "molecular map" of a physiological region. Measurements can be taken at any location in the gastrointestinal tract. For

example, in one aspect, analytes from samples from one or more of the duodenum, jejunum, ileum, ascending colon, transverse colon or descending colon can be measured to create a molecular map of the small and large intestine. In one aspect, the sample is from the duodenum. In one aspect, the sample is from the jejunum. In one aspect, the sample is from the ileum. In one aspect, the sample is from the ascending colon. In one aspect, the sample is from the transverse colon. In one aspect, the sample is from the descending colon.

In another aspect, a series of measurements can be taken over a shorter distance of the gastrointestinal tract (e.g., the ileum) to create a higher resolution molecular map. In some embodiments, previous endoscopic imaging may identify a diseased area for molecular mapping. For example, a gastroenterologist may use imaging (e.g., an endoscope equipped with a camera) to identify the presence of Crohn's Disease in the ileum and cecum of a patient, and the methods and techniques herein may be used to measure inflammation-associated analytes in this diseased area of the patient. In a related embodiment, the inflammation-associated analytes, or any analyte, may be measured every one or more days to monitor disease flare-ups, or response to therapeutics.

Analytes

The compositions and methods described herein can be used to detect, analyze, and/or quantitate a variety of analytes in a human subject. "Analyte" as used herein refers to a compound or composition to be detected in a sample. Exemplary analytes suitable for use herein include those described in U.S. Patent 6,251,581, which is incorporated by reference herein in its entirety. Broadly speaking, an analyte can be any substance (e.g., a substance with one or more antigens) capable of being detected. An exemplary and non-limiting list of analytes includes ligands, proteins, blood clotting factors, hormones, cytokines, polysaccharides, mucopolysaccharides, microorganisms (e.g., bacteria), microbial antigens, and therapeutic agents (including fragments and metabolites thereof).

For instance, the analyte may be a ligand, which is monovalent (monoepitopic) or polyvalent (polyepitopic), usually antigenic or haptenic, and is a single compound or plurality of compounds which share at least one common epitopic or determinant site. The analyte can be a part of a cell such as bacteria or a cell bearing a blood group antigen such as A, B, D, etc., a human leukocyte antigen (HLA), or other cell surface antigen, or a microorganism, *e.g.*, bacterium (e.g. a pathogenic bacterium), a fungus, protozoan, or a virus (*e.g.*, a protein, a nucleic acid, a lipid, or a hormone). In some embodiments, the analyte can be a part of an exosome (*e.g.*, a bacterial exosome). In some embodiments, the analyte is derived from a subject (e.g., a human

subject). In some embodiments, the analyte is derived from a microorganism present in the subject. In some embodiments, the analyte is a nucleic acid (e.g., a DNA molecule or a RNA molecule), a protein (e.g., a soluble protein, a cell surface protein), or a fragment thereof, that can be detected using any of the devices and methods provided herein.

The polyvalent ligand analytes will normally be poly(amino acids), *i.e.*, a polypeptide (*i.e.*, protein) or a peptide, polysaccharides, nucleic acids (e.g., DNA or RNA), and combinations thereof. Such combinations include components of bacteria, viruses, chromosomes, genes, mitochondria, nuclei, cell membranes, and the like.

In some embodiments, the polyepitopic ligand analytes have a molecular weight of at least about 5,000 Da, more usually at least about 10,000 Da. In the poly(amino acid) category, the poly(amino acids) of interest may generally have a molecular weight from about 5,000 Da to about 5,000,000 Da, more usually from about 20,000 Da to 1,000,000 Da; among the hormones of interest, the molecular weights will usually range from about 5,000 Da to 60,000 Da.

In some embodiments, the monoepitopic ligand analytes generally have a molecular weight of from about 100 to 2,000 Da, more usually from 125 to 1,000 Da.

A wide variety of proteins may be considered as to the family of proteins having similar structural features, proteins having particular biological functions, proteins related to specific microorganisms, particularly disease causing microorganisms, etc. Such proteins include, for example, immunoglobulins, cytokines, enzymes, hormones, cancer antigens, nutritional markers, tissue specific antigens, etc.

In some embodiments, the analyte is a protein. In some embodiments, the analyte is a protein, e.g., an enzyme (e.g., a hemolysin, a protease, a phospholipase), a soluble protein, an exotoxin. In some embodiments, the analyte is a fragment of a protein, a peptide, or an antigen. In some embodiments, the analyte is a peptide of at least 5 amino acids (e.g., at least 6, at least 7, at least 8, at least 9, at least 10, at least 25, at least, 50, or at least 100 amino acids). Exemplary lengths include 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75, or 100 amino acids. Exemplary classes of protein analytes include, but are not limited to: protamines, histones, albumins, globulins, scleroproteins, phosphoproteins, mucoproteins, chromoproteins, lipoproteins, nucleoproteins, glycoproteins, T-cell receptors, proteoglycans, cell surface receptors, membrane-anchored proteins, transmembrane proteins, secreted proteins, HLA, and unclassified proteins.

In some embodiments, the analyte is an affimer (see, e.g., Tiede *et al.* (2017) *eLife* 6: e24903, which is expressly incorporated herein by reference).

Exemplary analytes include: Prealbumin, Albumin, α_1-Lipoprotein, α_1-Antitrypsin, α_1-Glycoprotein, Transcortin, 4.6S-Postalbumin, α_1-glycoprotein, α_{1X}-Glycoprotein, Thyroxin-binding globulin, Inter-α-trypsin-inhibitor, Gc-globulin (Gc 1-1, Gc 2-1, Gc 2-2), Haptoglobin (Hp 1-1, Hp 2-1, Hp 2-2), Ceruloplasmin, Cholinesterase, α_2-Lipoprotein(s), Myoglobin, C-Reactive Protein, α_2-Macroglobulin, α_2-HS-glycoprotein, Zn-α_2-glycoprotein, α_2-Neuramino-glycoprotein, Erythropoietin, β-lipoprotein, Transferrin, Hemopexin, Fibrinogen, Plasminogen, β_2-glycoprotein I, β_2-glycoprotein II, Immunoglobulin G (IgG) or γG-globulin, Immunoglobulin A (IgA) or γA-globulin, Immunoglobulin M (IgM) or γM-globulin, Immunoglobulin D (IgD) or γD-Globulin (γD), Immunoglobulin E (IgE) or γE-Globulin (γE), Free κ and λ light chains, and Complement factors: C′1, (C′1q, C′1r, C′1s, C′2, C′3 (β_1A, α_2D), C′4, C′5, C′6, C′7, C′8, C′9.

Additional examples of analytes include tumor necrosis factor-α (TNFα), interleukin-12 (IL-12), IL-23, IL-6, α2β1 integrin, α1β1 integrin, α4β7 integrin, integrin α4β1 (VLA-4), E-selectin, ICAM-1, α5β1 integrin, α4β1 integrin, VLA-4, α2β1 integrin, α5β3 integrin, α5β5 integrin, αIIbβ3 integrin, MAdCAM-1, SMAD7, JAK1, JAK2, JAK3, TYK-2, CHST15, IL-1, IL-1α, IL-1β, IL-18, IL-36α, IL-36β, IL-36γ, IL-38, IL-33, IL-13, CD40L, CD40, CD3γ, CD3δ, CD3ε, CD3ζ, TCR, TCRα, TCRβ, TCRδ, TCRγ, CD14, CD20, CD25, IL-2, IL-2 β chain, IL-2 γ chain, CD28, CD80, CD86, CD49, MMP1, CD89, IgA, CXCL10, CCL11, an ELR chemokine, CCR2, CCR9, CXCR3, CCR3, CCR5, CCL2, CCL8, CCL16, CCL25, CXCR1m CXCR2m CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and CXCL8, and a nucleic acid (e.g., mRNA) encoding any of the same.

In some embodiments, the analyte is a blood clotting factor. Exemplary blood clotting factors include, but are not limited to:

International designation	Name
I	Fibrinogen
II	Prothrombin
IIa	Thrombin
III	Tissue thromboplastin
V and VI	Proaccelerin, accelerator globulin
VII	Proconvertin
VIII	Antihemophilic globulin (AHG)
IX	Christmas factor plasma thromboplastin component (PTC)
X	Stuart-Prower factor, autoprothrombin III
XI	Plasma thromboplastin antecedent (PTA)
XII	Hagemann factor
XIII	Fibrin-stabilizing factor

In some embodiments, the analyte is a hormone. Exemplary hormones include, but are not limited to: Peptide and Protein Hormones, Parathyroid hormone, (parathromone), Thyrocalcitonin, Insulin, Glucagon, Relaxin, Erythropoietin, Melanotropin (melancyte-stimulating hormone; intermedin), Somatotropin (growth hormone), Corticotropin (adrenocorticotropic hormone), Thyrotropin, Follicle-stimulating hormone, Luteinizing hormone (interstitial cell-stimulating hormone), Luteomammotropic hormone (luteotropin, prolactin), Gonadotropin (chorionic gonadotropin), Secretin, Gastrin, Angiotensin I and II, Bradykinin, and Human placental lactogen, thyroxine, cortisol, triiodothyronine, testosterone, estradiol, estrone, progestrone, luteinizing hormone-releasing hormone (LHRH), and immunosuppressants such as cyclosporin, FK506, mycophenolic acid, and so forth.

In some embodiments, the analyte is a peptide hormone (e.g., a peptide hormone from the neurohypophysis). Exemplary peptide hormones from the neurohypophysis include, but are not limited to: Oxytocin, Vasopressin, and releasing factors (RF) (e.g., corticotropin releasing factor (CRF), luteinizing hormone releasing factor (LRF), thyrotropin releasing factor (TRF), Somatotropin-RF, growth hormone releasing factor (GRF), follicle stimulating hormone-releasing factor (FSH-RF), prolactin inhibiting factor (PIF), and melanocyte stimulating hormone inhibiting factor (MIF)).

In some embodiments, the analyte is a cytokine or a chemokine. Exemplary cytokines include, but are not limited to: interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), epidermal growth factor (EGF), tumor necrosis factor (TNF, e.g., TNF-α or TNF-β), and nerve growth factor (NGF).

In some embodiments, the analyte is a cancer antigen. Exemplary cancer antigens include, but are not limited to: prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), α-fetoprotein, Acid phosphatase, CA19.9, and CA125.

In some embodiments, the analyte is a tissue-specific antigen. Exemplary tissue specific antigens include, but are not limited to: alkaline phosphatase, myoglobin, CPK-MB, calcitonin, and myelin basic protein.

In some embodiments, the analyte is a mucopolysaccharide or a polysaccharide.

In some embodiments, the analyte is a microorganism, or a molecule derived from or produced by a microorganism (e.g., a bacteria, a virus, prion, or a protozoan). For example, in some embodiments, the analyte is a molecule (e.g., an protein or a nucleic acid) that is specific for a particular microbial genus, species, or strain (e.g., a specific bacterial genus, species, or strain). In some embodiments, the microorganism is pathogenic (*i.e.*, causes disease). In some embodiments, the microorganism is non-pathogenic (*e.g.*, a commensal microorganism). Exemplary microorganisms include, but are not limited to:

Corynebacteria	
Corynebacterium diphtheria	
Pneumococci	
Diplococcus pneumoniae	
Streptococci	
Streptococcus pyrogenes	
Streptococcus salivarus	
Staphylococci	
Staphylococcus aureus	
Staphylococcus albus	
Neisseria	
Neisseria meningitidis	
Neisseria gonorrhea	
Enterobacteriaciae	
Escherichia coli	
Aerobacter aerogenes	**The coliform**

Klebsiella pneumoniae	**bacteria**
Salmonella typhosa	
Salmonella choleraesuis	**The Salmonellae**
Salmonella typhimurium	
Shigella dysenteria	
Shigella schmitzii	
Shigella arabinotarda	
	The Shigellae
Shigella flexneri	
Shigella boydii	
Shigella sonnei	
Other enteric bacilli	
Proteus vulgaris	
Proteus mirabilis	**Proteus species**
Proteus morgani	
Pseudomonas aeruginosa	
Alcaligenes faecalis	
Vibrio cholerae	
Hemophilus-Bordetella group	***Rhizopus oryzae***
Hemophilus influenza, ***H. ducryi***	***Rhizopus arrhizua***
	Phycomycetes
Hemophilus hemophilus	***Rhizopus nigricans***
Hemophilus aegypticus	***Sporotrichum schenkii***
Hemophilus parainfluenza	***Flonsecaea pedrosoi***
Bordetella pertussis	***Fonsecacea compact***
Pasteurellae	***Fonsecacea dermatidis***
Pasteurella pestis	***Cladosporium carrionii***
Pasteurella tulareusis	***Phialophora verrucosa***
Brucellae	***Aspergillus nidulans***

Brucella melltensis

Brucella abortus

Brucella suis

Aerobic Spore-forming Bacilli

Bacillus anthracis

Bacillus subtilis

Bacillus megaterium

Bacillus cereus

Anaerobic Spore-forming Bacilli

Clostridium botulinum

Clostridium tetani

Clostridium perfringens

Clostridium novyi

Clostridium septicum

Clostridium histoyticum

Clostridium tertium

Clostridium bifermentans

Clostridium sporogenes

Mycobacteria

Mycobacterium tuberculosis **hominis**

Mycobacterium bovis

Mycobacterium avium

Mycobacterium leprae

Mycobacterium paratuberculosis

Actinomycetes (fungus-ike bacteria)

Actinomyces Isaeli

Actinomyces bovis

Actinomyces naeslundii

Nocardia asteroides

Madurella mycetomi

Madurella grisea

Allescheria boydii

Phialophora jeanselmei

Microsporum gypseum

Trichophyton mentagrophytes

Keratinomyces ajelloi

Microsporum canis

Trichophyton rubrum

Microsporum adouini

Viruses

Adenoviruses

Herpes Viruses

Herpes simplex

Varicella (Chicken pox)

Herpes Zoster (Shingles)

Virus B

Cytomegalovirus

Pox Viruses

Variola (smallpox)

Vaccinia

Poxvirus bovis

Paravaccinia

Molluscum contagiosum

Picornaviruses

Poliovirus

Coxsackievirus

Echoviruses

Rhinoviruses

Nocardia brasiliensis

The Spirochetes

Treponema pallidum

Treponema pertenue

Spirillum minus

Streptobacillus monoiliformis

Treponema carateum

Borrelia recurrentis

Leptospira icterohemorrhagiae

Leptospira canicola

Trypanasomes

Mycoplasmas

Mycoplasma pneumoniae

Other pathogens

Listeria monocytogenes

Erysipeothrix rhusiopathiae

Streptobacillus moniliformis

Donvania granulomatis

Entamoeba histolytica

Plasmodium falciparum

Plasmodium japonicum

Bartonella bacilliformis

Rickettsia (bacteria-like parasites)

Rickettsia prowazekii

Rickettsia mooseri

Rickettsia rickettsii

Rickettsia conori

Myxoviruses

Influenza(A, B, and C)

Parainfluenza (1-4)

Mumps Virus

Newcastle Disease Virus

Measles Virus

Rinderpest Virus

Canine Distemper Virus

Respiratory Syncytial Virus

Rubella Virus

Arboviruses

Eastern Equine Encephalitis Virus

Western Equine Encephalitis Virus

Sindbis Virus

Chikugunya Virus

Semliki Forest Virus

Mayora Virus

St. Louis Encephalitis

California Encephalitis Virus

Colorado Tick Fever Virus

Yellow Fever Virus

Dengue Virus

Reoviruses

Reovirus Types 1-3

Retroviruses

Rickettsia australis	**Human Immunodeficiency**
Rickettsia sibiricus	**Viruses I and II (HTLV)**
Rickettsia akari	**Human T-cell Lymphotrophic**
Rickettsia tsutsugamushi	**Virus I & II (HIV)**
Rickettsia burnetti	**Hepatitis**
Rickettsia quintana	**Hepatitis A Virus**
Chlamydia (unclassifiable parasites bacterial/viral)	**Hepatitis B Virus**
	Hepatitis C Virus
Chlamydia agents (naming uncertain)	**Tumor Viruses**
Chlamydia trachomatis	
Fungi	**Rauscher Leukemia Virus**
Cryptococcus neoformans	**Gross Virus**
Blastomyces dermatidis	**Maloney Leukemia Virus**
Histoplasma capsulatum	
Coccidioides immitis	**Human Papilloma Virus**
Paracoccidioides brasliensis	
Candida albicans	
Aspergillus fumigatus	
***Mucor corymbifer* (*Absidia corymbifera*)**	

In some embodiments, the analyte is a bacterium. Exemplary bacteria include, but are not limited to: *Escherichia coli* (or *E. coli*), *Bacillus anthracis, Bacillus cereus, Clostridium botulinum, Clostridium difficile, Yersinia pestis, Yersinia enterocolitica, Francisella tularensis, Brucella* species, *Clostridium perfringens, Burkholderia mallei, Burkholderia pseudomallei, Staphylococcus* species, *Mycobacterium* species, Group A *Streptococcus*, Group B *Streptococcus, Streptococcus pneumoniae, Helicobacter pylori, Salmonella enteritidis, Mycoplasma hominis, Mycoplasma orale, Mycoplasma salivarium, Mycoplasma fermentans, Mycoplasma pneumoniae, Mycobacterium bovis, Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium leprae, Rickettsia rickettsii, Rickettsia akari, Rickettsia prowazekii,*

Rickettsia canada, Bacillus subtilis, Bacillus subtilis niger, Bacillus thuringiensis, Coxiella burnetti, *Faecalibacterium prausnitzii* (also known as *Bacteroides praussnitzii*), *Roseburia hominis*, *Eubacterium rectale*, *Dialister invisus*, *Ruminococcus albus*, *Ruminococcus callidus*, and *Ruminococcus bromii*. Additional exemplary bacteria include bacteria of the phyla Firmicutes (e.g., *Clostridium* clusters XIVa and IV), bacteria of the phyla Bacteroidetes (e.g., *Bacteroides fragilis* or *Bacteroides vulgatus*), and bacteria of the phyla Actinobacteria (e.g., *Coriobacteriaceae* spp. or *Bifidobacterium adolescentis*). Bacteria of the *Clostridium* cluster XIVa includes species belonging to, for example, the *Clostridium*, *Ruminococcus*, *Lachnospira*, *Roseburia*, *Eubacterium*, *Coprococcus*, *Dorea*, and *Butyrivibrio* genera. Bacteria of the *Clostridium* cluster IV includes species belonging to, for example, the *Clostridium*, *Ruminococcus*, *Eubacterium* and *Anaerofilum* genera. In some embodiments, the analyte is *Candida*, *e.g., Candida albicans*. In some embodiments, the analyte is a byproduct from a bacterium or other microorganism, e.g., helminth ova, enterotoxin (*Clostridium difficile* toxin A; TcdA) or cytotoxin (*Clostridium difficile* toxin B; TcdB).

In some embodiments, the bacterium is a pathogenic bacterium. Non-limiting examples of pathogenic bacteria belong to the genera *Bacillus, Bordetella, Borrelia, Brucella, Campylobacter, Chlamydia, Chlamydophila, Clostridium, Corynebacterium, Enterobacter, Enterococcus, Escherichia, Francisella, Haemophilus, Helicobacter, Legionella, Leptospira, Listeria, Mycobacterium, Mycoplasma, Neisseria, Pseudomonas, Rickettsia, Salmonella, Shigella, Staphylococcus, Streptococcus, Treponema, Vibrio*, and *Yersinia*. Non-limiting examples of specific pathogenic bacterial species include a strain of *Bacillus anthracis*, a strain of a strain of *Bordetella pertussis*, a strain of a strain of *Borrelia burgdorferi*, a strain of a strain of *Brucella abortus*, a strain of a strain of *Brucella canis*, a strain of a strain of *Brucella melitensis*, a strain of a strain of *Brucella suis*, a strain of a strain of *Campylobacter jejuni*, a strain of *Chlamydia pneumoniae*, a strain of *Chlamydia trachomatis*, a strain of *Chlamydophila psittaci*, a strain of *Clostridium botulinum*, a strain of *Clostridium difficile*, a strain of *Clostridium perfringens*, a strain of *Clostridium tetani*, a strain of *Corynebacterium diphtheria*, a strain of *Enterobacter sakazakii*, a strain of *Enterococcus faecalis*, a strain of *Enterococcus faecium*, a strain of *Escherichia coli* (e.g., *E. coli* O157 H7), a strain of *Francisella tularensis*, a strain of *Haemophilus influenza*, a strain of *Helicobacter pylori*, a strain of *Legionella pneumophila*, a strain of *Leptospira interrogans*, a strain of *Listeria monocytogenes*, a strain of *Mycobacterium leprae*, a strain of *Mycobacterium tuberculosis*, a strain of *Mycobacterium ulcerans*, a strain of *Mycoplasma pneumonia*, a strain of *Neisseria gonorrhoeae*, a strain of

Neisseria meningitides, a strain of *Pseudomonas aeruginosa*, a strain of *Rickettsia rickettsia*, a strain of *Salmonella typhi* and *Salmonella typhimurium*, a strain of *Shigella sonnei*, a strain of *Staphylococcus aureus*, a strain of *Staphylococcus epidermidis*, a strain of *Staphylococcus saprophyticus*, a strain of *Streptococcus agalactiae*, a strain of *Streptococcus pneumonia*, a strain of *Streptococcus pyogenes*, a strain of *Treponema pallidum*, a strain of *Vibrio cholera*, a strain of *Yersinia enterocolitica*, and, a strain of *Yersinia pestis.*

In some embodiments, the bacterium is a commensal bacterium (e.g., a probiotic). In some embodiments, the bacterium has been previously administered to a subject, e.g., as a live biotherapeutic agent. Exemplary commensal bacteria include, but are not limited to, *Faecalibacterium prausnitzii* (also referred to as *Bacteroides praussnitzii*), *Roseburia hominis*, *Eubacterium rectale*, *Dialister invisus*, *Ruminococcus albus*, *Ruminococcus gnavus*, *Ruminococcus torques, Ruminococcus callidus*, and *Ruminococcus bromii*.

In some embodiments, the analyte is a virus. In some embodiments, the virus is a pathogenic virus. Non-limiting examples of pathogenic viruses belong to the families Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, and Togaviridae.

In some embodiments, the analyte is a fungus. In some embodiments, the fungi is a pathogenic fungus. Non-limiting examples of pathogenic fungi belong to the genera *Asperfillus, Canidia, Cryptococcus, Histoplasma, Pneumocystis*, and *Stachybotrys*. Non-limiting examples of specific pathogenic fungi species include a strain of *Aspergillus clavatus, Aspergillus fumigatus, Aspergillus flavus, Canidia albicans, Cryptococcus albidus, Cryptococcus gattii, Cryptococcus laurentii, Cryptococcus neoformans, Histoplasma capsulatum, Pneumocystis jirovecii, Pneumocystis carinii*, and *Stachybotrys chartarum.*

In some embodiments, the analyte is a protozoan. In some embodiments, the analyte is a pathogenic protozoan. Non-limiting examples of pathogenic protozoa belong to the genera *Acanthamoeba, Balamuthia, Cryptosporidium, Dientamoeba, Endolimax, Entamoeba, Giardia, Iodamoeba, Leishmania, Naegleria, Plasmodium, Sappinia, Toxoplasma, Trichomonas*, and *Trypanosoma*. Non-limiting examples of specific pathogenic protozoa species include a strain of *Acanthamoeba* spp., *Balamuthia mandrillaris, Cryptosporidium canis, Cryptosporidium felis, Cryptosporidium hominis, Cryptosporidium meleagridis, Cryptosporidium muris, Cryptosporidium parvum, Dientamoeba fragilis, Endolimax nana, Entamoeba dispar, Entamoeba hartmanni, Entamoeba histolytica, Entamoeba coli, Entamoeba moshkovskii, Giardia*

lamblia, Iodamoeba butschlii, Leishmania aethiopica, Leishmania braziliensis, Leishmania chagasi, Leishmania donovani, Leishmania infantum, Leishmania major, Leishmania mexicana, Leishmania tropica, Naegleria fowleri, Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, Sappinia diploidea, Toxoplasma gondii, Trichomonas vaginalis, Trypanosoma brucei, and *Trypanosoma cruzi.*

In some embodiments, the analyte is secreted by or expressed on the cell surface of a microorganism (e.g., a bacterium, a colonic bacterium, a viable bacterium, a dead bacterium, a parasite (e.g., *Giardia lamblia, Cryptosporidium, Cystoisosporiasis belli,* and *Balantidium coli*), a virus (e.g., a herpes virus, a cytomegalovirus, a herpes simplex virus, an Epstein-Barr virus, a human papilloma virus, a rotavirus, a human herpesvirus-8; Goodgame (1999) Curr. Gastroenterol. Rep. 1(4): 292-300). In some embodiments, the analyte is secreted by or expressed on the cell surface of a Gram-negative bacterium (e.g., *E. coli, Helicobacter pylori*). In some embodiments, the analyte is secreted by or expressed on the cell surface (e.g., a bacterial surface epitope) of a Gram-positive bacterium (e.g., *Staphylococcus aureus, Clostridium botulinum, Clostridium difficile*).

In some embodiments, the analyte is a molecule expressed on the surface of a bacterial cell (e.g., a bacterial cell surface protein). In some embodiments, the analyte is a bacterial toxin (e.g., TcdA and/or TcdB from *Clostridium difficile*). In some embodiments, the analyte is CFA/I fimbriae, flagella, lipopolysaccharide (LPS), lipoteichoic acid, or a peptidoglycan. Non-limiting examples of bacterium that may express an analyte that can be detected using any of the devices and methods described herein include: *Bacillus anthracis, Bacillus cereus, Clostridium botulinum, Clostridium difficile, Escherichia coli, Yersinia pestis, Yersinia enterocolitica, Francisella tularensis, Brucella* species, *Clostridium perfringens, Burkholderia mallei, Burkholderia pseudomallei, Helicobacter pylori, Staphylococcus* species, *Mycobacterium* species, Group A *Streptococcus,* Group B *Streptococcus, Streptococcus pneumoniae, Francisella tularensis, Salmonella enteritidis, Mycoplasma hominis, Mycoplasma orale, Mycoplasma salivarium, Mycoplasma fermentans, Mycoplasma pneumoniae, Mycobacterium bovis, Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium leprae, Rickettsia rickettsii, Rickettsia akari, Rickettsia prowazekii, Rickettsia canada, Bacillus subtilis, Bacillus subtilis niger, Bacillus thuringiensis, Coxiella bumetti, Candida albicans, Bacteroides fragilis, Leptospira interrogans, Listeria monocytogenes, Pasteurella multocida, Salmonella typhi, Salmonella typhimurium, Shigella dysenteriae, Shigella flexneria, Shigella sonnei, Vibrio cholera,* and *Vibrio parahaemolyticus*.

In some embodiments, the analyte is a byproduct from a bacterium or another microorganism, e.g., helminth ova, enterotoxin (*Clostridium difficile* toxin A; TcdA), cytotoxin (*Clostridium difficile toxin* B; TcdB), ammonia. In some embodiments, the analyte is an antigen from a microorganism (e.g., a bacteria, virus, prion, fungus, protozoan or a parasite).

In some embodiments, the analytes include drugs, metabolites, pesticides, pollutants, and the like. Included among drugs of interest are the alkaloids. Among the alkaloids are morphine alkaloids, which includes morphine, codeine, heroin, dextromethorphan, their derivatives and metabolites; cocaine alkaloids, which include cocaine and benzyl ecgonine, their derivatives and metabolites; ergot alkaloids, which include the diethylamide of lysergic acid; steroid alkaloids; iminazoyl alkaloids; quinazoline alkaloids; isoquinoline alkaloids; quinoline alkaloids, which include quinine and quinidine; diterpene alkaloids, their derivatives and metabolites.

In some embodiments, the analyte is a steroid selected from the estrogens, androgens, andreocortical steroids, bile acids, cardiotonic glycosides and aglycones, which includes digoxin and digoxigenin, saponins and sapogenins, their derivatives and metabolites. Also included are the steroid mimetic substances, such as diethylstilbestrol.

In some embodiments, the analyte is a bile acid. In some embodiments, the presence, absence, and/or a specific level of one or more bile acids in the GI tract of a subject is indicative of a condition or disease state (e.g., a GI disorder and/or a non-GI disorder (e.g., a systemic disorder). For example, in some embodiments, the compositions and methods described herein may be used to detect and/or quantify a bile acid in the GI tract of the subject to diagnose a condition such as bile acid malabsorption (also known as bile acid diarrhea). In some embodiments, the analyte is a metabolite in the serotonin, tryptophan and/or kynurenine pathways, including but not limited to, serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), 5-hydroxytryptophan (5-HTP), kynurenine (K), kynurenic acid (KA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid, anthranilic acid, and combinations thereof. 5-HT is a molecule that plays a role in the regulation of gastrointestinal motility, secretion, and sensation. Imbalances in the levels of 5-HT are associated with several diseases including inflammatory bowel syndrome (IBS), autism, gastric ulcer formation, non-cardiac chest pain, and functional dyspepsia (see, *e.g.*, Faure *et al.* (2010) *Gastroenterology* 139(1): 249-58 and Muller *et al.* (2016) *Neuroscience* 321: 24-41, and International Publication No. WO 2014/188377, each of which are incorporated herein by reference). Conversion of metabolites within the serotonin, tryptophan and/or kynurenine pathways affects the levels of 5-HT in a subject. Therefore, measuring the levels of one or more of the metabolites in this pathway may

be used for the diagnosis, management and treatment of a disease or disorder associated with 5-HT imbalance including but not limited to IBS, autism, carcinoid syndrome, depression, hypertension, Alzheimer's disease, constipation, migraine, and serotonin syndrome. One or more analytes in the serotonin, tryptophan and/or kynurenine pathways can be detected and/or quantitated using, for example, methods and analyte-binding agents that bind to these metabolites including, e.g., antibodies, known in the art (see, e.g., International Publication No. WO2014/188377, the entire contents of which are expressly incorporated herein by reference).

In some embodiments, the analyte is a lactam having from 5 to 6 annular members selected from barbituates, *e.g.*, phenobarbital and secobarbital, diphenylhydantonin, primidone, ethosuximide, and metabolites thereof.

In some embodiments, the analyte is an aminoalkylbenzene, with alkyl of from 2 to 3 carbon atoms, selected from the amphetamines; catecholamines, which includes ephedrine, L-dopa, epinephrine; narceine; papaverine; and metabolites thereof.

In some embodiments, the analyte is a benzheterocyclic selected from oxazepam, chlorpromazine, tegretol, their derivatives and metabolites, the heterocyclic rings being azepines, diazepines and phenothiazines.

In some embodiments, the analyte is a purine selected from theophylline, caffeine, their metabolites and derivatives.

In some embodiments, the analyte is marijuana, cannabinol or tetrahydrocannabinol.

In some embodiments, the analyte is a vitamin such as vitamin A, vitamin B, *e.g.* vitamin B_{12}, vitamin C, vitamin D, vitamin E and vitamin K, folic acid, thiamine.

In some embodiments, the analyte is selected from prostaglandins, which differ by the degree and sites of hydroxylation and unsaturation.

In some embodiments, the analyte is a tricyclic antidepressant selected from imipramine, dismethylimipramine, amitriptyline, nortriptyline, protriptyline, trimipramine, chlomipramine, doxepine, and desmethyldoxepin.

In some embodiments, the analyte is selected from anti-neoplastics, including methotrexate.

In some embodiments, the analyte is an antibiotic as described herein, including, but not limited to, penicillin, chloromycetin, actinomycetin, tetracycline, terramycin, and metabolites and derivatives.

In some embodiments, the analyte is a nucleoside and nucleotide selected from ATP, NAD, FMN, adenosine, guanosine, thymidine, and cytidine with their appropriate sugar and phosphate substituents.

In some embodiments, the analyte is selected from methadone, meprobamate, serotonin, meperidine, lidocaine, procainamide, acetylprocainamide, propranolol, griseofulvin, valproic acid, butyrophenones, antihistamines, chloramphenicol, anticholinergic drugs, such as atropine, their metabolites and derivatives.

In some embodiments, the analyte is a metabolite related to a diseased state. Such metabolites include, but are not limited to spermine, galactose, phenylpyruvic acid, and porphyrin Type 1.

In some embodiments, the analyte is an aminoglycoside, such as gentamicin, kanamicin, tobramycin, or amikacin.

In some embodiments, the analyte is a pesticide. Among pesticides of interest are polyhalogenated biphenyls, phosphate esters, thiophosphates, carbamates, polyhalogenated sulfenamides, their metabolites and derivatives.

In some embodiments, the analyte has a molecular weight of about 500 Da to about 1,000,000 Da (e.g., about 500 to about 500,000 Da, about 1,000 to about 100,000 Da).

In some embodiments, the analyte is a receptor, with a molecular weight ranging from 10,000 to 2 x 10^8 Da, more usually from 10,000 to 10^6 Da. For immunoglobulins, IgA, IgG, IgE and IgM, the molecular weights will generally vary from about 160,000 Da to about 10^6 Da. Enzymes will normally range in molecular weight from about 10,000 Da to about 1,000,000 Da. Natural receptors vary widely, generally having a molecular weight of at least about 25,000 Da and may be 10^6 or higher Da, including such materials as avidin, DNA, RNA, thyroxine binding globulin, thyroxine binding prealbumin, transcortin, etc.

In some embodiments, the term "analyte" further includes polynucleotide analytes such as those polynucleotides defined below. These include m-RNA, r-RNA, t-RNA, DNA, DNA-RNA duplexes, etc. The term analyte also includes polynucleotide-binding agents, such as, for example, restriction enzymes, trascription factors, transcription activators, transcription repressors, nucleases, polymerases, histones, DNA repair enzymes, intercalating gagents, chemotherapeutic agents, and the like.

In some embodiments, the analyte may be a molecule found directly in a sample such as a body fluid from a host. The sample can be examined directly or may be pretreated to render the analyte more readily detectible. Furthermore, the analyte of interest may be determined by

detecting an agent probative of the analyte of interest (*i.e.*, an analyte-binding agent), such as a specific binding pair member complementary to the analyte of interest, whose presence will be detected only when the analyte of interest is present in a sample. Thus, the agent probative of the analyte becomes the analyte that is detected in an assay.

In some embodiments, the analyte a nucleic acid (e.g., a bacterial DNA molecule or a bacterial RNA molecule (e.g., a bacterial tRNA, a transfer-messenger RNA (tmRNA)). See, e.g., Sjostrom et al. (2015) Scientific Reports 5: 15329; Ghosal (2017) Microbial Pathogenesis 104: 161-163; Shen et al. (2012) Cell Host Microbe. 12(4): 509-520.

In some embodiments, the analyte is a component of an outer membrane vesicle (OMV) (e.g., an OmpU protein, Elluri et al. (2014) PloS One 9: e106731). See, e.g., Kulp and Kuehn (2010) Annual Review of microbiology 64: 163-184; Berleman and Auer (2013) Environmental microbiology 15: 347-354; Wai et al. (1995) Microbiology and immunology 39: 451-456; Lindmark et al. (2009) BMC microbiology 9: 220; Sjostrom et al. (2015) Scientific Reports 5: 15329.

In some embodiments, the analyte is G-CSF, which can stimulate the bone marrow to produce granulocytes and stem cells and release them into the bloodstream.

In some embodiments, the analyte is an enzyme such as glutathione S-transferase. For example, the ingestible device can include P28GST, a 28 kDa helminth protein from *Schistosoma* with potent immunogenic and antioxidant properties. P28GST prevents intestinal inflammation in experimental colitis through a Th2-type response with mucosal eosinophils and can be recombinantly produced (e.g., in *S. cerevisiae*). See, for example, U.S. Patent No. 9,593,313, Driss *et al.*, *Mucosal Immunology*, 2016 9, 322–335; and Capron *et al.*, *Gastroenterology*, 146(5):S-638.

In some embodiments, the analyte is a metabolite in the serotonin, tryptophan and/or kynurenine pathways, including but not limited to, serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), 5-hydroxytryptophan (5-HTP), kynurenine (K), kynurenic acid (KA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid, anthranilic acid, and combinations thereof.

In some embodiments, analytes are therapeutic agents or drugs. In some embodiments, analytes are biomarkers. The therapeutic agents disclosed herein are can also be analytes. Examples of biomarkers are provided herein.

In some embodiments, analytes are therapeutic agents, fragments thereof, and metabolites thereof (e.g., antibiotics). In some embodiments, the analytes are antibodies. In some

embodiments, the analytes are antibiotics. Additional exemplary analytes (e.g., antibodies and antibiotics) are provided below.

a. Antibodies

In some embodiments, the analyte or the analyte-binding agent is an antibody. An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain (ScFv) and domain antibodies), and fusion proteins including an antibody portion, and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site. The term antibody includes antibody fragments (e.g., antigen-binding fragments) such as an Fv fragment, a Fab fragment, a F(ab')2 fragment, and a Fab' fragment. Additional examples of antigen-binding fragments include an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g., an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM). An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies including the population

are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Patent No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature 348:552-554, for example.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) that contain hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (*i.e.*, Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-Lazikani et al, 1997, J. Molec. Biol. 273:927-948). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

As known in the art, a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

A "derivative" refers to any polypeptide (*e.g.*, an antibody) having a substantially identical amino acid sequence to the naturally occurring polypeptide, in which one or more amino acids have been modified at side groups of the amino acids (*e.g.*, an biotinylated protein or antibody). The term "derivative" shall also include any polypeptide (*e.g.*, an antibody) which has one or more amino acids deleted from, added to, or substituted from the natural polypeptide

sequence, but which retains a substantial amino acid sequence homology to the natural sequence. A substantial sequence homology is any homology greater than 50 percent.

In some embodiments, the antibody can be a humanized antibody, a chimeric antibody, a multivalent antibody, or a fragment thereof. In some embodiments, an antibody can be a scFv-Fc (Sokolowska-Wedzina et al., *Mol. Cancer Res.* 15(8):1040-1050, 2017), a VHH domain (Li et al., *Immunol. Lett.* 188:89-95, 2017), a VNAR domain (Hasler et al., *Mol. Immunol.* 75:28-37, 2016), a $(scFv)_2$, a minibody (Kim et al., *PLoS One* 10(1):e113442, 2014), or a BiTE. In some embodiments, an antibody can be a DVD-Ig (Wu et al., *Nat. Biotechnol.* 25(11):1290-1297, 2007; WO 08/024188; WO 07/024715), and a dual-affinity re-targeting antibody (DART) (Tsai et al., *Mol. Ther. Oncolytics* 3:15024, 2016), a triomab (Chelius et al., *MAbs* 2(3):309-319, 2010), kih IgG with a common LC (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), a crossmab (Regula et al., *EMBO Mol. Med.* 9(7):985, 2017), an ortho-Fab IgG (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), a 2-in-1-IgG (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), IgG-scFv (Cheal et al., *Mol. Cancer Ther.* 13(7):1803-1812, 2014), scFv2-Fc (Natsume et al., *J. Biochem.* 140(3):359-368, 2006), a bi-nanobody (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), tanden antibody (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), a DART-Fc (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), a scFv-HSA-scFv (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), DNL-Fab3 (Kontermann et al., *Drug Discovery Today* 20(7):838-847, 2015), DAF (two-in-one or four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair antibody, Fab-arm exchange antibody, SEEDbody, Triomab, LUZ-Y, Fcab, kλ-body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)-IgG, IgG (L,H)-Fc, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG, nanobody (e.g., antibodies derived from *Camelus bactriamus*, *Calelus dromaderius*, or *Lama paccos*) (U.S. Patent No. 5,759,808; Stijlemans et al., *J. Biol. Chem.* 279:1256-1261, 2004; Dumoulin et al., *Nature* 424:783-788, 2003; and Pleschberger et al., *Bioconjugate Chem.* 14:440-448, 2003), nanobody-HSA, a diabody (e.g., Poljak, *Structure* 2(12):1121-1123, 1994; Hudson et al., *J. Immunol. Methods* 23(1-2):177-189, 1999), a TandAb (Reusch et al., *mAbs* 6(3):727-738, 2014), scDiabody (Cuesta et al., *Trends in Biotechnol.* 28(7):355-362, 2010), scDiabody-CH3 (Sanz et al., *Trends in Immunol.* 25(2):85-91, 2004), Diabody-CH3 (Guo et al.), Triple Body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')2-scFV2, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc,

intrabody (Huston et al., *Human Antibodies* 10(3-4):127-142, 2001; Wheeler et al., *Mol. Ther.* 8(3):355-366, 2003; Stocks, *Drug Discov. Today* 9(22):960-966, 2004), dock and lock bispecific antibody, ImmTAC, HSAbody, scDiabody-HSA, tandem scFv, IgG-IgG, Cov-X-Body, and scFv1-PEG-scFv2.

In some embodiments, an antibody can be an IgNAR, a bispecific antibody (Milstein and Cuello, *Nature* 305:537-539, 1983; Suresh et al., *Methods in Enzymology* 121:210, 1986; WO 96/27011; Brennan et al., *Science* 229:81, 1985; Shalaby et al., *J. Exp. Med.* 175:217-225, 1992; Kolstelny et al., *J. Immunol.* 148(5):1547-1553, 1992; Hollinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-6448, 1993; Gruber et al., *J. Immunol.* 152:5368, 1994; Tutt et al., *J. Immunol.* 147:60, 1991), a bispecific diabody, a triabody (Schoonooghe et al., *BMC Biotechnol.* 9:70, 2009), a tetrabody, scFv-Fc knobs-into-holes, a scFv-Fc-scFv, a (Fab'scFv)$_2$, a V-IgG, a IvG-V, a dual V domain IgG, a heavy chain immunoglobulin or a camelid (Holt et al., *Trends Biotechnol.* 21(11):484-490, 2003), an intrabody, a monoclonal antibody (e.g., a human or humanized monoclonal antibody), a heteroconjugate antibody (e.g., U.S. Patent No. 4,676,980), a linear antibody (Zapata et al., *Protein Eng.* 8(10:1057-1062, 1995), a trispecific antibody (Tutt et al., *J. Immunol.* 147:60, 1991), a Fabs-in-Tandem immunoglobulin (WO 15/103072), or a humanized camelid antibody.

In some embodiments, the antibody binds specifically to a metabolite in the serotonin, tryptophan and/or kynurenine pathways, including but not limited to, serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), 5-hydroxytryptophan (5-HTP), kynurenine (K), kynurenic acid (KA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid, anthranilic acid. Exemplary antibodies that bind to metabolites in these pathways are disclosed, for example, in International Publication No. WO2014/188377, the entire contents of which are incorporated herein by reference.

In some embodiments, the antibody is specific for a particular genus, species, or strain of a microorganism, and may therefore be used for the detection, analysis and/or quantitation of the microorganism using the detection methods described below. In some embodiments, the antibody specifically binds to a surface-specific biomolecule (e.g., a pilus subunit or a flagella protein) present in a particular genus, species or strain of microorganism, and does not cross-react with other microorganisms. In some embodiments, these antibodies may be used in the methods described herein to diagnose a subject with a particular infection or disease, or to monitor an infection (e.g., during or after treatment). In some embodiments, the antibody specifically binds to an antigen present in a particular genera, species or strain of a

microorganism. Exemplary antigens, the corresponding microorganism that can be detected, and the disease caused by the microorganism (in parentheticals) include: outer membrane protein A OmpA (*Acinetobacter baumannii, Acinetobacter* infections)); HIV p24 antigen, HIV Eenvelope proteins (Gp120, Gp41, Gp160) (HIV (Human immunodeficiency virus), AIDS (Acquired immunodeficiency syndrome)); galactose-inhibitable adherence protein GIAP, 29 kDa antigen Eh29, GaVGaINAc lectin, protein CRT, 125 kDa immunodominant antigen, protein M17, adhesin ADH112, protein STIRP (*Entamoeba histolytica*, Amoebiasis); protective Antigen PA, edema factor EF, lethal facotor LF, the S-layer homology proteins SLH (*Bacillus anthracis*, Anthrax); nucleocapsid protein NP, glycoprotein precursor GPC, glycoprotein GP1, glycoprotein GP2 (Junin virus, Argentine hemorrhagic fever); 41 kDa allergen Asp v13, allergen Asp f3, major conidial surface protein rodlet A, protease Pep1p, GPI-anchored protein Gel1p, GPI-anchored protein Crf1p (*Aspergillus* genus, Aspergillosis); outer surface protein A OspA, outer surface protein OspB, outer surface protein OspC, decorin binding protein A DbpA, flagellar filament 41 kDa core protein Fla, basic membrane protein A precursor BmpA (Immunodominant antigen P39), outer surface 22 kDa lipoprotein precursor (antigen IPLA7), variable surface lipoprotein vIsE (*Borrelia* genus, *Borrelia* infection); OmpA-like transmembrane domain-containing protein Omp31, immunogenic 39-kDa protein M5 P39, 25 kDa outer-membrane immunogenic protein precursor Omp25, outer membrane protein MotY Omp16, conserved outer membrane protein D15, malate dehydrogenase Mdh, component of the Type-IV secretion system (T4SS) VirJ, lipoprotein of unknown function BAB1_0187 (*Brucella* genus, Brucellosis); major outer membrane protein PorA, flagellin FIaA, surface antigen CjaA, fibronectin binding protein CadF, aspartate/glutamate-binding ABC transporter protein Peb1A, protein FspA1, protein FspA2 (*Campylobacter* genus, Campylobacteriosis); glycolytic enzyme enolase, secreted aspartyl proteinases SAP1-10, glycophosphatidylinositol (GPI)-linked cell wall protein, adhesin Als3p, cell surface hydrophobicity protein CSH (usually *Candida albicans* and other *Candida* species, Candidiasis); envelope glycoproteins (gB, gC, gE, gH, gI, gK, gL) (Varicella zoster virus (VZV), Chickenpox); major outer membrane protein MOMP, probable outer membrane protein PMPC, outer membrane complex protein B OmcB (*Chlamydia trachomatis, Chlamydia*); major outer membrane protein MOMP, outer membrane protein 2 Omp2, (*Chlamydophila pneumoniae, Chlamydophila pneumoniae* infection); outer membrane protein U Porin ompU, (*Vibrio cholerae*, Cholera); surface layer proteins SLPs, Cell Wall Protein CwpV, flagellar protein FliC, flagellar protein FliD (*Clostridium difficile, Clostridium difficile* infection); acidic ribosomal protein P2 CpP2, mucin antigens Muc1, Muc2, Muc3 Muc4, Muc5, Muc6, Muc7, surface adherence protein

CP20, surface adherence protein CP23, surface protein CP12, surface protein CP21, surface protein CP40, surface protein CP60, surface protein CP15, surface-associated glycopeptides gp40, surface-associated glycopeptides gp15, oocyst wall protein AB, profilin PRF, apyrase (*Cryptosporidium* genus, Cryptosporidiosis); membrane protein pp15, capsid-proximal tegument protein pp150 (Cytomegalovirus, Cytomegalovirus infection); prion protein (vCJD prion, Variant Creutzfeldt-Jakob disease (vCJD, nvCJD)); cyst wall proteins CWP1, CWP2, CWP3, variant surface protein VSP, VSP1, VSP2, VSP3, VSP4, VSP5, VSP6, 56 kDa antigen (*Giardia intestinalis*, Giardiasis); minor pilin-associated subunit pilC, major pilin subunit and variants pilE, pilS (*Neisseria gonorrhoeae*, Gonorrhea); outer membrane protein A OmpA, outer membrane protein C OmpC, outer membrane protein K17 OmpK17 (*Klebsiella granulomatis, Granuloma inguinale* (Donovanosis)); fibronectin-binding protein Sfb (*Streptococcus pyogenes*, Group A streptococcal infection); outer membrane protein P6 (*Haemophilus influenzae, Haemophilus influenzae* infection); integral membrane proteins, aggregation-prone proteins, O-antigen, toxin-antigens Stx2B, toxin-antigen Stx1B, adhesion-antigen fragment Int28, protein EspA, protein EspB, Intimin, protein Tir, protein IntC300, protein Eae (*Escherichia coli O*157:H7, O111 and O104:H4, Hemolytic-uremic syndrome (HUS)); hepatitis A surface antigen HBAg (Hepatitis A Virus, Hepatitis A); hepatitis B surface antigen HBsAg (Hepatitis B Virus, Hepatitis B); envelope glycoprotein E1 gp32 gp35, envelope glycoprotein E2 NS1 gp68 gp70, capsid protein C, (Hepatitis C Virus, Hepatitis C); type IV pilin PilE, outer membrane protein MIP, major outer membrane protein MompS (*Legionella pneumophila*, Legionellosis (Legionnaires' disease, Pontiac fever)); minor pilin-associated subunit pilC, major pilin subunit and variants pilE, pilS (*Neisseria meningitidis*, Meningococcal disease); adhesin P1, adhesion P30 (*Mycoplasma pneumoniae, Mycoplasma* pneumonia); F1 capsule antigen, outer membrane protease Pla, (*Yersinia pestis*, Plague); surface adhesin PsaA, cell wall surface anchored protein psrP (*Streptococcus pneumoniae*, Pneumococcal infection); flagellin FliC, invasion protein SipC, glycoprotein gp43, outer membrane protein LamB, outer membrane protein PagC, outer membrane protein TolC, outer membrane protein NmpC, outer membrane protein FadL, transport protein SadA (*Salmonella* genus, Salmonellosis); collagen adhesin Cna, fibronectin-binding protein A FnbA, secretory antigen SssA (*Staphylococcus* genus, Staphylococcal food poisoning); collagen adhesin Can (*Staphylococcus* genus, *Staphylococcal* infection); fibronectin-binding protein A FbpA (Ag85A), fibronectin-binding protein D FbpD, fibronectin-binding protein C FbpC1, heat-shock protein HSP65, protein PST-S (*Mycobacterium tuberculosis*, Tuberculosis); and outer membrane protein FobA, outer membrane protein FobB, type IV pili

glycosylation protein, outer membrane protein tolC, protein TolQ (*Francisella tularensis*, Tularemia). Additional exemplary microorganisms and corresponding antigens are disclosed, e.g., in U.S. Publication No. 2015/0118264, the entire contents of which are expressly incorporated herein by reference.

In some embodiments, a plurality of antibodies (e.g.,2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, or more antibodies) are used as analyte-binding agents in any of the methods described herein (e.g., to detect the presence of one or more analytes in a sample). In some embodiments, the plurality of antibodies bind to the same analyte (e.g., an antigen). In some embodiments, the plurality of antibodes bind to the same epitope present on the analyte (e.g., an antigen). In some embodiments, the plurality of antibodies bind to different epitopes present on the same analyte. In some embodiments, the plurality of antibodies bind to overlapping epitopes present on the same analyte. In some embodiments, the plurality of antibodies bind to non-overlapping epitopes present on the same analyte.

b. *Antibiotics*

In some embodiments, the analyte or analyte-binding agent is an antibiotic. An "antibiotic" or "antibiotic agent" refers to a substance that has the capacity to inhibit or slow down the growth of, or to destroy bacteria and/or other microorganisms. In some embodiments, the antibiotic agent is a bacteriostatic antibiotic agent. In some embodiments, the antibiotic is a bacteriolytic antibiotic agent. Exemplary antibiotic agents are set forth in the U.S. Patent Publication US 2006/0269485, which is hereby incorporated by reference herein in its entirety.

In some embodiments, the antibiotic agent is selected from the classes consisting of beta-lactam antibiotics, aminoglycosides, ansa-type antibiotics, anthraquinones, antibiotic azoles, antibiotic glycopeptides, macrolides, antibiotic nucleosides, antibiotic peptides, antibiotic polyenes, antibiotic polyethers, quinolones, antibiotic steroids, sulfonamides, tetracycline, dicarboxylic acids, antibiotic metals, oxidizing agents, substances that release free radicals and/or active oxygen, cationic antimicrobial agents, quaternary ammonium compounds, biguanides, triguanides, bisbiguanides and analogs and polymers thereof and naturally occurring antibiotic compounds. In some embodiments, the antibiotic is rifaximin.

Beta-lactam antibiotics include, but are not limited to, 2-(3-alanyl)clavam, 2-hydroxymethylclavam, 8-epi-thienamycin, acetyl-thienamycin, amoxicillin, amoxicillin sodium, amoxicillin trihydrate, amoxicillin-potassium clavulanate combination, ampicillin, ampicillin sodium, ampicillin trihydrate, ampicillin-sulbactam, apalcillin, aspoxicillin, azidocillin,

azlocillin, aztreonam, bacampicillin, biapenem, carbenicillin, carbenicillin disodium, carfecillin, carindacillin, carpetimycin, cefacetril, cefaclor, cefadroxil, cefalexin, cefaloridine, cefalotin, cefamandole, cefamandole, cefapirin, cefatrizine, cefatrizine propylene glycol, cefazedone, cefazolin, cefbuperazone, cefcapene, cefcapene pivoxil hydrochloride, cefdinir, cefditoren, cefditoren pivoxil, cefepime, cefetamet, cefetamet pivoxil, cefixime, cefinenoxime, cefinetazole, cefminox, cefminox, cefmolexin, cefodizime, cefonicid, cefoperazone, ceforanide, cefoselis, cefotaxime, cefotetan, cefotiam, cefoxitin, cefozopran, cefpiramide, cefpirome, cefpodoxime, cefpodoxime proxetil, cefprozil, cefquinome, cefradine, cefroxadine, cefsulodin, ceftazidime, cefteram, cefteram pivoxil, ceftezole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuroxime axetil, cephalosporin, cephamycin, chitinovorin, ciclacillin, clavulanic acid, clometocillin, cloxacillin, cycloserine, deoxy pluracidomycin, dicloxacillin, dihydro pluracidomycin, epicillin, epithienamycin, ertapenem, faropenem, flomoxef, flucloxacillin, hetacillin, imipenem, lenampicillin, loracarbef, mecillinam, meropenem, metampicillin, meticillin, mezlocillin, moxalactam, nafcillin, northienamycin, oxacillin, panipenem, penamecillin, penicillin, phenethicillin, piperacillin, tazobactam, pivampicillin, pivcefalexin, pivmecillinam, pivmecillinam hydrochloride, pluracidomycin, propicillin, sarmoxicillin, sulbactam, sulbenicillin, talampicillin, temocillin, terconazole, thienamycin, ticarcillin and analogs, salts and derivatives thereof.

Aminoglycosides include, but are not limited to, 1,2′-N-DL-isoseryl-3′,4′-dideoxykanamycin B, 1,2′-N-DL-isoseryl-kanamycin B, 1,2′-N-[(S)-4-amino-2-hydroxybutyryl]-3′,4′-dideoxykanamycin B, 1,2′-N-[(S)-4-amino-2-hydroxybutyryl]-kanamycin B, 1-N-(2-Aminobutanesulfonyl) kanamycin A, 1-N-(2-aminoethanesulfonyl)3′,4′-dideoxyribostamycin, 1-N-(2-Aminoethanesulfonyl)3′-deoxyribostamycin, 1-N-(2-aminoethanesulfonyl)3′4′-dideoxykanamycin B, 1-N-(2-aminoethanesulfonyl)kanamycin A, 1-N-(2-aminoethanesulfonyl)kanamycin B, 1-N-(2-aminoethanesulfonyl)ribostamycin, 1-N-(2-aminopropanesulfonyl)3′-deoxykanamycin B, 1-N-(2-aminopropanesulfonyl)3′4′-dideoxykanamycin B, 1-N-(2-aminopropanesulfonyl)kanamycin A, 1-N-(2-aminopropanesulfonyl)kanamycin B, 1-N-(L-4-amino-2-hydroxy-butyryl)2,′3′-dideoxy-2′-fluorokanamycin A, 1-N-(L-4-amino-2-hydroxy-propionyl)2,′3′-dideoxy-2′-fluorokanamycin A, 1-N-DL-3′,4′-dideoxy-isoserylkanamycin B, 1-N-DL-isoserylkanamycin, 1-N-DL-isoserylkanamycin B, 1-N-[L-(−)-(alpha-hydroxy-gamma-aminobutyryl)]-XK-62-2,2′,3′-dideoxy-2′-fluorokanamycin A,2-hydroxygentamycin A3,2-hydroxygentamycin B, 2-hydroxygentamycin B1, 2-hydroxygentamycin JI-20A, 2-hydroxygentamycin JI-20B, 3″-N-

methyl-4″-C-methyl-3′,4′-dodeoxy kanamycin A, 3″-N-methyl-4″-C-methyl-3′,4′-dodeoxy kanamycin B, 3″-N-methyl-4″-C-methyl-3′,4′-dodeoxy-6′-methyl kanamycin B, 3′,4′-Dideoxy-3′-eno-ribostamycin,3′,4′-dideoxyneamine,3′,4′-dideoxyribostamycin, 3′-deoxy-6′-N-methyl-kanamycin B,3′-deoxyneamine,3′-deoxyribostamycin, 3′-oxysaccharocin,3,3′-nepotrehalosadiamine, 3-demethoxy-2″-N-formimidoylistamycin B disulfate tetrahydrate, 3-demethoxyistamycin B,3-O-demethyl-2-N-formimidoylistamycin B, 3-O-demethylistamycin B,3-trehalosamine,4″,6″-dideoxydibekacin, 4-N-glycyl-KA-6606VI, 5″-Amino-3′,4′,5″-trideoxy-butirosin A, 6″-deoxydibekacin,6′-epifortimicin A, 6-deoxy-neomycin (structure 6-deoxy-neomycin B),6-deoxy-neomycin B, 6-deoxy-neomycin C, 6-deoxy-paromomycin, acmimycin, AHB-3′,4′-dideoxyribostamycin, AHB-3′-deoxykanamycin B, AHB-3′-deoxyneamine, AHB-3′-deoxyribostamycin, AHB-4″-6″-dideoxydibekacin, AHB-6″-deoxydibekacin, AHB-dideoxyneamine, AHB-kanamycin B, AHB-methyl-3′-deoxykanamycin B, amikacin, amikacin sulfate, apramycin, arbekacin, astromicin, astromicin sulfate, bekanamycin, bluensomycin, boholmycin, butirosin, butirosin B, catenulin, coumamidine gamma1, coumamidine gamma2,D,L-1-N-(alpha-hydroxy-beta-aminopropionyl)-XK-62-2, dactimicin, de-O-methyl-4-N-glycyl-KA-6606VI, de-O-methyl-KA-6606I, de-O-methyl-KA-7038I, destomycin A, destomycin B, di-N6′,O3-demethylistamycin A, dibekacin, dibekacin sulfate, dihydrostreptomycin, dihydrostreptomycin sulfate, epi-formamidoylglycidylfortimicin B, epihygromycin, formimidoyl-istamycin A, formimidoyl-istamycin B, fortimicin B, fortimicin C, fortimicin D, fortimicin KE, fortimicin KF, fortimicin KG, fortimicin KG1 (stereoisomer KG1/KG2), fortimicin KG2 (stereoisomer KG1/KG2), fortimicin KG3, framycetin, framycetin sulphate, gentamicin, gentamycin sulfate, globeomycin, hybrimycin A1, hybrimycin A2, hybrimycin B1, hybrimycin B2, hybrimycin C1, hybrimycin C2, hydroxystreptomycin, hygromycin, hygromycin B, isepamicin, isepamicin sulfate, istamycin, kanamycin, kanamycin sulphate, kasugamycin, lividomycin, marcomycin, micronomicin, micronomicin sulfate, mutamicin, myomycin, N-demethyl-7-O-demethylcelesticetin, demethylcelesticetin, methanesulfonic acid derivative of istamycin, nebramycin, nebramycin, neomycin, netilmicin, oligostatin, paromomycin, quintomycin, ribostamycin, saccharocin, seldomycin, sisomicin, sorbistin, spectinomycin, streptomycin, tobramycin, trehalosmaine, trestatin, validamycin, verdamycin, xylostasin, zygomycin and analogs, salts and derivatives thereof.

Ansa-type antibiotics include, but are not limited to, 21-hydroxy-25-demethyl-25-methylth ioprotostreptovaricin, 3-methylth iorifamycin, ansamitocin, atropisostreptovaricin, awamycin, halomicin, maytansine, naphthomycin, rifabutin, rifamide, rifampicin, rifamycin,

rifapentine, rifaximin (*e.g.*, Xifaxan®), rubradirin, streptovaricin, tolypomycin and analogs, salts and derivatives thereof.

Antibiotic anthraquinones include, but are not limited to, auramycin, cinerubin, ditrisarubicin, ditrisarubicin C, figaroic acid fragilomycin, minomycin, rabelomycin, rudolfomycin, sulfurmycin and analogs, salts and derivatives thereof.

Antibiotic azoles include, but are not limited to, azanidazole, bifonazole, butoconazol, chlormidazole, chlormidazole hydrochloride, cloconazole, cloconazole monohydrochloride, clotrimazol, dimetridazole, econazole, econazole nitrate, enilconazole, fenticonazole, fenticonazole nitrate, fezatione, fluconazole, flutrimazole, isoconazole, isoconazole nitrate, itraconazole, ketoconazole, lanoconazole, metronidazole, metronidazole benzoate, miconazole, miconazole nitrate, neticonazole, nimorazole, niridazole, omoconazol, ornidazole, oxiconazole, oxiconazole nitrate, propenidazole, secnidazol, sertaconazole, sertaconazole nitrate, sulconazole, sulconazole nitrate, tinidazole, tioconazole, voriconazol and analogs, salts and derivatives thereof.

Antibiotic glycopeptides include, but are not limited to, acanthomycin, actaplanin, avoparcin, balhimycin, bleomycin B (copper bleomycin), chloroorienticin, chloropolysporin, demethylvancomycin, enduracidin, galacardin, guanidylfungin, hachimycin, demethylvancomycin, N-nonanoyl-teicoplanin, phleomycin, platomycin, ristocetin, staphylocidin, talisomycin, teicoplanin, vancomycin, victomycin, xylocandin, zorbamycin and analogs, salts and derivatives thereof.

Macrolides include, but are not limited to, acetylleucomycin, acetylkitasamycin, angolamycin, azithromycin, bafilomycin, brefeldin, carbomycin, chalcomycin, cirramycin, clarithromycin, concanamycin, deisovaleryl-niddamycin, demycinosyl-mycinamycin, Di-O-methyltiacumicidin, dirithromycin, erythromycin, erythromycin estolate, erythromycin ethyl succinate, erythromycin lactobionate, erythromycin stearate, flurithromycin, focusin, foromacidin, haterumalide, haterumalide, josamycin, josamycin ropionate, juvenimycin, juvenimycin, kitasamycin, ketotiacumicin, lankavacidin, lankavamycin, leucomycin, machecin, maridomycin, megalomicin, methylleucomycin, methymycin, midecamycin, miocamycin, mycaminosyltylactone, mycinomycin, neutramycin, niddamycin, nonactin, oleandomycin, phenylacetyideltamycin, pamamycin, picromycin, rokitamycin, rosaramicin, roxithromycin, sedecamycin, shincomycin, spiramycin, swalpamycin, tacrolimus, telithromycin, tiacumicin, tilmicosin, treponemycin, troleandomycin, tylosin, venturicidin and analogs, salts and derivatives thereof.

Antibiotic nucleosides include, but are not limited to, amicetin, angustmycin, azathymidine, blasticidin S, epiroprim, flucytosine, gougerotin, mildiomycin, nikkomycin, nucleocidin, oxanosine, oxanosine, puromycin, pyrazomycin, showdomycin, sinefungin, sparsogenin, spicamycin, tunicamycin, uracil polyoxin, vengicide and analogs, salts and derivatives thereof.

Antibiotic peptides include, but are not limited to, actinomycin, aculeacin, alazopeptin, amfomycin, amythiamycin, antifungal from *Zalerion arboricola*, antrimycin, apid, apidaecin, aspartocin, auromomycin, bacileucin, bacillomycin, bacillopeptin, bacitracin, bagacidin, beminamycin, beta-alanyl-L-tyrosine, bottromycin, capreomycin, caspofungine, cepacidine, cerexin, cilofungin, circulin, colistin, cyclodepsipeptide, cytophagin, dactinomycin, daptomycin, decapeptide, desoxymulundocandin, echanomycin, echinocandin B, echinomycin, ecomycin, enniatin, etamycin, fabatin, ferrimycin, ferrimycin, ficellomycin, fluoronocathiacin, fusaricidin, gardimycin, gatavalin, globopeptin, glyphomycin, gramicidin, herbicolin, iomycin, iturin, iyomycin, izupeptin, janiemycin, janthinocin, jolipeptin, katanosin, killertoxin, lipopeptide antibiotic, lipopeptide from *Zalerion* sp., lysobactin, lysozyme, macromomycin, magainin, melittin, mersacidin, mikamycin, mureidomycin, mycoplanecin, mycosubtilin, neopeptifluorin, neoviridogrisein, netropsin, nisin, nocathiacin, nocathiacin 6-deoxyglycoside, nosiheptide, octapeptin, pacidamycin, pentadecapeptide, peptifluorin, permetin, phytoactin, phytostreptin, planothiocin, plusbacin, polcillin, polymyxin antibiotic complex, polymyxin B, polymyxin B1, polymyxin F, preneocarzinostatin, quinomycin, quinupristin-dalfopristin, safracin, salmycin, salmycin, salmycin, sandramycin, saramycetin, siomycin, sperabillin, sporamycin, a *Streptomyces* compound, subtilin, teicoplanin aglycone, telomycin, thermothiocin, thiopeptin, thiostrepton, tridecaptin, tsushimycin, tuberactinomycin, tuberactinomycin, tyrothricin, valinomycin, viomycin, virginiamycin, zervacin and analogs, salts and derivatives thereof.

In some embodiments, the antibiotic peptide is a naturally-occurring peptide that possesses an antibacterial and/or an antifungal activity. Such peptide can be obtained from an herbal or a vertebrate source.

Polyenes include, but are not limited to, amphotericin, amphotericin, aureofungin, ayfactin, azalomycin, blasticidin, candicidin, candicidin methyl ester, candimycin, candimycin methyl ester, chinopricin, filipin, flavofungin, fradicin, hamycin, hydropricin, levorin, lucensomycin, lucknomycin, mediocidin, mediocidin methyl ester, mepartricin, methylamphotericin, natamycin, niphimycin, nystatin, nystatin methyl ester, oxypricin, partricin,

pentamycin, perimycin, pimaricin, primycin, proticin, rimocidin, sistomycosin, sorangicin, trichomycin and analogs, salts and derivatives thereof.

Polyethers include, but are not limited to, 20-deoxy-epi-narasin, 20-deoxysalinomycin, carriomycin, dianemycin, dihydrolonomycin, etheromycin, ionomycin, iso-lasalocid, lasalocid, lenoremycin, lonomycin, lysocellin, monensin, narasin, oxolonomycin, a polycyclic ether antibiotic, salinomycin and analogs, salts and derivatives thereof.

Quinolones include, but are not limited to, an alkyl-methylendioxy-4(1H)-oxocinnoline-3-carboxylic acid, alatrofloxacin, cinoxacin, ciprofloxacin, ciprofloxacin hydrochloride, danofloxacin, dermofongin A, enoxacin, enrofloxacin, fleroxacin, flumequine, gatifloxacin, gemifloxacin, grepafloxacin, levofloxacin, lomefloxacin, lomefloxacin, hydrochloride, miloxacin, moxifloxacin, nadifloxacin, nalidixic acid, nifuroquine, norfloxacin, ofloxacin, orbifloxacin, oxolinic acid, pazufloxacine, pefloxacin, pefloxacin mesylate, pipemidic acid, piromidic acid, premafloxacin, rosoxacin, rufloxacin, sparfloxacin, temafloxacin, tosufloxacin, trovafloxacin and analogs, salts and derivatives thereof.

Antibiotic steroids include, but are not limited to, aminosterol, ascosteroside, cladosporide A, dihydrofusidic acid, dehydro-dihydrofusidic acid, dehydrofusidic acid, fusidic acid, squalamine and analogs, salts and derivatives thereof.

Sulfonamides include, but are not limited to, chloramine, dapsone, mafenide, phthalylsulfathiazole, succinylsulfathiazole, sulfabenzamide, sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadiazine silver, sulfadicramide, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfalene, sulfamazone, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfamoxol, sulfanilamide, sulfaperine, sulfaphenazol, sulfapyridine, sulfaquinoxaline, sulfasuccinamide, sulfathiazole, sulfathiourea, sulfatolamide, sulfatriazin, sulfisomidine, sulfisoxazole, sulfisoxazole acetyl, sulfacarbamide and analogs, salts and derivatives thereof.

Tetracyclines include, but are not limited to, dihydrosteffimycin, demethyltetracycline, aclacinomycin, akrobomycin, baumycin, bromotetracycline, cetocyclin, chlortetracycline, clomocycline, daunorubicin, demeclocycline, doxorubicin, doxorubicin hydrochloride, doxycycline, lymecyclin, marcellomycin, meclocycline, meclocycline sulfosalicylate, methacycline, minocycline, minocycline hydrochloride, musettamycin, oxytetracycline, rhodirubin, rolitetracycline, rubomycin, serirubicin, steffimycin, tetracycline and analogs, salts and derivatives thereof.

Dicarboxylic acids, having between about 6 and about 14 carbon atoms in their carbon atom skeleton are particularly useful in the treatment of disorders of the skin and mucosal membranes that involve microbial. Suitable dicarboxylic acid moieties include, but are not limited to, adipic acid, pimelic acid, suberic acid, azelaic acid, sebacic acid, 1,11-undecanedioic acid, 1,12-dodecanedioic acid, 1,13-tridecanedioic acid and 1,14-tetradecanedioic acid. Thus, in one or more embodiments of the present disclosure, dicarboxylic acids, having between about 6 and about 14 carbon atoms in their carbon atom skeleton, as well as their salts and derivatives (*e.g.*, esters, amides, mercapto-derivatives, anhydraides), are useful immunomodulators in the treatment of disorders of the skin and mucosal membranes that involve inflammation. Azelaic acid and its salts and derivatives are preferred. It has antibacterial effects on both aerobic and anaerobic organisms, particularly *Propionibacterium acnes* and *Staphylococcus epidermidis*, normalizes keratinization, and has a cytotoxic effect on malignant or hyperactive melanocytes. In a preferred embodiment, the dicarboxylic acid is azelaic acid in a concentration greater than 10%. Preferably, the concentration of azelaic acid is between about 10% and about 25%. In such concentrates, azelaic acid is suitable for the treatment of a variety of skin disorders, such as acne, rosacea and hyperpigmentation.

In some embodiments, the antibiotic agent is an antibiotic metal. A number of metals ions have been shown to possess antibiotic activity, including silver, copper, zinc, mercury, tin, lead, bismutin, cadmium, chromium and ions thereof. It has been theorized that these antibiotic metal ions exert their effects by disrupting respiration and electron transport systems upon absorption into bacterial or fungal cells. Anti-microbial metal ions of silver, copper, zinc, and gold, in particular, are considered safe for *in vivo* use. Anti-microbial silver and silver ions are particularly useful due to the fact that they are not substantially absorbed into the body. Thus, in one or more embodiment, the antibiotic metal consists of an elemental metal, selected from the group consisting of silver, copper, zinc, mercury, tin, lead, bismutin, cadmium, chromium and gold, which is suspended in the composition as particles, microparticles, nanoparticles or colloidal particles. The antibiotic metal can further be intercalated in a chelating substrate.

In further embodiments, the antibiotic metal is ionic. The ionic antibiotic metal can be presented as an inorganic or organic salt (coupled with a counterion), an organometallic complex or an intercalate. Non-binding examples of counter inorganic and organic ions are sulfadiazine, acetate, benzoate, carbonate, iodate, iodide, lactate, laurate, nitrate, oxide, and palmitate, a negatively charged protein. In preferred embodiments, the antibiotic metal salt is a silver salt,

such as silver acetate, silver benzoate, silver carbonate, silver iodate, silver iodide, silver lactate, silver laurate, silver nitrate, silver oxide, silver palmitate, silver protein, and silver sulfadiazine.

In one or more embodiments, the antibiotic metal or metal ion is embedded into a substrate, such as a polymer, or a mineral (such as zeolite, clay and silica).

In one or more embodiments, the antibiotic agent includes strong oxidants and free radical liberating compounds, such as oxygen, hydrogen peroxide, benzoyl peroxide, elemental halogen species, as well as oxygenated halogen species, bleaching agents (*e.g.*, sodium, calcium or magnesium hypochloride and the like), perchlorite species, iodine, iodate, and benzoyl peroxide. Organic oxidizing agents, such as quinones, are also included. Such agents possess a potent broad-spectrum activity.

In one or more embodiments, the antibiotic agent is a cationic antimicrobial agent. The outermost surface of bacterial cells universally carries a net negative charge, making them sensitive to cationic substances. Examples of cationic antibiotic agents include: quaternary ammonium compounds (QAC's)—QAC's are surfactants, generally containing one quaternary nitrogen associated with at least one major hydrophobic moiety; alkyltrimethyl ammonium bromides are mixtures of where the alkyl group is between 8 and 18 carbons long, such as cetrimide (tetradecyltrimethylammonium bromide); benzalkonium chloride, which is a mixture of n-alkyldimethylbenzyl ammonium chloride where the alkyl groups (the hydrophobic moiety) can be of variable length; dialkylmethyl ammonium halides; dialkylbenzyl ammonium halides; and QAC dimmers, which bear bi-polar positive charges in conjunction with interstitial hydrophobic regions.

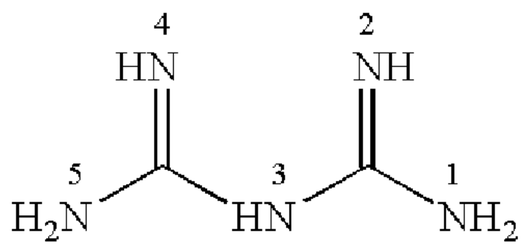
In one or more embodiments, the cationic antimicrobial agent is a polymer. Cationic antimicrobial polymers include, for example, guanide polymers, biguanide polymers, or polymers having side chains containing biguanide moieties or other cationic functional groups, such as benzalkonium groups or quarternium groups (e.g., quaternary amine groups). It is understood that the term "polymer" as used herein includes any organic material including three or more repeating units, and includes oligomers, polymers, copolymers, block copolymers, terpolymers, etc. The polymer backbone may be, for example a polyethylene, ploypropylene or polysilane polymer.

In one or more embodiments, the cationic antimicrobial polymer is a polymeric biguanide compound. When applied to a substrate, such a polymer is known to form a barrier film that can engage and disrupt a microorganism. An exemplary polymeric biguanide compound is polyhexamethylene biguanide (PHMB) salts. Other exemplary biguanide polymers include, but

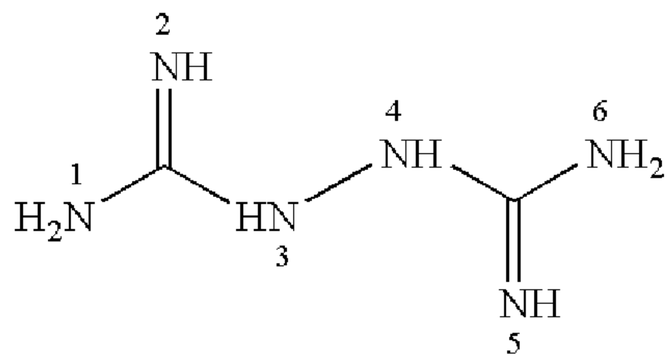
are not limited to poly(hexamethylenebiguanide), poly(hexamethylenebiguanide) hydrochloride, poly(hexamethylenebiguanide) gluconate, poly(hexamethylenebiguanide) stearate, or a derivative thereof. In one or more embodiments, the antimicrobial material is substantially water-insoluble.

In some embodiments, the antibiotic agent is selected from the group of biguanides, triguanides, bisbiguanides and analogs thereof.

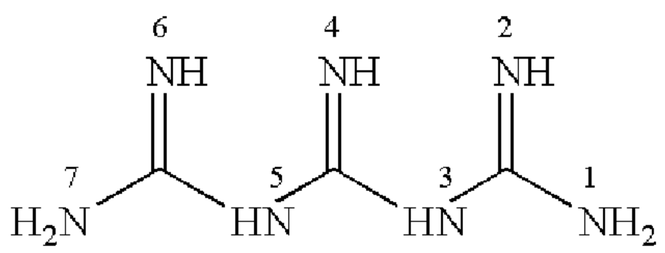
Guanides, biguanides, biguanidines and triguanides are unsaturated nitrogen containing molecules that readily obtain one or more positive charges, which make them effective antimicrobial agents. The basic structures a guanide, a biguanide, a biguanidine and a triguanide are provided below.



Biguanide



Biguanidine



Triguanide

In some embodiments, the guanide, biguanide, biguanidine or triguanide, provide bi-polar configurations of cationic and hydrophobic domains within a single molecule.

Examples of guanides, biguanides, biguanidines and triguanides that are currently been used as antibacterial agents include chlorhexidine and chlorohexidine salts, analogs and derivatives, such as chlorhexidine acetate, chlorhexidine gluconate and chlorhexidine hydrochloride, picloxydine, alexidine and polihexanide. Other examples of guanides, biguanides, biguanidines and triguanides that can conceivably be used according to the present disclosure are chlorproguanil hydrochloride, proguanil hydrochloride (currently used as antimalarial agents), mefformin hydrochloride, phenformin and buformin hydrochloride (currently used as antidiabetic agents).

Yet, in one or more embodiments, the antibiotic is a non-classified antibiotic agent, including, without limitation, aabomycin, acetomycin, acetoxycycloheximide, acetylnanaomycin, an *Actinoplanes* sp. compound, actinopyrone, aflastatin, albacarcin, albacarcin, albofungin, albofungin, alisamycin, alpha-R,S-methoxycarbonylbenzylmonate, altromycin, amicetin, amycin, amycin demanoyl compound, amycine, amycomycin, anandimycin, anisomycin, anthramycin,

anti-syphilis immune substance, anti-tuberculosis immune substance, an antibiotic from *Escherichia coli*, an antibiotic from *Streptomyces refuineus*, anticapsin, antimycin, aplasmomycin, aranorosin, aranorosinol, arugomycin, ascofuranone, ascomycin, ascosin, *Aspergillus flavus* antibiotic, asukamycin, aurantinin, an Aureolic acid antibiotic substance, aurodox, avilamycin, azidamfenicol, azidimycin, bacillaene, a *Bacillus larvae* antibiotic, bactobolin, benanomycin, benzanthrin, benzylmonate, bicozamycin, bravomicin, brodimoprim, butalactin, calcimycin, calvatic acid, candiplanecin, carumonam, carzinophilin, celesticetin, cepacin, cerulenin, cervinomycin, chartreusin, chloramphenicol, chloramphenicol palmitate, chloramphenicol succinate sodium, chlorflavonin, chlorobiocin, chlorocarcin, chromomycin, ciclopirox, ciclopirox olamine, citreamicin, cladosporin, clazamycin, clecarmycin, clindamycin, coliformin, collinomycin, copiamycin, corallopyronin, corynecandin, coumermycin, culpin, cuprimyxin, cyclamidomycin, cycloheximide, dactylomycin, danomycin, danubomycin, delaminomycin, demethoxyrapamycin, demethylscytophycin, dermadin, desdamethine, dexylosyl-benanomycin, pseudoaglycone, dihydromocimycin, dihydronancimycin, diumycin, dnacin, dorrigocin, dynemycin, dynemycin triacetate, ecteinascidin, efrotomycin, endomycin, ensanchomycin, equisetin, ericamycin, esperamicin, ethylmonate, everninomicin, feldamycin, flambamycin, flavensomycin, florfenicol, fluvomycin, fosfomycin, fosfonochlorin, fredericamycin, frenolicin, fumagillin, fumifungin, funginon, fusacandin, fusafungin, gelbecidine, glidobactin, grahamimycin, granaticin, griseofulvin, griseoviridin, grisonomycin, hayumicin, hayumicin, hazymicin, hedamycin, heneicomycin, heptelicid acid, holomycin, humidin, isohematinic acid, karnatakin, kazusamycin, kristenin, L-dihydrophenylalanine, a L-isoleucyl-L-2-amino-4-(4′-amino-2′,5′-cyclohexadienyl) derivative, lanomycin, leinamycin, leptomycin, libanomycin, lincomycin, lomofungin, lysolipin, magnesidin, manumycin, melanomycin, methoxycarbonylmethylmonate, methoxycarbonylethylmonate, methoxycarbonylphenylmonate, methyl pseudomonate, methylmonate, microcin, mitomalcin, mocimycin, moenomycin, monoacetyl cladosporin, monomethyl cladosporin, mupirocin, mupirocin calcium, mycobacidin, myriocin, myxopyronin, pseudoaglycone, nanaomycin, nancimycin, nargenicin, neocarcinostatin, neoenactin, neothramycin, nifurtoinol, nocardicin, nogalamycin, novobiocin, octylmonate, olivomycin, orthosomycin, oudemansin, oxirapentyn, oxoglaucine methiodide, pactacin, pactamycin, papulacandin, paulomycin, phaeoramularia fungicide, phenelfamycin, phenyl, cerulenin, phenylmonate, pholipomycin, pirlimycin, pleuromutilin, a polylactone derivative, polynitroxin, polyoxin, porfiromycin, pradimicin, prenomycin, prop-2-enylmonate, protomycin,

Pseudomonas antibiotic, pseudomonic acid, purpuromycin, pyrinodemin, pyrroInitrin, pyrrolomycin, amino, chloro pentenedioic acid, rapamycin, rebeccamycin, resistomycin, reuterin, reveromycin, rhizocticin, roridin, rubiflavin, naphthyridinomycin, saframycin, saphenamycin, sarkomycin, sarkomycin, sclopularin, selenomycin, siccanin, spartanamicin, spectinomycin, spongistatin, stravidin, streptolydigin, *Streptomyces arenae* antibiotic complex, streptonigrin, streptothricins, streptovitacin, streptozotocine, a strobilurin derivative, stubomycin, sulfamethoxazol-trimethoprim, sakamycin, tejeramycin, terpentecin, tetrocarcin, thermorubin, thermozymocidin, thiamphenicol, thioaurin, thiolutin, thiomarinol, thiomarinol, tirandamycin, tolytoxin, trichodermin, trienomycin, trimethoprim, trioxacarcin, tyrissamycin, umbrinomycin, unphenelfamycin, urauchimycin, usnic acid, uredolysin, variotin, vermisporin, verrucarin and analogs, salts and derivatives thereof.

In one or more embodiments, the antibiotic agent is a naturally occurring antibiotic compound. As used herein, the term "naturally-occurring antibiotic agent" includes all antibiotics that are obtained, derived or extracted from plant or vertebrate sources. Non-limiting examples of families of naturally-occurring antibiotic agents include phenol, resorcinol, antibiotic aminoglycosides, anamycin, quinines, anthraquinones, antibiotic glycopeptides, azoles, macrolides, avilamycin, agropyrene, cnicin, aucubin antibioticsaponin fractions, berberine (isoquinoline alkaloid), arctiopicrin (sesquiterpene lactone), lupulone, humulone (bitter acids), allicin, hyperforin, echinacoside, coniosetin, tetramic acid, imanine and novoimanine.

Ciclopirox and ciclopiroxolamine possess fungicidal, fungistatic and sporicidal activity. They are active against a broad spectrum of dermatophytes, yeasts, moulds and other fungi, such as *Trichophytons* species, *Microsporum* species, *Epidermophyton* species and yeasts (*Candida albicans*, *Candida glabrata*, other candida species and *Cryptococcus neoformans*). Some *Aspergillus* species are sensitive to ciclopirox as are some *Penicillium*. Likewise, ciclopirox is effective against many Gram-positive and Gram-negative bacteria (e.g., *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus* and *Streptococcus* species), as well as *Mycoplasma* species, *Trichomonas vaginalis* and *Actinomyces*.

Plant oils and extracts which contain antibiotic agents are also useful. Non-limiting examples of plants that contain agents include thyme, *Perilla*, lavender, tea tree, *Terfezia clayeryi*, *Micromonospora*, *Putterlickia verrucosa*, *Putterlickia pyracantha*, *Putterlickia retrospinosa*, *Maytenus ilicifolia*, *Maytenus evonymoides*, *Maytenus aquifolia*, *Faenia interjecta*, *Cordyceps sinensis*, couchgrass, holy thistle, plantain, burdock, hops, echinacea, buchu,

chaparral, myrrh, red clover and yellow dock, garlic, and St. John's wort.Mixtures of the antibiotic agents as described herein may also be employed.

Combination Detection:

Any combination of the analytes disclosed herein can be detected using any of the methods described herein. In particular, any combination disclosed herein can be detected using any of the methods described herein.

A "photosensitizer" as used herein refers to a sensitizer for generation of singlet oxygen usually by excitation with light. Exemplary photosensitizers suitable for use include those described in U.S. Patent Nos. 6,251,581, 5,516,636, 8,907,081, 6,545,012, 6,331,530, 8,247,180, 5,763,602, 5,705,622, 5,516,636, 7,217,531, and U.S. Patent Publication No. 2007/0059316, all of which are herein expressly incorporated by reference in their entireties. The photosensitizer can be photoactivatable (*e.g.*, dyes and aromatic compounds) or chemiactivated (*e.g.*, enzymes and metal salts). When excited by light the photosensitizer is usually a compound comprised of covalently bonded atoms, usually with multiple conjugated double or triple bonds. The compound should absorb light in the wavelength range of 200-1100 nm, usually 300-1000 nm, *e.g.*, 450-950 nm, with an extinction coefficient at its absorbance maximum greater than 500 $M^{-1}cm^{-1}$, *e.g.*, at least 5000 $M^{-1}cm^{-1}$, or at least 50,000 $M^{-1}cm^{-1}$ at the excitation wavelength. The lifetime of an excited state produced following absorption of light in the absence of oxygen will usually be at least 100 nsec, *e.g.*, at least 1 μsec. In general, the lifetime must be sufficiently long to permit energy transfer to oxygen, which will normally be present at concentrations in the range of 10^{-5} to 10^{31} ^{3}M depending on the medium. The sensitizer excited state will usually have a different spin quantum number (S) than its ground state and will usually be a triplet (S=l) when, as is usually the case, the ground state is a singlet (S=O). In some embodiments, the sensitizer will have a high intersystem crossing yield. That is, photoexcitation of a sensitizer will produce the long lived state (usually triplet) with an efficiency of at least 10%, at least 40%, *e.g.*, greater than 80%. The photosensitizer will usually be at most weakly fluorescent under the assay conditions (quantum yield usually less that 0.5, or less that 0.1).

Photosensitizers that are to be excited by light will be relatively photostable and will not react efficiently with singlet oxygen. Several structural features are present in most useful sensitizers. Most sensitizers have at least one and frequently three or more conjugated double or triple bonds held in a rigid, frequently aromatic structure. They will frequently contain at least one group that accelerates intersystem crossing such as a carbonyl or imine group or a heavy

atom selected from rows 3-6 of the periodic table, especially iodine or bromine, or they may have extended aromatic structures. Typical sensitizers include acetone, benzophenone, 9-thioxanthone, eosin, 9,10-dibromoanthracene, methylene blue, metallo-porphyrins, such as hematoporphyrin, phthalocyanines, chlorophylls, rose bengal, buckminsterfullerene, etc., and derivatives of these compounds having substituents of 1 to 50 atoms for rendering such compounds more lipophilic or more hydrophilic and/or as attaching groups for attachment. Examples of other photosensitizers that may be utilized are those that have the above properties and are enumerated in N. J. Turro, "Molecular Photochemistry," page 132, W. A. Benjamin Inc., N.Y. 1965.

In some embodiments, the photosensitizers are relatively non-polar to assure dissolution into a lipophilic member when the photosensitizer is incorporated in an oil droplet, liposome, latex particle, etc.

In some embodiments, the photosensitizers suitable for use herein include other substances and compositions that can produce singlet oxygen with or without activation by an external light source. Thus, for example, molybdate ($MoO_4^{=}$) salts and chloroperoxidase and myeloperoxidase plus bromide or chloride ion (Kanofsky, *J. Biol. Chem.* (1983) 259 5596) have been shown to catalyze the conversion of hydrogen peroxide to singlet oxygen and water. Either of these compositions can, for example, be included in particles and used in the assay method wherein hydrogen peroxide is included as an ancillary reagebly, chloroperoxidase is bound to a surface and molybdate is incorporated in the aqueous phase of a liposome. Also included within the scope of the invention as photosensitizers are compounds that are not true sensitizers but which on excitation by heat, light, or chemical activation will release a molecule of singlet oxygen. The best known members of this class of compounds includes the endoperoxides such as 1,4-biscarboxyethyl-1,4-naphthalene endoperoxide, 9,10-diphenylanthracene-9,10-endoperoxide and 5,6,11,12-tetraphenyl naphthalene 5,12-endoperoxide. Heating or direct absorption of light by these compounds releases singlet oxygen.

A "chemiluminescent compound" as used herein refers to a substance that undergoes a chemical reaction with singlet oxygen to form a metastable intermediate that can decompose with the simultaneous or subsequent emission of light within the wavelength range of 250 to 1200 nm. Exemplary chemiluminescent compounds suitable for use include those described in U.S. Patent Nos. 6,251,581 and 7,709,273, and Patent Cooperatio Treaty (PCT) International Application Publication No. WO1999/042838. Examplery chemiluminescent compound includes the following:

Chemiluminescer	Half-Life	Emission Max
Thioxene + Diphenyl anthracence:	0.6 seconds	430 nm
Thioxene + Umbelliferone derivative	0.6 seconds	500 nm
Thioxene + Europium chelate	0.6 seconds	615 nm
Thioxene + Samarium Chelate	0.6 seconds	648 nm
Thioxene + terbium Chelate	0.6 seconds	540nm
N-Phenyl Oxazine + Umbelliferone derivative	30 seconds	500 nm
N-Phenyl Oxazine + Europium chelate	30 seconds	613nm
N-phenyl Oxazine + Samarium Chelate	30 seconds	648 nm
N-phenyl Oxazine + terbium Chelate	30 seconds	540nm
Dioxene + Umbelliferone derivative	300 seconds	500 nm
Dioxene + Europium chelate	300 seconds	613nm
Dioxene + Samarium Chelate	300 seconds	648 nm
N-phenyl Oxazine + terbium Chelate	300 seconds	540nm

All of the above mentioned applications are herey expressly incorporated by reference herein in their entireties. Emission will usually occur without the presence of an energy acceptor or catalyst to cause decomposition and light emission. In some embodiments, the intermediate decomposes spontaneously without heating or addition of ancillary reagents following its formation. However, addition of a reagent after formation of the intermediate or the use of elevated temperature to accelerate decomposition will be required for some chemiluminescent compounds. The chemiluminescent compounds are usually electron rich compounds that react with singlet oxygen, frequently with formation of dioxetanes or dioxetanones. Exemplary of such compounds are enol ethers, enamines, 9-alkylidenexanthans, 9-alkylidene-N-alkylacridans, aryl vinyl ethers, dioxenes, arylimidazoles and lucigenin. Other chemiluminescent compounds give intermediates upon reaction with singlet oxygen, which subsequently react with another reagent with light emission. Exemplary compounds are hydrazides such as luminol and oxalate esters.

The chemiluminescent compounds of interest will generally emit at wavelengths above 300 nanometers and usually above 400 nm. Compounds that alone or together with a fluorescent molecule emit light at wavelengths beyond the region where serum components absorb light will be of particular use. The fluorescence of serum drops off rapidly above 500 nm and becomes relatively unimportant above 550 nm. Therefore, when the analyte is in serum, chemiluminescent compounds that emit light above 550 nm, *e.g.*, above 600 nm may be suitable

for use. In order to avoid autosensitization of the chemiluminescent compound, in some embodiments, the chemiluminescent compounds do not absorb light used to excite the photosensitizer. In some embodiments, the sensitizer is excited with light wavelengths longer than 500 nm, it will therefore be desirable that light absorption by the chemiluminescent compound be very low above 500 nm.

Where long wave length emission from the chemiluminescent compound is desired, a long wavelength emitter such as a pyrene, bound to the chemiluminescent compound can be used. Alternatively, a fluorescent molecule can be included in the medium containing the chemiluminescent compound. In some embodiments, fluorescent molecules will be excited by the activated chemiluminescent compound and emit at a wavelength longer than the emission wavelength of the chemiluminescent compound, usually greater that 550 nm. It is usually also desirable that the fluorescent molecules do not absorb at the wavelengths of light used to activate the photosensitizer. Examples of useful dyes include rhodamine, ethidium, dansyl, $Eu(fod)_3$, $Eu(TTA)_3$, $Ru(bpy)_3^{++}$ (wherein bpy=2,2′-dipyridyl, etc. In general, these dyes act as acceptors in energy transfer processes and in some embodiments, have high fluorescent quantum yields and do not react rapidly with singlet oxygen. They can be incorporated into particles simultaneously with the incorporation of the chemiluminescent compound into the particles.

In some embodiments, the disclosure provides diffractive optics detection technology that can be used with, for example, ingestible device technology. In certain embodiments, an ingestible device includes the diffractive optics technology (e.g., diffractive optics detection system). In certain embodiments, the disclosure provides diffractive optics technology (e.g., diffractive optics detection systems) that are used outside the body of subject. As an example, an ingestible device can be used to obtain one more samples in the body (e.g., in the gastrointestinal tract) of a subject, and the diffractive optics technology can be used to analyze the sample(s). Such analysis can be performed *in vivo* (e.g., when the ingestible device contains the diffractive optics).

Diffraction is a phenomenon that occurs due to the wave nature of light. When light hits an edge or passes through a small aperture, it is scattered in different directions. But light waves can interfere to add (constructively) and subtract (destructively) from each other, so that if light hits a non-random pattern of obstacles, the subsequent constructive and destructive interference will result in a clear and distinct diffraction pattern. A specific example is that of a diffraction grating, which is of uniformly spaced lines, typically prepared by ruling straight, parallel grooves on a surface. Light incident on such a surface produces a pattern of evenly spaced spots of high

light intensity. This is called Bragg scattering, and the distance between spots (or 'Bragg scattering peaks') is a unique function of the diffraction pattern and the wavelength of the light source. Diffraction gratings, like focusing optics, can be operated in both transmission and reflection modes.

In general, the light used in the diffractive optics can be of any appropriate wavelength. Exemplary wavelengths include visible light, infrared red (IR) and ultraviolet (UV). Optionally, the light can be monochromatic or polychromatic. The light can be coherent or incoherent. The light can be collimated or non-collimated. In some embodiments, the light is coherent and collimated. Generally, any appropriate light source may be used, such as, for example, a laser (e.g., a laser diode) or a light emitting diode. In some embodiments, the light source is a laser diode operating at 670 nm wavelength, e.g., at 3 mWatts power. Optionally, an operating wavelength of a laser diode can be 780 nm, e.g., when larger grating periods are used. In certain embodiments, the light source is a laser, such as, for example, a He-Ne laser, a Nd:YVO4 laser, or an argon-ion laser. In some embodiments, the light source is a low power, continuous waver laser.

The diffracted light can be detected using any appropriate light detector(s). Examples of light detectors include photodetectors, such as, for example, position sensitive photodiodes, photomultiplier tubes (PMTs), photodiodes (PDs), avalanche photodiodes (APDs), charged-coupled device (CCD) arrays, and CMOS detectors. In some embodiments, the diffracted light is detected via one or more individual photodiodes.

In general, the diffraction grating is made of a material that is transparent in the wavelength of the radiation used to illuminate the sensor. Any appropriate material may be used for the diffraction grating substrate, such as glass or a polymer. Exemplary polymers include polystyrene polymers (PSEs), cyclo-olefin polymers (COPs), polycarbonate polymers, polymethyl methacrylates, and methyl methacrylate styrene copolymers. Exemplary COPs include Zeonex (e.g., Zeonex E48R, Zeonex F52R).

The light may be incident on the diffraction grating any appropriate angle. In some embodiments, the light is incident on the diffraction grating with an angle of incidence of from 30° to 80° (e.g., from 40° to 80°, from 50° to 70°, from 55° to 65°, 60°). Optionally, the system is configured so that that diffractive grating and light source can move relative to each other

In general, the light detector can be positioned with respect to the diffractive grating so that the diffraction grating can be illuminated at a desired angle of incidence and/or so that

diffracted light can be detected at a desired angle and/or so that diffracted light of a desired order can be detected.

The period P of the diffraction grating can be selected as desired. In some embodiments, the period P is from 0.5 microns to 50 microns (e.g., from one micron to 15 microns, from one micron to five microns). In some embodiments, the grating is a repeating patter of 1.5 micron and 4.5 micron lines with a period of 15 microns.

The height h of the diffraction grating can be selected as desired. In certain embodiments, the height h is from one nanometer to about 1000 nanometers (e.g., from about five nanometers to about 250 nanometers, from five nanometers to 100 nanometers).

In general, the diffractive optics can be prepared using any appropriate method, such as, for example, surface ablation, photolithograph (e.g., UV photolithography), laser etching, electron beam etching, nano-imprint molding, or microcontact printing.

Optionally, the diffractive optics system can include one or more additional optical elements, such as, for example, one or more mirrors, filters and/or lenses. Such optical elements can, for example, be arranged between the light source and the diffractive grating and/or between the diffractive grating and the detector.

In some of the embodiments of the devices described herein, a primary binding partner specifically binds to a secondary binding partner through non-covalent interactions (e.g., electrostatic, van der Waals, hydrophobic effect). In some embodiments, a primary binding partner specifically binds to a secondary binding partner via a covalent bond (e.g., a polar covalent bond or a non-polar covalent bond). In some embodiments of any of the devices described herein, the primary and the secondary binding partner can be interchanged. For example, the primary binding partner can be biotin, or a derivative thereof, and the secondary binding partner is avidin, or a derivative thereof. In other examples, the primary binding partner can be avidin, or a derivative thereof, and the secondary binding partner is biotin.

In some embodiments, the binding of the primary and the secondary binding partner is essentially irreversible. In some embodiments, the binding of the primary and the secondary binding partner is reversible. In some embodiments, the primary binding partner is CaptAvidin™ biotin-binding protein and the secondary binding partner is biotin, or vice versa. In some embodiments, the primary binding partner is DSB-X™ biotin and the secondary binding partner is avidin, or vice versa. In some embodiments, the primary binding partner is desthiobiotin and the secondary binding partner is avidin, or vice versa (Hirsch et al., *Anal Biochem.* 308(2):343-357, 2002). In some embodiments, the primary binding partner is

glutathione (GSH) or a derivative thereof, and the secondary binding partner is glutathione-S-transferase (GST).

In some embodiments, the primary binding partner can bind to a target analyte that is a nucleic acid (e.g., a DNA molecule, a RNA molecule). In some embodiments, the primary binding partner comprises a portion of a nucleic acid that is complementary to the nucleic acid sequence of the target analyte.

In some embodiments of any of the devices described herein, the device can include a label that binds to the target analyte and does not prevent binding of the target analyte to the primary binding partner. In some embodiments, the label can amplify the diffraction signal of the target analyte.

In some embodiments, the label is from about 1 nm to 200 nm (e.g., about 50 nm to about 200 nm).

In some embodiments, the label (e.g., any of the labels described herein) includes one or more antibodies (e.g., any of the antibodies and/or antibody fragments described herein).

In some embodiments, the label is a nanoparticle (e.g., a gold nanoparticle) that includes the primary binding partner that has a nucleic acid sequence that is complementary to the target analyte, and is covalently linked to the nanoparticle.

One or more additional steps can be performed in any of the methods described herein. In some embodiments, the one or more additional steps are performed: prior to the binding of the primary binding partner to the secondary binding partner, after the binding of the primary binding partner to the secondary binding partner, prior to the binding of the primary binding partner to the target analyte, or after the binding of the primary binding partner to the target analyte.

In some embodiments of any of the methods described herein, the determining step (during which the primary binding partner binds to the target analyte is detected) can occur in at least 15 seconds. In some embodiments, the binding of the primary binding partner to the target analyte can occur during a period of time of, for example, five at least seconds.

In some embodiments, the one or more additional steps can include: a blocking of the sensors step, at least one wash step, a capturing step, and/or a filtering step. In some embodiments, the blocking step can include blocking a sensor within the ingestible device with a solution comprising at least 1% bovine serum albumin (BSA) in a buffered solution (e.g., phosphate buffered saline (PBS), Tris buffered saline (TBS)). In some embodiments, the at least one wash step can include washing with a buffered solution (e.g., phosphate buffered saline

(PBS), Tris buffered saline (TBS)). In general, blocking is performed during capsule manufacture, rather than in vivo.

In some embodiments, the capturing step includes enriching the target analyte. In some embodiments, the capturing step includes physically separating the target analyte from the remaining sample using a filter, a pore, or a magnetic bead. In some embodiments, the target analyte is captured by size exclusion.

In some embodiments, the disclosure provides methods of obtaining, culturing, and/or detecting target cells and/or target analytes *in vivo* within the gastrointestinal (GI) tract or reproductive tract of a subject. Associated devices are also disclosed. The methods and devices described provide a number of advantages for obtaining and/or analyzing fluid samples from a subject. In some embodiments, diluting the fluid sample increases the dynamic range of analyte detection and/or reduces background signals or interference within the sample. For example, interference may be caused by the presence of non-target analytes or non-specific binding of a dye or label within the sample. In some embodiments, culturing the sample increases the concentration of target cells and/or target analytes produced by the target cells thereby facilitating their detection and/or characterization.

In certain embodiments, the methods and devices a described herein may be used to obtain information regarding bacteria populations in the GI tract of a subject. This has a number of advantages and is less invasive than surgical procedures such as intubation or endoscopy to obtain fluid samples from the GI tract. The use of an ingestible device as described herein also allows for fluid samples to be obtained and data to be generated on bacterial populations from specific regions of the GI tract.

In some embodiments, the methods and devices described herein may be used to generate data such as by analyzing the fluid sample, dilutions thereof or cultured samples for one or more target cells and/or target analytes. The data may include, but is not limited to, the types of bacteria present in the fluid sample or the concentration of bacteria in specific regions of the GI tract. Such data may be used to determine whether a subject has an infection, such as Small Intestinal Bacterial Overgrowth (SIBO), or to characterize bacterial populations within the GI tract for diagnostic or other purposes. Thus, in some embodiments, analytes disclosed herein are indicative of disorders of the gastrointestinal tract associated with anomalous bacterial populations.

For example, in one aspect, the data may include, but is not limited to, the concentration of bacteria in a specific region of the GI tract that is one or more of the duodenum, jejunum,

ileum, ascending colon, transverse colon or descending colon. . In one aspect, the specific region of the GI tract is the duodenum. In one aspect, the specific region of the GI tract is the jejunum. In one aspect, the specific region of the GI tract is the ileum. In one aspect, the specific region of the GI tract is the ascending colon. In one aspect, the specific region of the GI tract is the transverse colon. In one aspect, the specific region of the GI tract is the descending colon. In a related embodiment, the data may be generated every one or more days to monitor disease flare-ups, or response to the therapeutic agents disclosed herein.

Data may be generated after the device has exited the subject, or the data may be generated *in vivo* and stored on the device and recovered *ex vivo*. Alternatively, the data can be transmitted wirelessly from the device while the device is passing through the GI tract of the subject or in place within the reproductive tract of the subject.

In some embodiments, a method comprises: providing a device comprising one or more dilution chambers and dilution fluid; transferring all or part of a fluid sample obtained from the GI tract or reproductive tract of the subject into the one or more dilution chambers *in vivo*; and combining the fluid sample and the dilution fluid to produce one or more diluted samples in the one or more dilution chambers.

In certain embodiments, a method comprises: providing an ingestible device comprising one or more dilution chambers; transferring all or part of a fluid sample obtained from the GI tract into the one or more dilution chambers comprising sterile media; culturing the sample in vivo within the one or more dilution chambers to produce one or more cultured samples; and detecting bacteria in the one or more cultured samples.

In some embodiments, a method comprises: providing a device comprising one or more dilution chambers; transferring all or part of a fluid sample obtained from the GI tract or reproductive tract into the one or more dilution chambers; combining all or part of the fluid sample with a dilution fluid in the one or more dilution chambers; and detecting the target analyte in the one or more diluted samples.

In certain embodiments, a device comprises: one or more dilution chambers for diluting a fluid sample obtained from the GI tract or reproductive tract; and dilution fluid for diluting the sample within the one or more dilution chambers.

In some embodiments, the device comprises: one or more dilution chambers for culturing a fluid sample obtained from the GI tract; sterile media for culturing the sample within the one or more dilution chambers; and a detection system for detecting bacteria.

In certain embodiments, a device comprises: one or more dilution chambers for culturing a fluid sample obtained from the GI tract; sterile media for culturing the sample within the one or more dilution chambers; and a detection system for detecting bacteria.

Also provided is the use of a device as described herein for diluting one or more samples obtained from the GI tract or reproductive tract of a subject. In one embodiment, there is provided the use of an ingestible device as described herein for detecting target cells and/or target analytes *in vivo* within the gastrointestinal (GI) tract of a subject.

Further provided is a system comprising a device as described herein and a base station. In one embodiment, the device transmits data to the base station, such as data indicative of the concentration and/or types of bacteria in the GI tract of the subject. In one embodiment, the device receives operating parameters from the base station. Some embodiments described herein provide an ingestible device for obtaining one or more samples from the GI tract or reproductive tract of a subject and diluting and/or culturing all or part of the one or more samples. The ingestible device includes a cylindrical rotatable element having a port on the wall of the cylindrical rotatable element. The ingestible device further includes a shell element wrapping around the cylindrical rotatable element to form a first dilution chamber between the cylindrical rotatable element and the shell element. The shell element has an aperture that exposes a portion of the wall of the cylindrical rotatable element to an exterior of the ingestible device.

In certain embodiments, the medical device comprises one or more dilution chambers for receiving a fluid sample from the GI tract or reproductive tract of a subject or a dilution thereof. In some embodiments, one or more dilutions of the fluid sample are cultured in one or more dilution chambers. In certain embodiments, the dilution chambers each define a known volume, optionally the same volume or different volumes. In some embodiments, the dilution chambers define a fluid volume ranging from about 10 μL to about 1 mL. The dilution chambers may define a fluid volume less than or equal to about 500 μL, less than or equal to about 250 μL, less than or equal to about 100 μL, or less than or equal to about 50 μL. In certain embodiments, the dilution chambers define a fluid volume of greater than or equal to about 10 μL, greater than or equal to about 20 μL, greater than or equal to about 30 μL, or greater than or equal to about 50 μL. In some embodiments, the dilution chambers define a fluid volume between about 10 μL and 500 μL, between about 20 μL and 250 μL, between about 30 μL and 100 μL or about 50 μL.

In some embodiments, dilution fluid in the device is combined with all or part of the fluid sample, or dilution thereof, to produce one or more dilutions. In certain embodiments, the

dilution fluid is sterile media suitable for culturing one or more target cells within the dilution chambers.

In certain embodiments, the one or more dilution chambers may be filled with the dilution fluid prior to a patient ingesting the ingestible device. In some embodiments, the dilution fluid may be added into the one or more dilution chambers *in vivo* from a reservoir of the ingestible device. Sampling and dilution of the GI fluid sample may take place *in vivo.* For example, an actuator of the ingestible device may pump the dilution fluid from the reservoir into a dilution chamber when it is determined that the ingestible device is located at a predetermined location within the GI tract. In some embodiments, the dilution chambers each contain a volume of sterile media suitable for culturing a fluid sample from the GI tract or reproductive tract. In certain embodiments, the dilution chambers are at least 95%, at least 97%, at least 98%, or at least 99% full of sterile media. In some embodiments, the dilution chambers each contain oxygen to facilitate aerobic bacteria growth. In certain embodiments, a non-dilution chamber comprises oxygen and is added to one or more of the dilution chambers to facilitate aerobic bacteria growth.

In some embodiments, the culturing may take place *in vivo* immediately after the GI fluid sample has been diluted. Or alternatively, the culturing may take place *ex vivo,* e.g., when the ingestible device has been evacuated and recovered such that the dilution chamber containing the diluted GI fluid sample may be extracted and the culturing may be performed in a laboratory. The recovery of the ingestible device may be performed in a similar manner as embodiments described in U.S. Provisional Application No. 62/434,188, filed on December 14, 2016, which is herein expressly incorporated by reference in its entirety.

As used herein "culturing" refers to maintaining target cells in an environment that allows a population of one or more target cells to increase in number through cell division. For example, in some embodiments, "culturing" may include combining the cells with media in an dilution chamber at a temperature that permits cell growth, optionally a temperature found *in vivo* within the GI tract or reproductive tract of a subject. In certain embodiments, the cells are cultured at a temperature between about 35 °C and 42 °C.

As used herein "dilution fluid" refers to a fluid within the device for diluting a fluid sample from the GI tract or reproductive tract. In some embodiments, the dilution fluid is an aqueous solution. In certain embodiments, the dilution fluid comprises one or more agents that promote or inhibit the growth of an organism, such as a fungus or bacteria. In some embodiments, the dilution fluid comprises one or more agents that facilitate the detection of a target analyte, such as dyes or binding agents for target analytes.

In some embodiments, the dilution fluid is a sterile media. As used herein, "sterile media" refers to media that does not contain any viable bacteria or other cells that would grow and increase in number through cell division. Media may be rendered sterile by various techniques known in the art such as, but not limited to, autoclaving and/or preparing the media using aseptic techniques. In certain embodiments, the media is a liquid media. Examples of media suitable for culturing bacteria include nutrient broth, Lysogeny Broth (LB) (also known as Luria Broth), Wilkins chalgren, and Tryptic Soy Broth (TSB), Other growth or culture media known in the art may also be used in the methods and devices described herein. In some embodiments, the media has a carbon source, such as glucose or glycerol, a nitrogen source such as ammonium salts or nitrates or amino acids, as well as salts and/or trace elements and vitamins required for microbial growth. In certain embodiments, the media is suitable for maintaining eukaryotic cells. In some embodiments, the media comprises one or more agents that promote or inhibit the growth of bacteria, optionally agents that promote or inhibit the growth of specific types of bacteria.

In certain embodiments, the media is a selective media. As used herein, "selective media" refers to a media that allows certain types of target cells to grow and inhibits the growth of other organisms. Accordingly, the growth of cells in a selective media indicates the presence of certain types of cells within the cultured sample. For example, in some embodiments, the media is selective for gram-positive or gram-negative bacteria. In certain embodiments, the media contains crystal violet and bile salts (such as found in MacConkey agar) that inhibit the growth of gram-positive organisms and allows for the selection and isolation of gram-negative bacteria. In some embodiments, the media contains a high concentration of salt (NaCl) (such as found in Mannitol salt agar) and is selective for Gram-positive bacteria. In some embodiments, the media selectively kills eukaryotic cells or only grows prokaryotic cells, for example, using a media comprising Triton™ X-100. In certain embodiments, the media selectively kills prokaryotic cells (or alternatively only grows eukaryotic cells), for example, using a media that comprises antibiotics.

In some embodiments, the media is an indicator media. As used herein, "indicator media" refers to a media that contains specific nutrients or indicators (such as, but not limited to neutral red, phenol red, eosin y, or methylene blue) that produce a detectable signal when a certain type of cell is cultured in the indicator media.

In some embodiments, the disclosure provides a composition comprising a dye and optionally a reagent for selective lysis of eukaryotic cells. In certain embodiments, the

composition comprises both a dye and a reagent for selective lysis of eukaryotic cells. In some embodiments, the composition further comprises one or more reagents independently selected from the group consisting of: a second reagent for selective lysis of eukaryotic cells (*e.g.*, Triton X-100), an electrolyte (*e.g.*, $MgCl_2$), an anti-fungi reagent (*e.g.*, amphotericin-B), and an antibiotic. In some embodiments, the composition comprises water and is in the form of an aqueous solution. In some embodiments, the composition is a solid or semi-solid. In some embodiments, the compositions described here are suitable for use in a kit or device for detecting or quantifying viable bacterial cells in a sample. In some embodiments, such a device is an ingestible device for detecting or quantifying viable bacterial cells *in vivo* (*e.g.*, in the GI tract). In some embodiments, viable bacterial cells in a sample are detected or quantified in the presence of one or more antibiotics to determine antibiotic resistance of the bacteria in the sample. In some embodiments, anomalous bacterial populations in a sample may be detected or quantified, for example through the use of one a composition comprising a dye as disclosed herein, to determine whether a subject has an infection, such as Small Intestinal Bacterial Overgrowth (SIBO), or to characterize bacterial populations within the GI tract for diagnostic or other purposes.

In some embodiments, a method comprises: (a) contacting the sample with a composition as described herein; and (b) measuring total fluorescence or rate of change of fluorescence as a function of time of said sample, thereby detecting viable bacterial cells in said sample. In some embodiments, a control as described herein may be employed in the method. In some embodiments, the total fluorescence or the rate of change of fluorescence as a function of time of the sample is measured over multiple time points for an extended period of time in step (b), thereby detecting viable bacterial cells in said sample. In some embodiments, the method further comprises correlating the total fluorescence or the rate of change of fluorescence as a function of time determined in step (b) to the number of viable bacterial cells in the sample. In some embodiments, the rate of change of fluorescence as a function of time of the sample measured over multiple time points is determined and compared to the rate of change of fluorescence as a function of time of a control measured over the same time points to determine the number of viable bacterial cells in the sample. In some embodiments, the method does not require *ex vivo* plating or culturing. In some embodiments, the method does not require aspiration. In some embodiments, the method is performed *in vivo* (*e.g.*, in an ingestible device *in vivo*). In some embodiments, the method comprises communicating the results of the onboard assay(s) to an *ex vivo* receiver.

In certain embodiments, a kit comprises a composition as described herein and instructions, *e.g.*, for detecting or quantifying viable bacterial cells in a sample. In some embodiments, a device comprises a composition as described herein, *e.g.*, for detecting or quantifying viable bacterial cells in a sample. The detection of live cells, as opposed to the detection of bacterial components (such as endotoxins) which can be present in the sample environment and lead to conflicting results, is the gold standard of viable plate counting and represents one of the advantages of the compositions and methods described herein.

The systems employ methods, compositions and detection systems found to accurately and reliably correlate fluorescence to total bacteria count (TBC) in an autonomous, ingestible device, or other similarly-sized device. The compositions include novel combinations of dyes, buffers and detergents that allow for the selective staining of viable bacterial cells in samples that comprise non-bacterial cells and other components that otherwise make detecting or quantifying live bacterial cells challenging. In some embodiments, the systems allow for bacteria to be quantified in near real-time and the results to be shared telemetrically outside of the device.

In certain embodiments, the disclosure provides a method of assessing or monitoring the need to treat a subject suffering from or at risk of overgrowth of bacterial cells in the gastrointestinal tract, which comprises: (a) obtaining a sample from the gastrointestinal tract of said subject; (b) contacting the sample with a composition as described herein; (c) measuring total fluorescence or rate of change of fluorescence as a function of time of said sample; and (d) correlating the total fluorescence or the rate of change of fluorescence as a function of time measured in step (c) to the number of viable bacterial cells in the sample, wherein the number of the viable bacterial cells determined in step (e) greater than about 105 CFU/mL indicates a need for treatment, *e.g.*, with an antibiotic agent as described herein. In some embodiments, a control as described herein may be employed in the method. In some embodiments, the total fluorescence or the rate of change of fluorescence as a function of time of the sample is measured over multiple time points for an extended period of time in step (c). In some embodiments, the rate of change of fluorescence as a function of time of the sample measured over multiple time points is determined and compared to the rate of change of fluorescence as a function of time of a control measured over the same time points to determine the number of viable bacterial cells in the sample. In some embodiments, the method does not require ex vivo plating or culturing. In some embodiments, the method does not require aspiration. In some embodiments, the method is performed *in vivo* (*e.g.*, in an ingestible device *in vivo*). In some embodiments, the method comprises communicating the results of the onboard assay(s) to an *ex vivo* receiver. In some

embodiments, the method may be further used to monitor the subject after the treatment (*e.g.*, with an antibiotic). In some embodiments, the method may be used to assess the efficacy of the treatment. For example, efficacious treatment may be indicated by the decrease of the number of viable bacterial cells in a sample from the GI tract of the subject post-treatment. Efficacy of the treatment may be evaluated by the rate of decrease of the number of viable bacterial cells in a sample from the GI tract of the subject post-treatment. In some embodiments, the method may be used to detect infection with antibiotic-resistant strains of bacteria in a subject. For instance, such infection may be indicated where the number of viable bacterial cells in a sample from the GI tract of the subject does not substantially decrease after antibiotic treatment.

In some embodiments, the disclosure provides an absorbable material, (*e.g.*, absorbable sponge), having absorbed therein a composition as described herein. In some embodiments, the absorbable sponge is Ahlstrom Grade 6613H (Lot 150191) or Porex PSU-567, having absorbed therein a composition as described herein. In some embodiments, the absorbable sponge may be prepared by injecting into the absorbable sponge an aqueous solution comprising a composition as described herein, and optionally further comprising a step of drying the resulting absorbable sponge.

In certain embodiments, the disclosure provides a method for detecting the presence of viable bacterial cells in a sample, which comprises: (a) fully or partially saturating an absorbable sponge as described herein, or an absorbable sponge prepared as described herein, with the sample; and (b) measuring total fluorescence or rate of change of fluorescence as a function of time of the fully or partially saturated sponge prepared in step (a), thereby detecting viable bacterial cells. In some embodiments, a control as described herein may be employed in the method. In some embodiments, the total fluorescence or the rate of change of fluorescence as a function of time of the fully or partially saturated sponge is measured over multiple time points for an extended period of time in step (b), thereby detecting viable bacterial cells in said sample. In some embodiments, the method further comprises correlating the total fluorescence or the rate of change of fluorescence as a function of time measured in step (b) to the number of viable bacterial cells in the sample. In some embodiments, the rate of change of fluorescence as a function of time of the fully or partially saturated sponge measured over multiple time points is determined and compared to the rate of change of fluorescence as a function of time of a control measured over the same time points to determine the number of viable bacterial cells in the sample. In some embodiments, the method does not require *ex vivo* plating or culturing. In some embodiments, the method does not require aspiration. In some embodiments, the method is

performed *in vivo* (*e.g.*, in an ingestible device *in vivo*). In some embodiments, the method comprises communicating the results of the onboard assay(s) to an *ex vivo* receiver.

In one aspect, provided herein is a kit comprising an absorbable sponge as described herein and instructions, *e.g.*, for detecting or quantifying viable bacterial cells in a sample. In another aspect, provided herein is a device comprising an absorbable sponge as described herein, *e.g.*, for detecting or quantifying viable bacterial cells in a sample.

In certain embodiments, the disclosure provides a method of assessing or monitoring the need to treat a subject suffering from or at risk of overgrowth of bacterial cells in the gastrointestinal tract, which comprises: (a) obtaining a sample from the gastrointestinal tract of said subject; (b) fully or partially saturating an absorbable sponge described herein, or an absorbable sponge prepared as described herein, with the sample; (c) measuring total fluorescence or rate of change of fluorescence as a function of time of the fully or partially saturated sponge prepared in step (b); (d) correlating the total fluorescence or the rate of change of fluorescence as a function of time measured in step (c) to the number of viable bacterial cells in the sample, wherein the number of the viable bacterial cells as determined in step (e) greater than about 10^5 CFU/mL indicates a need for treatment, *e.g.*, with an antibiotic agent as described herein. In some embodiments, a control as described herein may be employed in the method. In some embodiments, the total fluorescence or the rate of change of fluorescence as a function of time of the fully or partially saturated sponge is measured over multiple time points for an extended period of time in step (c). In some embodiments, the rate of change of fluorescence as a function of time of the fully or partially saturated sponge measured over multiple time points is determined and compared to the rate of change of fluorescence as a function of time of a control measured over the same time points to determine the number of viable bacterial cells in the sample. In some embodiments, the method does not require *ex vivo* plating or culturing. In some embodiments, the method does not require aspiration. In some embodiments, the method is performed *in vivo* (*e.g.*, in an ingestible device *in vivo*). In some embodiments, the method comprises communicating the results of the onboard assay(s) to an *ex vivo* receiver. In some embodiments, the method may be further used to monitor the subject after the treatment (*e.g.*, with an antibiotic). In some embodiments, the method may be used to assess the efficacy of the treatment. For example, efficacious treatment may be indicated by the decrease of the number of viable bacterial cells in a sample from the GI tract of the subject post-treatment. Efficacy of the treatment may be evaluated by the rate of decrease of the number of viable bacterial cells in a sample from the GI tract of the subject post-treatment. In some embodiments, the method may

be used to detect infection with antibiotic-resistant strains of bacteria in a subject. For instance, such infection may be indicated where the number of viable bacterial cells in a sample from the GI tract of the subject does not substantially decrease after antibiotic treatment

In certain embodiments, the disclosure provides and ingestible device comprising a housing; a first opening in the wall of the housing; a second opening in the first end of the housing; and a chamber connecting the first opening and the second opening, wherein at least a portion of the chamber forms a sampling chamber within the ingestible device. In some embodiments, the sampling chamber is configured to hold an absorbable sponge described herein. In some embodiments, the sampling chamber is configured to hold a sample obtained from a gastrointestinal (GI) tract of a body. In some embodiments, the ingestible device is individually calibrated (for example, by comparing to a positive or negative control as described herein), wherein the fluorescent properties of the absorbable sponge held in the sampling chamber of the device are determined prior to the introduction of the sample. The ingestible device as described herein is useful for detecting or quantifying viable bacterial cells *in vivo*. In some embodiments, provided herein is a method for detecting or quantifying viable bacterial cells in a GI tract sample *in vivo* using an ingestible device as described herein. In some embodiments, provided herein is a method of assessing or monitoring the need to treat a subject suffering from or at risk of overgrowth of bacterial cells in the GI tract *in vivo* using an ingestible device as described herein. In some embodiments, provided herein is a method of altering the treatment regimen of a subject suffering from or at risk of overgrowth of bacterial cells in the GI tract *in vivo* using an ingestible device as described herein. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the duodenum. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the jejunum. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the ileum. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the ascending colon. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the transverse colon. In one aspect, the subject is a subject suffering from or at risk of overgrowth of bacterial cells in the descending colon. In some embodiments, the method may be further used to monitor the subject after the treatment (*e.g.*, with an antibiotic). In some embodiments, the method may be used to assess the efficacy of the treatment. For example, efficacious treatment may be indicated by the decrease of the number of viable bacterial cells in a sample from the GI tract of the subject post-treatment. Efficacy of the treatment may be evaluated by the rate of decrease of the number of viable bacterial cells in a

sample from the GI tract of the subject post-treatment. In some embodiments, the method may be used to detect infection with antibiotic-resistant strains of bacteria in a subject. For instance, such infection may be indicated where the number of viable bacterial cells in a sample from the GI tract of the subject does not substantially decrease after antibiotic treatment. In some embodiments, the method is performed autonomously and does not require instructions, triggers or other inputs from outside the body after the device has been ingested.

"Eukaryotic" as recited herein relates to any type of eukaryotic organism excluding fungi, such as animals, in particular animals containing blood, and comprises invertebrate animals such as crustaceans and vertebrates. Vertebrates comprise both cold-blooded (fish, reptiles, amphibians) and warm blooded animal (birds and mammals). Mammals comprise in particular primates and more particularly humans

"Selective lysis" as used herein is obtained in a sample when the percentage of bacterial cells in that sample that remain intact is significantly higher (*e.g.* 2, 5, 10, 20, 50, 100, 250, 500, or 1,000 times more) than the percentage of the eukaryotic cells in that sample that remain intact, upon treatment of or contact with a composition or device as described herein.

In some embodiments, the dye suitable for use herein is a dye that is capable of being internalized by a viable cell, binding to or reacting with a target component of the viable cell, and having fluorescence properties that are measurably altered when the dye is bound to or reacted with the target component of the viable cell. In some embodiments, the dye herein is actively internalized by penetrating viable cells through a process other than passible diffusion across cell membranes. Such internalization includes, but is not limited to, internalization through cell receptors on cell surfaces or through channels in cell membranes. In some embodiments, the target component of a viable cell to which the dye is bound to or reacted with is selected from the group consisting of: nucleic acids, actin, tubulin, enzymes, nucleotide-binding proteins, ion-transport proteins, mitochondria, cytoplasmic components, and membrane components. In some embodiments, the dye suitable for use herein is a fluorogenic dye that is capable of being internalized and metabolized by a viable cell, and wherein said dye fluoresces when metabolized by the viable cell. In some embodiments, the dye is a chemiluminescent dye that is capable of being internalized and metabolized by a viable cell, and wherein said dye becomes chemiluminescent when metabolized by the viable cell.

In some embodiments, the composition comprises a dye that fluoresces when bond to nucleic acids. Examples of such dyes include, but are not limited to, acridine orange (U.S. Pat. No. 4,190,328); calcein-AM (U.S. Pat. No. 5,314,805); DAPI; Hoechst 33342; Hoechst 33258;

PicoGreen™; SYTO® 16; SYBR® Green I; Texas Red®; Redmond Red™; Bodipy® Dyes; Oregon Green™; ethidium bromide; and propidium iodide.

In some embodiments, the composition comprises a lipophilic dye that fluoresces when metabolized by a cell. In some embodiments, the dye fluoresces when reduced by a cell or a cell component. Examples of dyes that fluoresce when reduced include, but are not limited to, resazurin; C^{12}-resazurin; 7-hydroxy-9H-(1,3 dichloro-9,9-dimethylacridin-2-ol) N-oxide; 6-chloro-9-nitro-5-oxo-5H-benzo[a]phenoxazine; and tetrazolium salts. In some embodiment, the dye fluoresces when oxidized by a cell or a cell component. Examples of such dyes include, but are not limited to, dihydrocalcein AM; dihydrorhodamine 123; dihydroethidium; 2,3,4,5,6-pentafluorotetramethyldihydrorosamine; and 3′-(p-aminophenyl) fluorescein.

In some embodiments, the composition comprises a dye that becomes chemiluminescent when oxidized by a cell or a cell component, such as luminol.

In some embodiments, the composition comprises a dye that fluoresces when de-acetylated and/or oxidized by a cell or a cell component. Examples of such dyes include, but are not limited to, dihydrorhodamines; dihydrofluoresceins; 2',7'-dichlorodihydrofluorescein diacetate; 5-(and 6-)carboxy-2',7'-dichlorodihydrofluorescein diacetate; and chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester.

In some embodiments, the composition comprises a dye that fluoresces when reacted with a peptidase. Examples of such dyes include, but are not limited to, (CBZ-Ala-Ala-Ala-Ala)2-R110 elastase 2; (CBZ-Ala-Ala-Asp)2-R110 granzyme B; and 7-amino-4-methylcoumarin, N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide.

In some embodiments, the composition comprises a dye selected from the group consisting of resazurin, FDA, Calcein AM, and SYTO® 9. In some embodiments, the dye is FDA or SYTO® 9.

SYTO® 9, when used alone, labels the nucleic acid of bacteria cells. The excitation/emission wavelengths for SYTO® 9 is 480/500 nm, with the background remaining non-fluorescent. *See, e.g.*, J. Appl. Bacteriol. 72, 410 (1992); Lett. Appl. Microbiol. 13, 58 (1991); Curr. Microbiol. 4, 321 (1980); J. Microbiol. Methods 13, 87 (1991); and Microbiol. Rev. 51, 365 (1987); and J. Med. Microbiol. 39, 147 (1993).

FDA is a non-polar, non-fluorescent compound that can cross the membranes of mammalian and bacterial cells. The acetyl esterases (present only within viable cells) hydrolyze the FDA into the fluorescent compound fluorescein. Fluorescein is a fluorescent polar compound that is retained within these cells. Living cells can be visualized in a photospectrometer when

assayed with an excitation wavelength of 494 nm and an emission wavelength of 518 nm. *See, e.g.*, Brunius, G. (1980). *Technical aspects of the use of 3', 6' – Diacetyl fluorescein for vital fluorescent staining of bacteria*. Current Microbiol. 4: 321-323; Jones, K. H. and Senft, J. A. (1985). *An improved method to determine cellviability by simultaneous staining with fluorescein diacetate - propidium iodide*. J. Histochem. Cytochem. 33: 77-79; Ross, R. D. , Joneckis, C. C., Ordonez, J. V., Sisk, A. M., Wu, R. K., Hamburger, A. W., and Nora, R. E. (1989). *Estimation of cell survival by flow cytometric quantification of fluorescein diacetate/propidium iodide viable cell number*. Cancer Research. 49: 3776 - 3782.

Calcein-AM, which is an acetoxylmethyl ester of calcein, is highly lipophilic and cell permeable. Calcein-AM in itself is not fluorescent, but the calcein generated by esterase in a viable cell emits a green fluorescence with an excitation wavelength of 490 nm and an emission of 515 nm. Therefore, Calcein-AM can only stain viable cells. *See, e.g.*, Kimura, K., et al., *Neurosci. Lett.*, 208, 53 (1998); Shimokawa, I., et al., *J. Geronto.*, 51a, b49 (1998); Yoshida, S., et al., *Clin. Nephrol.*, 49, 273 (1998); and Tominaga, H., et al., *Anal. Commun.*, 36, 47 (1999).

Resazuirn (also known as Alamar Blue) is a blue compound that can be reduced to pink resorufin which is fluorescent. This dye is mainly used in viability assays for mammalian cells. C^{12} –resazurin has better cell permeability than resazurin. When lipohilic C^{12} –resazurin crosses the cell membranes, it is subsequently reduced by living cells to make a red fluorescent resorufin. The adsorption/emission of C^{12} –resazurin is 563/587 nm. *See, e.g.*, Appl Environ Microbiol 56, 3785 (1990); J Dairy Res 57, 239 (1990); J Neurosci Methods 70, 195 (1996); J Immunol Methods 210, 25 (1997); J Immunol Methods 213, 157 (1998); Antimicrob Agents Chemother 41, 1004 (1997).

In some embodiments, the composition optionally further comprises a reagent for selective lysis of eukaryotic cells. In some embodiments, the composition comprises a dye as described herein and a reagent for selective lysis of eukaryotic cells. In some embodiments, the reagent for selective lysis of eukaryotic cells is a detergent, such as a non-ionic or an ionic detergent. Examples of the reagent for selective lysis of eukaryotic cells include, but are not limited to, alkylglycosides, Brij 35 (C12E23 Polyoxyethyleneglycol dodecyl ether), Brij 58 (C16E20 Polyoxyethyleneglycol dodecyl ether), Genapol, glucanids such as MEGA-8, -9, -10, octylglucoside, Pluronic F127, Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$), Triton X-114 ($C_{24}H_{42}O_6$), Tween 20 (Polysorbate 20) and Tween 80 (Polysorbate 80), Nonidet P40, deoxycholate, reduced Triton X-100 and/or Igepal CA 630. In some embodiments, the composition comprises a dye as described herein and deoxycholate (*e.g.*, sodium deoxycholate) as a reagent for selective lysis of

eukaryotic cells. In some embodiments, the composition comprises deoxycholate at a concentration selected from 0.0001% to 1 wt%. In some embodiments, the composition comprises deoxycholate at a concentration of 0.005 wt%. In some embodiments, the composition may comprise more than one reagent for selective lysis of eukaryotic cells.

In some embodiments, the composition may comprise two different reagents for selective lysis of eukaryotic cells. In some instances, when more than one selective lysis reagents are used, more effective and/or complete selective lysis of eukaryotic cells in a sample may be achieved. For example, the composition may comprise deoxycholate (*e.g.*, sodium deoxycholate) and Triton X-100 as two different reagents for selective lysis of eukaryotic cells. In some embodiments, the composition comprises deoxycholate (*e.g.*, sodium deoxycholate) at a concentration selected from 0.0001% to 1 wt% (*e.g.*, 0.005 wt%) and Triton X-100 at a concentration selected from 0.1 to 0.05 wt%.

In some embodiments, after a sample (*e.g.*, a biological sample) is treated or contacted with a composition comprising a dye and one or more reagents for selective lysis of eukaryotic cells as described herein, the eukaryotic cells (*e.g.*, animal cells) in the sample are selectively lysed whereby a substantial percentage (*e.g.*, more than 20%, 40%, 60%, 80%, 90% or even more that 95%) of the bacterial cells in the same sample remains intact or alive.

In some embodiments, the composition does not comprise a reagent for selective lysis of eukaryotic cells, and such a composition is useful for detecting or quantifying viable bacterial cells in a sample (*e.g.*, an environmental sample such as a water sample) that does not contain any eukaryotic cells.

In some embodiments, the composition further comprises an electrolyte, such as a divalent electrolyte (*e.g.*, $MgCl_2$). In some embodiments, the composition comprises $MgCl_2$ at a concentration selected from 0.1 mM to 100 mM (*e.g.*, a concentration selected from 0.5 mM to 50 mM).

In some embodiments, the composition further comprises water and is in a form of an aqueous solution. In some embodiments, the composition has a pH selected from 5-8 (*e.g.*, a pH selected from 6-7.8, such as pH being 6.0). In some embodiments, the composition is a solid or a semi-solid.

In some embodiments, the composition further comprises an anti-fungal agent. Suitable anti-fungal agents for use herein include, but are not limited to, fungicidal and fungistatic agents including terbinafine, itraconazole, micronazole nitrate, thiapendazole, tolnaftate, clotrimazole

and griseofulvin. In some embodiments, the anti-fungal agent is a polyene anti-fungal agent, such as amphotericin-B, nystatin, and pimaricin.

In some embodiments, the composition does not contain any anti-fungal agent. In some embodiments, the composition contains broad spectrum antibiotics but not any anti-fungal agent. Such compositions that do not contain anti-fungal agents but contain broad spectrum antibiotics may be useful in detecting or quantifying fungi (*e.g.*, yeast) in a sample.

In some embodiments, the composition does not contain any anti-fungal agent, any antibiotics or any anti-mammalian agent. Such compositions that do not selectively lyse mammalian cells may be useful in detecting or quantifying mammalian cells (e.g., cells from the GI tract) in a sample since many dyes have a higher affinity for mammalian as compared to bacteria or fungi cells. In some embodiments, the composition contains broad spectrum antibiotics and one or more anti-fungal agents. Such compositions that contain anti-fungal agents and broad spectrum antibiotics may be useful in detecting or quantifying mammalian cells (e.g., cells from the GI tract) in a sample. The detection or quantification of mammalian cells may be useful for determining cell turnover in a subject. High cell turnover is sometimes associated with a GI injury (e.g., lesion), the presence of a tumor(s), or radiation-induced colitis or radiation enteropathy.

In some embodiments, the composition further comprises an antibiotic agent as described herein. Such a composition may be useful in detecting or quantifying antibiotic-resistant strains of bacteria in a sample.

In certain embodiments, the composition comprises Triton X-100, deoxycholate, resazurin, and $MgCl_2$. In some embodiments, the composition comprises Triton X-100, deoxycholate, resazurin, amphotericin-B and $MgCl_2$. In some embodiments, the composition comprises 0.1 wt% or 0.05 wt% Triton X-100; 0.005 wt% deoxycholate; 10 mM resazurin; 2.5 mg/L amphotericin-B and 50 mM $MgCl_2$. In some embodiments, the composition has a pH of 6.0.

In certain embodiments, the compositions are suitable for use in a kit or device, *e.g.*, for detecting or quantifying viable bacterial cells in a sample. In some embodiments, such a device is an ingestible device for detecting or quantifying viable bacterial cells *in vivo* (*e.g.*, in the GI tract).

FIG. 62 illustrates a nonlimiting example of a system for collecting, communicating and/or analyzing data about a subject, using an ingestible device as disclosed herein. For example, an ingestible device may be configured to communicate with an external base station.

As an example, an ingestible device can have a communications unit that communicates with an external base station which itself has a communications unit. FIG. 62 illustrates exemplary implementation of such an ingestible device. As shown in FIG. 62, a subject ingests an ingestible device as disclosed herein. Certain data about the subject (e.g., based on a collected sample) and/or the location of the ingestible device in the GI tract of the subject is collected or otherwise available and provided to a mobile device, which then forwards the data via the internet and a server/data store to a physician's office computer. The information collected by the ingestible device is communicated to a receiver, such as, for example, a watch or other object worn by the subject. The information is then communicated from the receiver to the mobile device which then forwards the data via the internet and a server/data store to a physician's office computer. The physician is then able to analyze some or all of the data about the subject to provide recommendations, such as, for example, delivery a therapeutic agent. While FIG. 62 shows a particular approach to collecting and transferring data about a subject, the disclosure is not limited. As an example, one or more of the receiver, mobile device, internet, and/or server/data store can be excluded from the data communication channel. For example, a mobile device can be used as the receiver of the device data, e.g., by using a dongle. In such embodiments, the item worn by the subject need not be part of the communication chain. As another example, one or more of the items in the data communication channel can be replaced with an alternative item. For example, rather than be provided to a physician's office computer, data may be provided to a service provider network, such as a hospital network, an HMO network, or the like. In some embodiments, subject data may be collected and/or stored in one location (e.g., a server/data store) while device data may be collected and/or stored in a different location (e.g., a different server/data store).

Locations of treatment

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the large intestine of the subject. In some embodiments, the location is in the proximal portion of the large intestine. In some embodiments, the location is in the distal portion of the large intestine.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ascending colon of the subject. In some embodiments, the location is in the proximal portion of the ascending colon. In some embodiments, the location is in the distal portion of the ascending colon.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the cecum of the subject. In some embodiments, the location is in the proximal portion of the cecum. In some embodiments, the location is in the distal portion of the cecum.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the sigmoid colon of the subject. In some embodiments, the location is in the proximal portion of the sigmoid colon. In some embodiments, the location is in the distal portion of the sigmoid colon.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the transverse colon of the subject. In some embodiments, the location is in the proximal portion of the transverse colon. In some embodiments, the location is in the distal portion of the transverse colon.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the descending colon of the subject. In some embodiments, the location is in the proximal portion of the descending colon. In some embodiments, the location is in the distal portion of the descending colon.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the small intestine of the subject. In some embodiments, the location is in the proximal portion of the small intestine. In some embodiments, the location is in the distal portion of the small intestine.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the duodenum of the subject. In some embodiments, the location is in the proximal portion of the duodenum. In some embodiments, the location is in the distal portion of the duodenum.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the jejunum of the subject. In some embodiments, the location is in the proximal portion of the jejunum. In some embodiments, the location is in the distal portion of the jejunum.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the duodenum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the duodenum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the duodenum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the duodenum of the subject and

is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the duodenum and a second site of disease is in the stomach and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal duodenum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal duodenum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the duodenum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal duodenum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the duodenum and a second site of disease is in the stomach and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the jejunum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the jejunum and a second site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the jejunum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the jejunum and a second site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the jejunum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the jejunum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the jejunum and a second site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ileum of the subject. In some embodiments, the location is in the proximal portion of the ileum. In some embodiments, the location is in the distal portion of the ileum.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ileum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and/or ascending colon, and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the ileum

of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the proximal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and/or ascending colon, and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the ileum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the ileum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the ileum and a second site of disease is in the cecum and/or ascending colon, and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the cecum of the subject and is not delivered at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the cecum of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a site of disease is in the cecum and/or ascending colon, and no site of disease is present at other locations in the gastrointestinal tract. In some embodiments, the live biotherapeutic, such as a stem cell, is delivered at a location in the distal portion of the ileum or the proximal portion of the ascending colon of the subject and is not delivered at other locations in the gastrointestinal tract, wherein a first site of disease is in the cecum and a second site of disease is in the ascending colon, and no site of disease is present at other locations in the gastrointestinal tract.

In some embodiments, a site of disease is in the colon and the live biotherapeutic such as a stem cell is released in the colon, such as in the cecum. In some embodiments, a site of disease is in the ascending colon and the live biotherapeutic such as a stem cell is released in the ascending colon, such as in the cecum. In some embodiments, a site of disease is in the ileum and the live biotherapeutic such as a stem cell is released in the ileum.

In some embodiments the subject is diagnosed with ileal Crohn's disease and the live biotherapeutic such as a stem cell is released in the ileum.

In some embodiments the subject is diagnosed with ileal colonic Crohn's disease and the live biotherapeutic such as a stem cell is released in both the ileum and the colon. In some more particular embodiments, the live biotherapeutic such as a stem cell is released in both the ileum and the colon from the same ingestble device. In some more particular embodiments, the live biotherapeutic such as a stem cell is released in the ileum from a first ingestble device and in the colon from a second ingestible device, wherein the first ingestble device and the second ingestible device are ingested at substantially the same time or at different times.

In some embodiments the subject is diagnosed with colitis throughout the colon and the live biotherapeutic such as a stem cell is released (a) in the cecum, (b) in the cecum and in the transverse colon, and/or release (c) in the descending colon.

In some embodiments the subject is diagnosed with right sided colitis and the live biotherapeutic such as a stem cell is released in the transverse colon or in the descending colon.

In some embodiments the subject is diagnosed with rectosigmoidal colitis and the live biotherapeutic such as a stem cell is released in the descending colon.

In some embodiments, the location at which the live biotherapeutic such as a stem cell is delivered is proximate to a site of disease. The site of disease may be, for example, an injury, inflamed tissue, or one or more lesions. In some embodiments, the location at which the live biotherapeutic such as a stem cell is delivered is proximate to one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 150 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 125 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 100 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 50 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 40 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 30 cm or less from the one

or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 20 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 10 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 5 cm or less from the one or more sites of disease. In some embodiments, the live biotherapeutic such as a stem cell is delivered 2 cm or less from the one or more sites of disease. In some embodiments, the method further comprises using an ingestible device to deliver the live biotherapeutic such as a stem cell and using localization methods disclosed herein (e.g., such as discussed in Example 14 below) to determine the location of the ingestible device within the GI tract (e.g., relative to the site of disease). In some embodiments, the method further comprises using an ingestible device to deliver the live biotherapeutic such as a stem cell and determining the period of time since the ingestible device was ingested to determine the location of the ingestible device within the GI tract (e.g., relative to the site of disease). In some embodiments, the method further comprises identifying the one or more sites of disease by a method comprising imaging of the gastrointestinal tract. In some embodiments, imaging of the gastrointestinal tract comprises video imaging. In some embodiments, imaging of the gastrointestinal tract comprises thermal imaging. In some embodiments, imaging of the gastrointestinal tract comprises ultrasound imaging. In some embodiments, imaging of the gastrointestinal tract comprises Doppler imaging.

In some embodiments the method does not comprise releasing more than 20 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease. In some embodiments the method does not comprise releasing more than 10 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease. In some embodiments the method does not comprise releasing more than 5 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease. In some embodiments the method does not comprise releasing more than 4 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease. In some embodiments the method does not comprise releasing more than 3 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease. In some embodiments the method does not comprise releasing more than 2 % of the live biotherapeutic, such as a stem cell, at a location that is not proximate to a site of disease.

In some embodiments the method comprises releasing at least 80% of the live biotherapeutic such as a stem cell at a location proximate to a site of disease. In some embodiments the method comprises releasing at least 90 % of the live biotherapeutic such as a

stem cell at a location proximate to a site of disease. In some embodiments the method comprises releasing at least 95 % of the live biotherapeutic such as a stem cell at a location proximate to a site of disease. In some embodiments the method comprises releasing at least 96% of the live biotherapeutic such as a stem cell at a location proximate to a site of disease. In some embodiments the method comprises releasing at least 97 % of the live biotherapeutic such as a stem cell at a location proximate to a site of disease. In some embodiments the method comprises releasing at least 98% of the live biotherapeutic such as a stem cell at a location proximate to a site of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 150 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 125 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 100 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 50 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 40 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 30 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 20 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 10 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 5 cm or less from the one or more sites of disease. In some embodiments, the at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, or at least 98% of the live biotherapeutic such as a stem cell is delivered 2 cm or less from the one or more sites of disease. In some embodiments, the method further comprises using an ingestible device to deliver the live biotherapeutic such as a stem cell and using localization methods disclosed herein (e.g., such as discussed in Example 14 below) to determine

the location of the ingestible device within the GI tract (e.g., relative to the site of disease). In some embodiments, the method further comprises using an ingestible device to deliver the live biotherapeutic such as a stem cell and determining the period of time since the ingestible device was ingested to determine the location of the ingestible device within the GI tract (e.g., relative to the site of disease).

In some embodiments, the amount of live biotherapeutic such as a stem cell that is delivered is a Human Equivalent Dose.

In some embodiments the method comprises releasing the live biotherapeutic, such as a stem cell, at a location that is proximate to a site of disease, wherein the live biotherapeutic, such as a stem cell, and, if applicable, any carriers, excipients or stabilizers admixed with the live biotherapeutic, such as a stem cell, are substantially unchanged, at the time of release of the live biotherapeutic at the location, relatively to the time of administration of the composition to the subject.

In some embodiments the method comprises releasing the live biotherapeutic, such as a stem cell, at a location that is proximate to a site of disease, wherein the live biotherapeutic, such as a stem cell, and, if applicable, any carriers, excipients or stabilizers admixed with the live biotherapeutic, are substantially unchanged by any physiological process (such as, but not limited to, degradation in the stomach), at the time of release of the live biotherapeutic, such as a stem cell, at the location, relatively to the time of administration of the composition to the subject.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered to the location by mucosal contact.

In some embodiments, a method of treatment disclosed herein includes determining the level of live biotherapeutic, such as a stem cell, at a site of disease or a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease. In some examples, a method of treatment as described herein can include determining the level of live biotherapeutic, such as a stem cell, at a site of disease or a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease within a time period of about 10 minutes to about 10 hours following administration of the device.

In some examples, a method of treatment disclosed herein includes determining the level of the live biotherapeutic, such as a stem cell, at a site of disease or a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease at a time point following administration of the device that is elevated as compared to a level of the stem cell at

the same site of disease or location at substantially the same time point in a subject following systemic administration of an equal amount of the stem cell.

As used herein, "GI tissue" refers to tissue in the gastrointestinal (GI) tract, such as tissue in one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum. In one particular embodiment, GI tissue refers to tissue in the proximal portion of one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, and sigmoid colon. In one particular embodiment, GI tissue refers to tissue in the distal portion of one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, and sigmoid colon. The GI tissue may be, for example, GI tissue proximate to one or more sites of disease. Accordingly, in some embodiments the live biotherapeuticcan penetrate the dudodenum tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the jejunum tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the ileum tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the cecum tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the ascending colon tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the transverse colon tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the descending colon tissue proximate to one or more sites of disease. In some embodiments the live biotherapeuticcan penetrate the sigmoid colon tissue proximate to one or more sites of disease. For example, a live biotherapeutic can penetrate one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa.

In some examples, administration of an live biotherapeutic using any of the compositions or devices described herein results in penetration (e.g., a detectable level of penetration) of GI tissue (e.g., one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa) within a time period of about 10 minutes to about 10 hours, about 10 minutes to about 9 hours, about 10 minutes to about 8 hours, about 10 minutes to about 7 hours, about 10 minutes to about 6 hours, about 10 minutes to about 5 hours, about 10 minutes to about 4.5 hours, about 10 minutes to about 4 hours, about 10 minutes to about 3.5 hours, about 10 minutes to about 3 hours, about 10 minutes to about 2.5 hours, about 10 minutes to about 2 hours, about 10 minutes to about 1.5 hours, about 10 minutes to about 1 hour, about 10 minutes to about 55 minutes, about 10 minutes to about 50 minutes,

about 10 minutes to about 45 minutes, about 10 minutes to about 40 minutes, about 10 minutes to about 35 minutes, about 10 minutes to about 30 minutes, about 10 minutes to about 25 minutes, about 10 minutes to about 20 minutes, about 10 minutes to about 15 minutes, about 15 minutes to about 10 hours, about 15 minutes to about 9 hours, about 15 minutes to about 8 hours, about 15 minutes to about 7 hours, about 15 minutes to about 6 hours, about 15 minutes to about 5 hours, about 15 minutes to about 4.5 hours, about 15 minutes to about 4 hours, about 15 minutes to about 3.5 hours, about 15 minutes to about 3 hours, about 15 minutes to about 2.5 hours, about 15 minutes to about 2 hours, about 15 minutes to about 1.5 hours, about 15 minutes to about 1 hour, about 15 minutes to about 55 minutes, about 15 minutes to about 50 minutes, about 15 minutes to about 45 minutes, about 15 minutes to about 40 minutes, about 15 minutes to about 35 minutes, about 15 minutes to about 30 minutes, about 15 minutes to about 25 minutes, about 15 minutes to about 20 minutes, about 20 minutes to about 10 hours, about 20 minutes to about 9 hours, about 20 minutes to about 8 hours, about 20 minutes to about 7 hours, about 20 minutes to about 6 hours, about 20 minutes to about 5 hours, about 20 minutes to about 4.5 hours, about 20 minutes to about 4 hours, about 20 minutes to about 3.5 hours, about 20 minutes to about 3 hours, about 20 minutes to about 2.5 hours, about 20 minutes to about 2 hours, about 20 minutes to about 1.5 hours, about 20 minutes to about 1 hour, about 20 minutes to about 55 minutes, about 20 minutes to about 50 minutes, about 20 minutes to about 45 minutes, about 20 minutes to about 40 minutes, about 20 minutes to about 35 minutes, about 20 minutes to about 30 minutes, about 20 minutes to about 25 minutes, about 25 minutes to about 10 hours, about 25 minutes to about 9 hours, about 25 minutes to about 8 hours, about 25 minutes to about 7 hours, about 25 minutes to about 6 hours, about 25 minutes to about 5 hours, about 25 minutes to about 4.5 hours, about 25 minutes to about 4 hours, about 25 minutes to about 3.5 hours, about 25 minutes to about 3 hours, about 25 minutes to about 2.5 hours, about 25 minutes to about 2 hours, about 25 minutes to about 1.5 hours, about 25 minutes to about 1 hour, about 25 minutes to about 55 minutes, about 25 minutes to about 50 minutes, about 25 minutes to about 45 minutes, about 25 minutes to about 40 minutes, about 25 minutes to about 35 minutes, about 25 minutes to about 30 minutes, about 30 minutes to about 10 hours, about 30 minutes to about 9 hours, about 30 minutes to about 8 hours, about 30 minutes to about 7 hours, about 30 minutes to about 6 hours, about 30 minutes to about 5 hours, about 30 minutes to about 4.5 hours, about 30 minutes to about 4 hours, about 30 minutes to about 3.5 hours, about 30 minutes to about 3 hours, about 30 minutes to about 2.5 hours, about 30 minutes to about 2 hours, about 30 minutes to about 1.5 hours, about 30 minutes to about 1 hour, about 30 minutes to about 55 minutes, about 30 minutes to about 50 minutes,

about 30 minutes to about 45 minutes, about 30 minutes to about 40 minutes, about 30 minutes to about 35 minutes, about 35 minutes to about 10 hours, about 35 minutes to about 9 hours, about 35 minutes to about 8 hours, about 35 minutes to about 7 hours, about 35 minutes to about 6 hours, about 35 minutes to about 5 hours, about 35 minutes to about 4.5 hours, about 35 minutes to about 4 hours, about 35 minutes to about 3.5 hours, about 35 minutes to about 3 hours, about 35 minutes to about 2.5 hours, about 35 minutes to about 2 hours, about 35 minutes to about 1.5 hours, about 35 minutes to about 1 hour, about 35 minutes to about 55 minutes, about 35 minutes to about 50 minutes, about 35 minutes to about 45 minutes, about 35 minutes to about 40 minutes, about 40 minutes to about 10 hours, about 40 minutes to about 9 hours, about 40 minutes to about 8 hours, about 40 minutes to about 7 hours, about 40 minutes to about 6 hours, about 40 minutes to about 5 hours, about 40 minutes to about 4.5 hours, about 40 minutes to about 4 hours, about 40 minutes to about 3.5 hours, about 40 minutes to about 3 hours, about 40 minutes to about 2.5 hours, about 40 minutes to about 2 hours, about 40 minutes to about 1.5 hours, about 40 minutes to about 1 hour, about 40 minutes to about 55 minutes, about 40 minutes to about 50 minutes, about 40 minutes to about 45 minutes, about 45 minutes to about 10 hours, about 45 minutes to about 9 hours, about 45 minutes to about 8 hours, about 45 minutes to about 7 hours, about 45 minutes to about 6 hours, about 45 minutes to about 5 hours, about 45 minutes to about 4.5 hours, about 45 minutes to about 4 hours, about 45 minutes to about 3.5 hours, about 45 minutes to about 3 hours, about 45 minutes to about 2.5 hours, about 45 minutes to about 2 hours, about 45 minutes to about 1.5 hours, about 45 minutes to about 1 hour, about 45 minutes to about 55 minutes, about 45 minutes to about 50 minutes, about 50 minutes to about 10 hours, about 50 minutes to about 9 hours, about 50 minutes to about 8 hours, about 50 minutes to about 7 hours, about 50 minutes to about 6 hours, about 50 minutes to about 5 hours, about 50 minutes to about 4.5 hours, about 50 minutes to about 4 hours, about 50 minutes to about 3.5 hours, about 50 minutes to about 3 hours, about 50 minutes to about 2.5 hours, about 50 minutes to about 2 hours, about 50 minutes to about 1.5 hours, about 50 minutes to about 1 hour, about 50 minutes to about 55 minutes, about 55 minutes to about 10 hours, about 55 minutes to about 9 hours, about 55 minutes to about 8 hours, about 55 minutes to about 7 hours, about 55 minutes to about 6 hours, about 55 minutes to about 5 hours, about 55 minutes to about 4.5 hours, about 55 minutes to about 4 hours, about 55 minutes to about 3.5 hours, about 55 minutes to about 3 hours, about 55 minutes to about 2.5 hours, about 55 minutes to about 2 hours, about 55 minutes to about 1.5 hours, about 55 minutes to about 1 hour, about 1 hour to about 10 hours, about 1 hour to about 9 hours, about 1 hour to about 8 hours, about 1 hour to about 7 hours, about 1 hour to

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or a luminescent agent (e.g., any suitable radioisotopes, heavy metals, fluorophores, or luminescent agents used for imaging known in the art).

While not wishing to be bound to a particular theory, the inventors contemplate that at or near the site of release a concentration gradient of the live biotherapeutic such as a stem cell is generated in the mucosa, and that administration of a live biotherapeutic such as a stem cell using a device as described herein advantageously results in a "reverse" concentration gradient when compared to the concentration gradient resulting from systemic administration. In such "reverse" concentration gradient, the drug concentration is highest from superficial to deep with respect to the mucosal surface. Systemic administration instead typically results in concentrations of the drug being highest from deep to superficial. A "reverse" concentration gradient as described above aligns more favorably with the pathophysiology of IBD.

In some embodiments, administration of a live biotherapeutic can provide for treatment (e.g., a reduction in the number, severity, and/or duration of one or more symptoms of any of the disorders described herein in a subject) for a time period of between about 1 hour to about 30 days, about 1 hour to about 28 days, about 1 hour to about 26 days, about 1 hour to about 24 days, about 1 hour to about 22 days, about 1 hour to about 20 days, about 1 hour to about 18 days, about 1 hour to about 16 days, about 1 hour to about 14 days, about 1 hour to about 12 days, about 1 hour to about 10 days, about 1 hour to about 8 days, about 1 hour to about 6 days, about 1 hour to about 5 days, about 1 hour to about 4 days, about 1 hour to about 3 days, about 1 hour to about 2 days, about 1 hour to about 1 day, about 1 hour to about 12 hours, about 1 hour to about 6 hours, about 1 hour to about 3 hours, about 3 hours to about 30 days, about 3 hours to about 28 days, about 3 hours to about 26 days, about 3 hours to about 24 days, about 3 hours to about 22 days, about 3 hours to about 20 days, about 3 hours to about 18 days, about 3 hours to about 16 days, about 3 hours to about 14 days, about 3 hours to about 12 days, about 3 hours to about 10 days, about 3 hours to about 8 days, about 3 hours to about 6 days, about 3 hours to about 5 days, about 3 hours to about 4 days, about 3 hours to about 3 days, about 3 hours to about 2 days, about 3 hours to about 1 day, about 3 hours to about 12 hours, about 3 hours to about 6 hours, about 6 hours to about 30 days, about 6 hours to about 28 days, about 6 hours to about 26 days, about 6 hours to about 24 days, about 6 hours to about 22 days, about 6 hours to about 20 days, about 6 hours to about 18 days, about 6 hours to about 16 days, about 6 hours to about 14 days, about 6 hours to about 12 days, about 6 hours to about 10 days, about 6 hours to about 8 days, about 6 hours to about 6 days, about 6 hours to about 5 days, about 6 hours to about 4 days, about 6 hours to about 3 days, about 6 hours to about 2 days, about 6 hours to about 1 day, about

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For example, treatment can result in a decrease (e.g., about 1% to about 99% decrease, about 1% to about 95% decrease, about 1% to about 90% decrease, about 1% to about 85% decrease, about 1% to about 80% decrease, about 1% to about 75% decrease, about 1% to about 70% decrease, about 1% to about 65% decrease, about 1% to about 60% decrease, about 1% to about 55% decrease, about 1% to about 50% decrease, about 1% to about 45% decrease, about 1% to about 40% decrease, about 1% to about 35% decrease, about 1% to about 30% decrease, about 1% to about 25% decrease, about 1% to about 20% decrease, about 1% to about 15%

decrease, about 1% to about 10% decrease, about 1% to about 5% decrease, about 5% to about 99% decrease, about 5% to about 95% decrease, about 5% to about 90% decrease, about 5% to about 85% decrease, about 5% to about 80% decrease, about 5% to about 75% decrease, about 5% to about 70% decrease, about 5% to about 65% decrease, about 5% to about 60% decrease, about 5% to about 55% decrease, about 5% to about 50% decrease, about 5% to about 45% decrease, about 5% to about 40% decrease, about 5% to about 35% decrease, about 5% to about 30% decrease, about 5% to about 25% decrease, about 5% to about 20% decrease, about 5% to about 15% decrease, about 5% to about 10% decrease, about 10% to about 99% decrease, about 10% to about 95% decrease, about 10% to about 90% decrease, about 10% to about 85% decrease, about 10% to about 80% decrease, about 10% to about 75% decrease, about 10% to about 70% decrease, about 10% to about 65% decrease, about 10% to about 60% decrease, about 10% to about 55% decrease, about 10% to about 50% decrease, about 10% to about 45% decrease, about 10% to about 40% decrease, about 10% to about 35% decrease, about 10% to about 30% decrease, about 10% to about 25% decrease, about 10% to about 20% decrease, about 10% to about 15% decrease, about 15% to about 99% decrease, about 15% to about 95% decrease, about 15% to about 90% decrease, about 15% to about 85% decrease, about 15% to about 80% decrease, about 15% to about 75% decrease, about 15% to about 70% decrease, about 15% to about 65% decrease, about 15% to about 60% decrease, about 15% to about 55% decrease, about 15% to about 50% decrease, about 15% to about 45% decrease, about 15% to about 40% decrease, about 15% to about 35% decrease, about 15% to about 30% decrease, about 15% to about 25% decrease, about 15% to about 20% decrease, about 20% to about 99% decrease, about 20% to about 95% decrease, about 20% to about 90% decrease, about 20% to about 85% decrease, about 20% to about 80% decrease, about 20% to about 75% decrease, about 20% to about 70% decrease, about 20% to about 65% decrease, about 20% to about 60% decrease, about 20% to about 55% decrease, about 20% to about 50% decrease, about 20% to about 45% decrease, about 20% to about 40% decrease, about 20% to about 35% decrease, about 20% to about 30% decrease, about 20% to about 25% decrease, about 25% to about 99% decrease, about 25% to about 95% decrease, about 25% to about 90% decrease, about 25% to about 85% decrease, about 25% to about 80% decrease, about 25% to about 75% decrease, about 25% to about 70% decrease, about 25% to about 65% decrease, about 25% to about 60% decrease, about 25% to about 55% decrease, about 25% to about 50% decrease, about 25% to about 45% decrease, about 25% to about 40% decrease, about 25% to about 35% decrease, about 25% to about 30% decrease, about 30% to about 99% decrease, about 30% to about 95%

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decrease, about 75% to about 99% decrease, about 75% to about 95% decrease, about 75% to about 90% decrease, about 75% to about 85% decrease, about 75% to about 80% decrease, about 80% to about 99% decrease, about 80% to about 95% decrease, about 80% to about 90% decrease, about 80% to about 85% decrease, about 85% to about 99% decrease, about 85% to about 95% decrease, about 85% to about 90% decrease, about 90% to about 99% decrease, about 90% to about 95% decrease, or about 95% to about 99% decrease) in one or more (e.g., two, three, four, five, six, seven, eight, or nine) of: the level of interferon-γ in GI tissue, the level of IL-1β in GI tissue, the level of IL-6 in GI tissue, the level of IL-22 in GI tissue, the level of IL-17A in the GI tissue, the level of TNFα in GI tissue, the level of IL-2 in GI tissue, and endoscopy score in a subject (e.g., as compared to the level in the subject prior to treatment or compared to a subject or population of subjects having a similar disease but receiving a placebo or a different treatment) (e.g., for a time period of between about 1 hour to about 30 days (e.g., or any of the subranges herein) following the first administration of an live biotherapeuticusing any of the compositions or devices described herein. Exemplary methods for determining the endoscopy score are described herein and other methods for determining the endoscopy score are known in the art. Exemplary methods for determining the levels of interferon-γ, IL-1β, IL-6, IL-22, IL-17A, TNFα, and IL-2 are described herein. Additional methods for determining the levels of these cytokines are known in the art.

In some examples, treatment can result in an increase (e.g., about 1% to about 500% increase, about 1% to about 400% increase, about 1% to about 300% increase, about 1% to about 200% increase, about 1% to about 150% increase, about 1% to about 100% increase, about 1% to about 90% increase, about 1% to about 80% increase, about 1% to about 70% increase, about 1% to about 60% increase, about 1% to about 50% increase, about 1% to about 40% increase, about 1% to about 30% increase, about 1% to about 20% increase, about 1% to about 10% increase, a 10% to about 500% increase, about 10% to about 400% increase, about 10% to about 300% increase, about 10% to about 200% increase, about 10% to about 150% increase, about 10% to about 100% increase, about 10% to about 90% increase, about 10% to about 80% increase, about 10% to about 70% increase, about 10% to about 60% increase, about 10% to about 50% increase, about 10% to about 40% increase, about 10% to about 30% increase, about 10% to about 20% increase, about 20% to about 500% increase, about 20% to about 400% increase, about 20% to about 300% increase, about 20% to about 200% increase, about 20% to about 150% increase, about 20% to about 100% increase, about 20% to about 90% increase, about 20% to about 80% increase, about 20% to about 70% increase, about 20% to about 60% increase, about 20% to

about 50% increase, about 20% to about 40% increase, about 20% to about 30% increase, about 30% to about 500% increase, about 30% to about 400% increase, about 30% to about 300% increase, about 30% to about 200% increase, about 30% to about 150% increase, about 30% to about 100% increase, about 30% to about 90% increase, about 30% to about 80% increase, about 30% to about 70% increase, about 30% to about 60% increase, about 30% to about 50% increase, about 30% to about 40% increase, about 40% to about 500% increase, about 40% to about 400% increase, about 40% to about 300% increase, about 40% to about 200% increase, about 40% to about 150% increase, about 40% to about 100% increase, about 40% to about 90% increase, about 40% to about 80% increase, about 40% to about 70% increase, about 40% to about 60% increase, about 40% to about 50% increase, about 50% to about 500% increase, about 50% to about 400% increase, about 50% to about 300% increase, about 50% to about 200% increase, about 50% to about 150% increase, about 50% to about 100% increase, about 50% to about 90% increase, about 50% to about 80% increase, about 50% to about 70% increase, about 50% to about 60% increase, about 60% to about 500% increase, about 60% to about 400% increase, about 60% to about 300% increase, about 60% to about 200% increase, about 60% to about 150% increase, about 60% to about 100% increase, about 60% to about 90% increase, about 60% to about 80% increase, about 60% to about 70% increase, about 70% to about 500% increase, about 70% to about 400% increase, about 70% to about 300% increase, about 70% to about 200% increase, about 70% to about 150% increase, about 70% to about 100% increase, about 70% to about 90% increase, about 70% to about 80% increase, about 80% to about 500% increase, about 80% to about 400% increase, about 80% to about 300% increase, about 80% to about 200% increase, about 80% to about 150% increase, about 80% to about 100% increase, about 80% to about 90% increase, about 90% to about 500% increase, about 90% to about 400% increase, about 90% to about 300% increase, about 90% to about 200% increase, about 90% to about 150% increase, about 90% to about 100% increase, about 100% to about 500% increase, about 100% to about 400% increase, about 100% to about 300% increase, about 100% to about 200% increase, about 100% to about 150% increase, about 150% to about 500% increase, about 150% to about 400% increase, about 150% to about 300% increase, about 150% to about 200% increase, about 200% to about 500% increase, about 200% to about 400% increase, about 200% to about 300% increase, about 300% to about 500% increase, about 300% to about 400% increase, or about 400% to about 500% increase) in one or both of stool consistency score and weight of a subject (e.g., as compared to the level in the subject prior to treatment or compared to a subject or population of subjects having a similar disease but receiving a placebo or a different

treatment) (e.g., for a time period of between about 1 hour to about 30 days (e.g., or any of the subranges herein) following the first administration of an live biotherapeutic using any of the compositions or devices described herein. Exemplary methods for determining stool consistency score are described herein. Additional methods for determining a stool consistency score are known in the art.

In some embodiments, administration of a live biotherapeutic using any of the devices or compositions described herein can result in a ratio of GI tissue concentration of the live biotherapeutic to the blood, serum, or plasma concentration of the live biotherapeutic that is higher than the same ratio when the live biotherapeutic is administered by traditional means (e.g., systemically or orally). Examples of a ratio of GI tissue concentration of the live biotherapeutic to the blood, serum, or plasma concentration of the live biotherapeutic include about 2 to about 600, about 2 to about 580, about 2 to about 560, about 2 to about 540, about 2 to about 520, about 2 to about 500, about 2 to about 480, about 2 to about 460, about 4 to about 440, about 2 to about 420, about 2 to about 400, about 2 to about 380, about 2 to about 360, about 2 to about 340, about 2 to about 320, about 2 to about 300, about 2 to about 280, about 2 to about 260, about 2 to about 240, about 2 to about 220, about 2 to about 200, about 2 to about 190, about 2 to about 180, about 2 to about 170, about 2 to about 160, about 2 to about 150, about 2 to about 140, about 2 to about 130, about 2 to about 120, about 2 to about 110, about 2 to about 100, about 2 to about 90, about 2 to about 80, about 2 to about 70, about 2 to about 60, about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, about 2 to about 15, about 2 to about 10, about 2 to about 5, about 5 to about 600, about 5 to about 580, about 5 to about 560, about 5 to about 540, about 5 to about 520, about 5 to about 500, about 5 to about 480, about 5 to about 460, about 5 to about 440, about 5 to about 420, about 5 to about 400, about 5 to about 380, about 5 to about 360, about 5 to about 340, about 5 to about 320, about 5 to about 300, about 5 to about 280, about 5 to about 260, about 5 to about 240, about 5 to about 220, about 5 to about 200, about 5 to about 190, about 5 to about 180, about 5 to about 170, about 5 to about 160, about 5 to about 150, about 5 to about 140, about 5 to about 130, about 5 to about 120, about 5 to about 110, about 5 to about 100, about 5 to about 90, about 5 to about 80, about 5 to about 70, about 5 to about 60, about 5 to about 50, about 5 to about 40, about 5 to about 30, about 5 to about 20, about 5 to about 15, about 5 to about 10, about 10 to about 600, about 10 to about 580, about 10 to about 560, about 10 to about 540, about 10 to about 520, about 10 to about 500, about 10 to about 480, about 10 to about 460, about 10 to about 440, about 10 to about 420, about 10 to about 400, about 10 to about 380, about 10 to about 360, about 10 to about 340, about 10 to about 320, about 10 to about 300, about

10 to about 280, about 10 to about 260, about 10 to about 240, about 10 to about 220, about 10 to about 200, about 10 to about 190, about 10 to about 180, about 10 to about 170, about 10 to about 160, about 10 to about 150, about 10 to about 140, about 10 to about 130, about 10 to about 120, about 10 to about 110, about 10 to about 100, about 10 to about 90, about 10 to about 80, about 10 to about 70, about 10 to about 60, about 10 to about 50, about 10 to about 40, about 10 to about 30, about 10 to about 20, about 10 to about 15, about 15 to about 600, about 15 to about 580, about 15 to about 560, about 15 to about 540, about 15 to about 520, about 15 to about 500, about 15 to about 480, about 15 to about 460, about 15 to about 440, about 15 to about 420, about 15 to about 400, about 15 to about 380, about 15 to about 360, about 15 to about 340, about 15 to about 320, about 15 to about 300, about 15 to about 280, about 15 to about 260, about 15 to about 240, about 15 to about 220, about 15 to about 200, about 15 to about 190, about 15 to about 180, about 15 to about 170, about 15 to about 160, about 15 to about 150, about 15 to about 140, about 15 to about 130, about 15 to about 120, about 15 to about 110, about 15 to about 100, about 15 to about 90, about 15 to about 80, about 15 to about 70, about 15 to about 60, about 15 to about 50, about 15 to about 40, about 15 to about 30, about 15 to about 20, about 20 to about 600, about 20 to about 580, about 20 to about 560, about 20 to about 540, about 20 to about 520, about 20 to about 500, about 20 to about 480, about 20 to about 460, about 20 to about 440, about 20 to about 420, about 20 to about 400, about 20 to about 380, about 20 to about 360, about 20 to about 340, about 20 to about 320, about 20 to about 300, about 20 to about 280, about 20 to about 260, about 20 to about 240, about 20 to about 220, about 20 to about 200, about 20 to about 190, about 20 to about 180, about 20 to about 170, about 20 to about 160, about 20 to about 150, about 20 to about 140, about 20 to about 130, about 20 to about 120, about 20 to about 110, about 20 to about 100, about 20 to about 90, about 20 to about 80, about 20 to about 70, about 20 to about 60, about 20 to about 50, about 20 to about 40, about 20 to about 30, about 30 to about 600, about 30 to about 580, about 30 to about 560, about 30 to about 540, about 30 to about 520, about 30 to about 500, about 30 to about 480, about 30 to about 460, about 30 to about 440, about 30 to about 420, about 30 to about 400, about 30 to about 380, about 30 to about 360, about 30 to about 340, about 30 to about 320, about 30 to about 300, about 30 to about 280, about 30 to about 260, about 30 to about 240, about 30 to about 220, about 30 to about 200, about 30 to about 190, about 30 to about 180, about 30 to about 170, about 30 to about 160, about 30 to about 150, about 30 to about 140, about 30 to about 130, about 30 to about 120, about 30 to about 110, about 30 to about 100, about 30 to about 90, about 30 to about 80, about 30 to about 70, about 30 to about 60, about 30 to about 50, about 30 to about 40, about 40 to about 600, about 40 to about 580, about 40 to about 560, about

40 to about 540, about 40 to about 520, about 40 to about 500, about 40 to about 480, about 40 to about 460, about 40 to about 440, about 40 to about 420, about 40 to about 400, about 40 to about 380, about 40 to about 360, about 40 to about 340, about 40 to about 320, about 40 to about 300, about 40 to about 280, about 40 to about 260, about 40 to about 240, about 40 to about 220, about 40 to about 200, about 40 to about 190, about 40 to about 180, about 40 to about 170, about 40 to about 160, about 40 to about 150, about 40 to about 140, about 40 to about 130, about 40 to about 120, about 40 to about 110, about 40 to about 100, about 40 to about 90, about 40 to about 80, about 40 to about 70, about 40 to about 60, about 40 to about 50, about 50 to about 600, about 50 to about 580, about 50 to about 560, about 50 to about 540, about 50 to about 520, about 50 to about 500, about 50 to about 480, about 50 to about 460, about 50 to about 440, about 50 to about 420, about 50 to about 400, about 50 to about 380, about 50 to about 360, about 50 to about 340, about 50 to about 320, about 50 to about 300, about 50 to about 280, about 50 to about 260, about 50 to about 240, about 50 to about 220, about 50 to about 200, about 50 to about 190, about 50 to about 180, about 50 to about 170, about 50 to about 160, about 50 to about 150, about 50 to about 140, about 50 to about 130, about 50 to about 120, about 50 to about 110, about 50 to about 100, about 50 to about 90, about 50 to about 80, about 50 to about 70, about 50 to about 60, about 60 to about 600, about 60 to about 580, about 60 to about 560, about 60 to about 540, about 60 to about 520, about 60 to about 500, about 60 to about 480, about 60 to about 460, about 60 to about 440, about 60 to about 420, about 60 to about 400, about 60 to about 380, about 60 to about 360, about 60 to about 340, about 60 to about 320, about 60 to about 300, about 60 to about 280, about 60 to about 260, about 60 to about 240, about 60 to about 220, about 60 to about 200, about 60 to about 190, about 60 to about 180, about 60 to about 170, about 60 to about 160, about 60 to about 150, about 60 to about 140, about 60 to about 130, about 60 to about 120, about 60 to about 110, about 60 to about 100, about 60 to about 90, about 60 to about 80, about 60 to about 70, about 70 to about 600, about 70 to about 580, about 70 to about 560, about 70 to about 540, about 70 to about 520, about 70 to about 500, about 70 to about 480, about 70 to about 460, about 70 to about 440, about 70 to about 420, about 70 to about 400, about 70 to about 380, about 70 to about 360, about 70 to about 340, about 70 to about 320, about 70 to about 300, about 70 to about 280, about 70 to about 260, about 70 to about 240, about 70 to about 220, about 70 to about 200, about 70 to about 190, about 70 to about 180, about 70 to about 170, about 70 to about 160, about 70 to about 150, about 70 to about 140, about 70 to about 130, about 70 to about 120, about 70 to about 110, about 70 to about 100, about 70 to about 90, about 70 to about 80, about 80 to about 600, about 80 to about 580, about 80 to about 560, about 80 to about 540, about 80 to about 520, about 80 to

about 500, about 80 to about 480, about 80 to about 460, about 80 to about 440, about 80 to about 420, about 80 to about 400, about 80 to about 380, about 80 to about 360, about 80 to about 340, about 80 to about 320, about 80 to about 300, about 80 to about 280, about 80 to about 260, about 80 to about 240, about 80 to about 220, about 80 to about 200, about 80 to about 190, about 80 to about 180, about 80 to about 170, about 80 to about 160, about 80 to about 150, about 80 to about 140, about 80 to about 130, about 80 to about 120, about 80 to about 110, about 80 to about 100, about 80 to about 90, about 90 to about 600, about 90 to about 580, about 90 to about 560, about 90 to about 540, about 90 to about 520, about 90 to about 500, about 90 to about 480, about 90 to about 460, about 90 to about 440, about 90 to about 420, about 90 to about 400, about 90 to about 380, about 90 to about 360, about 90 to about 340, about 90 to about 320, about 90 to about 300, about 90 to about 280, about 90 to about 260, about 90 to about 240, about 90 to about 220, about 90 to about 200, about 90 to about 190, about 90 to about 180, about 90 to about 170, about 90 to about 160, about 90 to about 150, about 90 to about 140, about 90 to about 130, about 90 to about 120, about 90 to about 110, about 90 to about 100, about 100 to about 600, about 100 to about 580, about 100 to about 560, about 100 to about 540, about 100 to about 520, about 100 to about 500, about 100 to about 480, about 100 to about 460, about 100 to about 440, about 100 to about 420, about 100 to about 400, about 100 to about 380, about 100 to about 360, about 100 to about 340, about 100 to about 320, about 100 to about 300, about 100 to about 280, about 100 to about 260, about 100 to about 240, about 100 to about 220, about 100 to about 200, about 100 to about 190, about 100 to about 180, about 100 to about 170, about 100 to about 160, about 100 to about 150, about 100 to about 140, about 100 to about 130, about 100 to about 120, about 100 to about 110, about 110 to about 600, about 110 to about 580, about 110 to about 560, about 110 to about 540, about 110 to about 520, about 110 to about 500, about 110 to about 480, about 110 to about 460, about 110 to about 440, about 110 to about 420, about 110 to about 400, about 110 to about 380, about 110 to about 360, about 110 to about 340, about 110 to about 320, about 110 to about 300, about 110 to about 280, about 110 to about 260, about 110 to about 240, about 110 to about 220, about 110 to about 200, about 110 to about 190, about 110 to about 180, about 110 to about 170, about 110 to about 160, about 110 to about 150, about 110 to about 140, about 110 to about 130, about 110 to about 120, about 120 to about 600, about 120 to about 580, about 120 to about 560, about 120 to about 540, about 120 to about 520, about 120 to about 500, about 120 to about 480, about 120 to about 460, about 120 to about 440, about 120 to about 420, about 120 to about 400, about 120 to about 380, about 120 to about 360, about 120 to about 340, about 120 to about 320, about 120 to about 300, about 120 to about 280, about 120 to about 260, about 120 to about

240, about 120 to about 220, about 120 to about 200, about 120 to about 190, about 120 to about 180, about 120 to about 170, about 120 to about 160, about 120 to about 150, about 120 to about 140, about 120 to about 130, about 130 to about 600, about 130 to about 580, about 130 to about 560, about 130 to about 540, about 130 to about 520, about 130 to about 500, about 130 to about 480, about 130 to about 460, about 130 to about 440, about 130 to about 420, about 130 to about 400, about 130 to about 380, about 130 to about 360, about 130 to about 340, about 130 to about 320, about 130 to about 300, about 130 to about 280, about 130 to about 260, about 130 to about 240, about 130 to about 220, about 130 to about 200, about 130 to about 190, about 130 to about 180, about 130 to about 170, about 130 to about 160, about 130 to about 150, about 130 to about 140, about 140 to about 600, about 140 to about 580, about 140 to about 560, about 140 to about 540, about 140 to about 520, about 140 to about 500, about 140 to about 480, about 140 to about 460, about 140 to about 440, about 140 to about 420, about 140 to about 400, about 140 to about 380, about 140 to about 360, about 140 to about 340, about 140 to about 320, about 140 to about 300, about 140 to about 280, about 140 to about 260, about 140 to about 240, about 140 to about 220, about 140 to about 200, about 140 to about 190, about 140 to about 180, about 140 to about 170, about 140 to about 160, about 140 to about 150, about 150 to about 600, about 150 to about 580, about 150 to about 560, about 150 to about 540, about 150 to about 520, about 150 to about 500, about 150 to about 480, about 150 to about 460, about 150 to about 440, about 150 to about 420, about 150 to about 400, about 150 to about 380, about 150 to about 360, about 150 to about 340, about 150 to about 320, about 150 to about 300, about 150 to about 280, about 150 to about 260, about 150 to about 240, about 150 to about 220, about 150 to about 200, about 150 to about 190, about 150 to about 180, about 150 to about 170, about 150 to about 160, about 160 to about 600, about 160 to about 580, about 160 to about 560, about 160 to about 540, about 160 to about 520, about 160 to about 500, about 160 to about 480, about 160 to about 460, about 160 to about 440, about 160 to about 420, about 160 to about 400, about 160 to about 380, about 160 to about 360, about 160 to about 340, about 160 to about 320, about 160 to about 300, about 160 to about 280, about 160 to about 260, about 160 to about 240, about 160 to about 220, about 160 to about 200, about 160 to about 190, about 160 to about 180, about 160 to about 170, about 170 to about 600, about 170 to about 580, about 170 to about 560, about 170 to about 540, about 170 to about 520, about 170 to about 500, about 170 to about 480, about 170 to about 460, about 170 to about 440, about 170 to about 420, about 170 to about 400, about 170 to about 380, about 170 to about 360, about 170 to about 340, about 170 to about 320, about 170 to about 300, about 170 to about 280, about 170 to about 260, about 170 to about 240, about 170 to about 220, about 170 to about

200, about 170 to about 190, about 170 to about 180, about 180 to about 600, about 180 to about 580, about 180 to about 560, about 180 to about 540, about 180 to about 520, about 180 to about 500, about 180 to about 480, about 180 to about 460, about 180 to about 440, about 180 to about 420, about 180 to about 400, about 180 to about 380, about 180 to about 360, about 180 to about 340, about 180 to about 320, about 180 to about 300, about 180 to about 280, about 180 to about 260, about 180 to about 240, about 180 to about 220, about 180 to about 200, about 180 to about 190, about 190 to about 600, about 190 to about 580, about 190 to about 560, about 190 to about 540, about 190 to about 520, about 190 to about 500, about 190 to about 480, about 190 to about 460, about 190 to about 440, about 190 to about 420, about 190 to about 400, about 190 to about 380, about 190 to about 360, about 190 to about 340, about 190 to about 320, about 190 to about 300, about 190 to about 280, about 190 to about 260, about 190 to about 240, about 190 to about 220, about 190 to about 200, about 200 to about 600, about 200 to about 580, about 200 to about 560, about 200 to about 540, about 200 to about 520, about 200 to about 500, about 200 to about 480, about 200 to about 460, about 200 to about 440, about 200 to about 420, about 200 to about 400, about 200 to about 380, about 200 to about 360, about 200 to about 340, about 200 to about 320, about 200 to about 300, about 200 to about 280, about 200 to about 260, about 200 to about 240, about 200 to about 220, about 220 to about 600, about 220 to about 580, about 220 to about 560, about 220 to about 540, about 220 to about 520, about 220 to about 500, about 220 to about 480, about 220 to about 460, about 220 to about 440, about 220 to about 420, about 220 to about 400, about 220 to about 380, about 220 to about 360, about 220 to about 340, about 220 to about 320, about 220 to about 300, about 220 to about 280, about 220 to about 260, about 220 to about 240, about 240 to about 600, about 240 to about 580, about 240 to about 560, about 240 to about 540, about 240 to about 520, about 240 to about 500, about 240 to about 480, about 240 to about 460, about 240 to about 440, about 240 to about 420, about 240 to about 400, about 240 to about 380, about 240 to about 360, about 240 to about 340, about 240 to about 320, about 240 to about 300, about 240 to about 280, about 240 to about 260, about 260 to about 600, about 260 to about 580, about 260 to about 560, about 260 to about 540, about 260 to about 520, about 260 to about 500, about 260 to about 480, about 260 to about 460, about 260 to about 440, about 260 to about 420, about 260 to about 400, about 260 to about 380, about 260 to about 360, about 260 to about 340, about 260 to about 320, about 260 to about 300, about 260 to about 280, about 280 to about 600, about 280 to about 580, about 280 to about 560, about 280 to about 540, about 280 to about 520, about 280 to about 500, about 280 to about 480, about 280 to about 460, about 280 to about 440, about 280 to about 420, about 280 to about 400, about 280 to about 380, about 280 to about

360, about 280 to about 340, about 280 to about 320, about 280 to about 300, about 300 to about 600, about 300 to about 580, about 300 to about 560, about 300 to about 540, about 300 to about 520, about 300 to about 500, about 300 to about 480, about 300 to about 460, about 300 to about 440, about 300 to about 420, about 300 to about 400, about 300 to about 380, about 300 to about 360, about 300 to about 340, about 300 to about 320, about 320 to about 600, about 320 to about 580, about 320 to about 560, about 320 to about 540, about 320 to about 520, about 320 to about 500, about 320 to about 480, about 320 to about 460, about 320 to about 440, about 320 to about 420, about 320 to about 400, about 320 to about 380, about 320 to about 360, about 320 to about 340, about 340 to about 600, about 340 to about 580, about 340 to about 560, about 340 to about 540, about 340 to about 520, about 340 to about 500, about 340 to about 480, about 340 to about 460, about 340 to about 440, about 340 to about 420, about 340 to about 400, about 340 to about 380, about 340 to about 360, about 360 to about 600, about 360 to about 580, about 360 to about 560, about 360 to about 540, about 360 to about 520, about 360 to about 500, about 360 to about 480, about 360 to about 460, about 360 to about 440, about 360 to about 420, about 360 to about 400, about 360 to about 380, about 380 to about 600, about 380 to about 580, about 380 to about 560, about 380 to about 540, about 380 to about 520, about 380 to about 500, about 380 to about 480, about 380 to about 460, about 380 to about 440, about 380 to about 420, about 380 to about 400, about 400 to about 600, about 400 to about 580, about 400 to about 560, about 400 to about 540, about 400 to about 520, about 400 to about 500, about 400 to about 480, about 400 to about 460, about 400 to about 440, about 400 to about 420, about 420 to about 600, about 420 to about 580, about 420 to about 560, about 420 to about 540, about 420 to about 520, about 420 to about 500, about 420 to about 480, about 420 to about 460, about 420 to about 440, about 440 to about 600, about 440 to about 580, about 440 to about 560, about 440 to about 540, about 440 to about 520, about 440 to about 500, about 440 to about 480, about 440 to about 460, about 460 to about 600, about 460 to about 580, about 460 to about 560, about 460 to about 540, about 460 to about 520, about 460 to about 500, about 460 to about 480, about 480 to about 600, about 480 to about 580, about 480 to about 560, about 480 to about 540, about 480 to about 520, about 480 to about 500, about 500 to about 600, about 500 to about 580, about 500 to about 560, about 500 to about 540, about 500 to about 520, about 520 to about 600, about 520 to about 580, about 520 to about 560, about 520 to about 540, about 540 to about 600, about 540 to about 580, about 540 to about 560, about 560 to about 600, about 560 to about 580, or about 580 to about 600.

Additional examples of a ratio of GI tissue concentration of the live biotherapeutic to the blood, serum, or plasma concentration of the live biotherapeutic include to 1.1 to 600, 1.2 to 600,

1.3 to 600, 1.4 to 600, 1.5 to 600, 1.6 to 600, 1.7 to 600, 1.8 to 600, or 1.9 to 600, such as 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or 1.9.

In some examples, administration of a live biotherapeutic using any of the devices or compositions described herein can result in a ratio of GI tissue concentration of the live biotherapeutic to the blood, serum, or plasma concentration of the live biotherapeutic of, e.g., about 2.8 to about 6.0, about 2.8 to about 5.8, about 2.8 to about 5.6, about 2.8 to about 5.4, about 2.8 to about 5.2, about 2.8 to about 5.0, about 2.8 to about 4.8, about 2.8 to about 4.6, about 2.8 to about 4.4, about 2.8 to about 4.2, about 2.8 to about 4.0, about 2.8 to about 3.8, about 2.8 to about 3.6, about 2.8 to about 3.4, about 2.8 to about 3.2, about 2.8 to about 3.0, about 3.0 to about 6.0, about 3.0 to about 5.8, about 3.0 to about 5.6, about 3.0 to about 5.4, about 3.0 to about 5.2, about 3.0 to about 5.0, about 3.0 to about 4.8, about 3.0 to about 4.6, about 3.0 to about 4.4, about 3.0 to about 4.2, about 3.0 to about 4.0, about 3.0 to about 3.8, about 3.0 to about 3.6, about 3.0 to about 3.4, about 3.0 to about 3.2, about 3.2 to about 6.0, about 3.2 to about 5.8, about 3.2 to about 5.6, about 3.2 to about 5.4, about 3.2 to about 5.2, about 3.2 to about 5.0, about 3.2 to about 4.8, about 3.2 to about 4.6, about 3.2 to about 4.4, about 3.2 to about 4.2, about 3.2 to about 4.0, about 3.2 to about 3.8, about 3.2 to about 3.6, about 3.2 to about 3.4, about 3.4 to about 6.0, about 3.4 to about 5.8, about 3.4 to about 5.6, about 3.4 to about 5.4, about 3.4 to about 5.2, about 3.4 to about 5.0, about 3.4 to about 4.8, about 3.4 to about 4.6, about 3.4 to about 4.4, about 3.4 to about 4.2, about 3.4 to about 4.0, about 3.4 to about 3.8, about 3.4 to about 3.6, about 3.6 to about 6.0, about 3.6 to about 5.8, about 3.6 to about 5.6, about 3.6 to about 5.4, about 3.6 to about 5.2, about 3.6 to about 5.0, about 3.6 to about 4.8, about 3.6 to about 4.6, about 3.6 to about 4.4, about 3.6 to about 4.2, about 3.6 to about 4.0, about 3.6 to about 3.8, about 3.8 to about 6.0, about 3.8 to about 5.8, about 3.8 to about 5.6, about 3.8 to about 5.4, about 3.8 to about 5.2, about 3.8 to about 5.0, about 3.8 to about 4.8, about 3.8 to about 4.6, about 3.8 to about 4.4, about 3.8 to about 4.2, about 3.8 to about 4.0, about 4.0 to about 6.0, about 4.0 to about 5.8, about 4.0 to about 5.6, about 4.0 to about 5.4, about 4.0 to about 5.2, about 4.0 to about 5.0, about 4.0 to about 4.8, about 4.0 to about 4.6, about 4.0 to about 4.4, about 4.0 to about 4.2, about 4.2 to about 6.0, about 4.2 to about 5.8, about 4.2 to about 5.6, about 4.2 to about 5.4, about 4.2 to about 5.2, about 4.2 to about 5.0, about 4.2 to about 4.8, about 4.2 to about 4.6, about 4.2 to about 4.4, about 4.4 to about 6.0, about 4.4 to about 5.8, about 4.4 to about 5.6, about 4.4 to about 5.4, about 4.4 to about 5.2, about 4.4 to about 5.0, about 4.4 to about 4.8, about 4.4 to about 4.6, about 4.6 to about 6.0, about 4.6 to about 5.8, about 4.6 to about 5.6, about 4.6 to about 5.4, about 4.6 to about 5.2, about 4.6 to about 5.0, about 4.6 to about 4.8, about 4.8 to about 6.0, about 4.8 to about 5.8, about 4.8 to

about 5.6, about 4.8 to about 5.4, about 4.8 to about 5.2, about 4.8 to about 5.0, about 5.0 to about 6.0, about 5.0 to about 5.8, about 5.0 to about 5.6, about 5.0 to about 5.4, about 5.0 to about 5.2, about 5.2 to about 6.0, about 5.2 to about 5.8, about 5.2 to about 5.6, about 5.2 to about 5.4, about 5.4 to about 6.0, about 5.4 to about 5.8, about 5.4 to about 5.6, about 5.6 to about 6.0, about 5.6 to about 5.8, or about 5.8 to about 6.0. Accordingly, in some embodiments, a method of treatment disclosed herein can include determining the ratio of the level of the live biotherapeutics in the GI tissue to the level of the live biotherapeutics in the blood, serum, or plasma of a subject at substantially the same time point following administration of the device is about 2.8 to about 6.0. Exemplary methods for measuring the concentration of a live biotherapeutic in the plasma or the GI tissue of a subject are described herein. Additional methods for measuring the concentration of a live biotherapeutic in the plasma or the GI tissue of a subject are known in the art.

Accordingly, in some embodiments, a method of treatment disclosed herein includes determining the level of the live biotherapeutic, such as a stem cell, in the GI tissue (e.g., one or more of any of the exemplary GI tissues described herein). In some embodiments, a method of treatment disclosed herein can include determining the level of live biotherapeutic, such as a stem cell, in one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa.

In some embodiments, a method of treatment disclosed herein includes determining that the level of the live biotherapeutic, such as a stem cell, in the GI tissue (e.g., one or more of any of the exemplary types of GI tissues described herein) at a time point following administration of the device is higher than the level of stem cell in the GI tissue at substantially the same time point following systemic administration of an equal amount of the stem cell. In some embodiments, a method of treatment disclosed herein can include determining that the level of the live biotherapeutic, such as a stem cell, in one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa at a time point following administration of the device is higher than the level of the live biotherapeutic, such as a stem cell, in one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa at substantially the same time point following systemic administration of an equal amount of the live biotherapeutic, such as a stem cell.

In some embodiments, a method of treatment disclosed herein includes determining the level of the live biotherapeutic, such as a stem cell, in the feces of the subject. In some

embodiments, a method of treatment disclosed herein includes determining the level of live biotherapeutic, such as a stem cell, in the GI tissue, e.g., in one or more (e.g., two, three, or four) of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa within a time period of about 10 minutes to about 10 hours following administration of the device.

In some embodiments, a method of treatment as disclosed herein comprises determining the level of the live biotherapeutic, such as a stem cell, at the location of disease following administration of the device.

In some embodiments, a method of treatment as disclosed herein comprises determining that the level of live biotherapeutic, such as a stem cell, at the location of disease at a time point following administration of the device is higher than the level of the live biotherapeutic, such as a stem cell, at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutic, such as a stem cell.

In some embodiments, a method of treatment as disclosed herein comprises determining that the level of live biotherapeutic, such as a stem cell, in plasma in a subject at a time point following administration of the device is lower than the level of the live biotherapeutic, such as a stem cell, in plasma in a subject at substantially the same time point following systemic administration of an equal amount of the live biotherapeutic, such as a stem cell.

In some embodiments, a method of treatment as disclosed herein comprises determining the level of the live biotherapeutic, such as a stem cell, in the tissue of the subject within a time period of about 10 minutes to 10 hours following administration of the device.

Some examples of any of the methods described herein can, e.g., result in a selective suppression of a local inflammatory response (e.g., an inflammatory response in local GI tissue), while maintaining the systemic immune response (e.g., blood). The GI tissue may be, for example, GI tissue proximate to one or more sites of disease. FAs used herein, "GI content" refers to the content of the gastrointestinal (GI) tract, such as the content of one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum, more particularly of the proximal portion of one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, and sigmoid colon, or of the distal portion of one or more of duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, and sigmoid colon. Accordingly, in some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the dudodenum tissue proximate to one or more sites of disease, while maintaining the systemic

immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the jejunum tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the ileum tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the cecum tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the ascending colon tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the transverse colon tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the descending colon tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some embodiments, the methods described herein can result in a selective suppression of the inflammatory response in the sigmoid colon tissue proximate to one or more sites of disease, while maintaining the systemic immune response. In some examples, the methods described herein can result in a 1% increase to 500% increase (e.g., a 1% increase to 450% increase, a 1% increase to 400% increase, a 1% increase to 350% increase, a 1% increase to 300% increase, a 1% increase to 250% increase, a 1% increase to 200% increase, a 1% increase to 190% increase, a 1% increase to 180% increase, a 1% increase to 170% increase, a 1% increase to 160% increase, a 1% increase to 150% increase, a 1% increase to 140% increase, a 1% increase to 130% increase, a 1% increase to 120% increase, a 1% increase to 110% increase, a 1% increase to 100% increase, a 1% increase to 90% increase, a 1% increase to 80% increase, a 1% increase to 70% increase, a 1% increase to 60% increase, a 1% increase to 50% increase, a 1% increase to 40% increase, a 1% increase to 30% increase, a 1% increase to 25% increase, a 1% increase to 20% increase, a 1% increase to 15% increase, a 1% increase to 10% increase, a 1% increase to 5% increase, a 5% increase to 500% increase, a 5% increase to 450% increase, a 5% increase to 400% increase, a 5% increase to 350% increase, a 5% increase to 300% increase, a 5% increase to 250% increase, a 5% increase to 200% increase, a 5% increase to 190% increase, a 5% increase to 180% increase, a 5% increase to 170% increase, a 5% increase to 160% increase, a 5% increase to 150% increase, a 5% increase to 140% increase, a

5% increase to 130% increase, a 5% increase to 120% increase, a 5% increase to 110% increase, a 5% increase to 100% increase, a 5% increase to 90% increase, a 5% increase to 80% increase, a 5% increase to 70% increase, a 5% increase to 60% increase, a 5% increase to 50% increase, a 5% increase to 40% increase, a 5% increase to 30% increase, a 5% increase to 25% increase, a 5% increase to 20% increase, a 5% increase to 15% increase, a 5% increase to 10% increase, a 10% increase to 500% increase, a 10% increase to 450% increase, a 10% increase to 400% increase, a 10% increase to 350% increase, a 10% increase to 300% increase, a 10% increase to 250% increase, a 10% increase to 200% increase, a 10% increase to 190% increase, a 10% increase to 180% increase, a 10% increase to 170% increase, a 10% increase to 160% increase, a 10% increase to 150% increase, a 10% increase to 140% increase, a 10% increase to 130% increase, a 10% increase to 120% increase, a 10% increase to 110% increase, a 10% increase to 100% increase, a 10% increase to 90% increase, a 10% increase to 80% increase, a 10% increase to 70% increase, a 10% increase to 60% increase, a 10% increase to 50% increase, a 10% increase to 40% increase, a 10% increase to 30% increase, a 10% increase to 25% increase, a 10% increase to 20% increase, a 10% increase to 15% increase, a 15% increase to 500% increase, a 15% increase to 450% increase, a 15% increase to 400% increase, a 15% increase to 350% increase, a 15% increase to 300% increase, a 15% increase to 250% increase, a 15% increase to 200% increase, a 15% increase to 190% increase, a 15% increase to 180% increase, a 15% increase to 170% increase, a 15% increase to 160% increase, a 15% increase to 150% increase, a 15% increase to 140% increase, a 15% increase to 130% increase, a 15% increase to 120% increase, a 15% increase to 110% increase, a 15% increase to 100% increase, a 15% increase to 90% increase, a 15% increase to 80% increase, a 15% increase to 70% increase, a 15% increase to 60% increase, a 15% increase to 50% increase, a 15% increase to 40% increase, a 15% increase to 30% increase, a 15% increase to 25% increase, a 15% increase to 20% increase, a 20% increase to 500% increase, a 20% increase to 450% increase, a 20% increase to 400% increase, a 20% increase to 350% increase, a 20% increase to 300% increase, a 20% increase to 250% increase, a 20% increase to 200% increase, a 20% increase to 190% increase, a 20% increase to 180% increase, a 20% increase to 170% increase, a 20% increase to 160% increase, a 20% increase to 150% increase, a 20% increase to 140% increase, a 20% increase to 130% increase, a 20% increase to 120% increase, a 20% increase to 110% increase, a 20% increase to 100% increase, a 20% increase to 90% increase, a 20% increase to 80% increase, a 20% increase to 70% increase, a 20% increase to 60% increase, a 20% increase to 50% increase, a 20% increase to 40% increase, a 20% increase to 30% increase, a 20% increase to 25%

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In some examples of any of the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of: the level of interferon-γ in GI tissue or GI content; the level of IL-1β in GI tissue or GI content; the level of IL-6 in GI tissue or GI content; the level of IL-22 in GI tissue or GI content; the level of IL-17A in GI tissue or GI content; the level of TNFα in GI tissue or GI content; and the level of IL-2 in GI tissue or GI content, e.g., as compared to the corresponding level in a subject not administered a treatment, or not administered a live biotherapeutic, such as a stem cell, locally as disclosed herein. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the

level of IL-17A; the level of TNFα; and the level of IL-2, in the duodenum tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the ileum tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the jejunum tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the cecum tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the ascending colon tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the transverse colon tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the level of TNFα; and the level of IL-2, in the descending colon tissue proximate to one or more sites of disease. Accordingly, in some embodiments, the methods described herein can result, e.g., in a 1% to 99% decrease (or any of the subranges of this range described herein) in one or more (e.g., two, three, four, five, six, or seven) of the level of interferon-γ; the level of IL-1β; the level of IL-6; the level of IL-22; the level of IL-17A; the

level of TNFα; and the level of IL-2, in the sigmoid colon tissue proximate to one or more sites of disease.

In some embodiments, the live biotherapeutic, such as a stem cell, is delivered to the location by a process that does not comprise systemic transport of the stem cell.

In some embodiments, the amount of the stem cells that is administered is about 20 million cells to about 500 million cells. In some embodiments, the amount of the stem cells that is administered is about 100 million cells to about 400 million cells. In some embodiments, the amount of the stem cells that is administered is about 120 million cells to about 300 million cells. In some embodiments, the amount of the stem cells that is administered is about 200 million cells. In some embodiments, the amount of the stem cells that is administered is from about 1 mg to about 500 mg. In some embodiments, the amount of the stem cells that is administered is from about 1 mg to about 100 mg. In some embodiments, the amount of the stem cells that is administered is from about 5 mg to about 40 mg. In some embodiments, the amount of the live biotherapeutic is administered as an escalating dose of 10 mg, followed by 20 mg, followed by 30 mg; or an escalating dose of 20 mg, followed by 30 mg, followed by 50 mg. In some embodiments, the amount of the live biotherapeutic is administered as an escalating dose of 10 mg, followed by 20 mg, followed by 30 mg; or an escalating dose of 20 mg, followed by 30 mg, followed by 50 mg.

In some embodiments, the amount of the live biotherapeutic is administered in a dose of, e.g., about 1 mg to about 300 mg, about 1 mg to about 250 mg, about 1 mg to about 200 mg, about 1 mg to about 195 mg, about 1 mg to about 190 mg, about 1 mg to about 185 mg, about 1 mg to about 180 mg, about 1 mg to about 175 mg, about 1 mg to about 170 mg, about 1 mg to about 165 mg, about 1 mg to about 160 mg, about 1 mg to about 155 mg, about 1 mg to about 150 mg, about 1 mg to about 145 mg, about 1 mg to about 140 mg, about 1 mg to about 135 mg, about 1 mg to about 130 mg, about 1 mg to about 125 mg, about 1 mg to about 120 mg, about 1 mg to about 115 mg, about 1 mg to about 110 mg, about 1 mg to about 105 mg, about 1 mg to about 100 mg, about 1 mg to about 95 mg, about 1 mg to about 90 mg, about 1 mg to about 85 mg, about 1 mg to about 80 mg, about 1 mg to about 75 mg, about 1 mg to about 70 mg, about 1 mg to about 65 mg, about 1 mg to about 60 mg, about 1 mg to about 55 mg, about 1 mg to about 50 mg, about 1 mg to about 45 mg, about 1 mg to about 40 mg, about 1 mg to about 35 mg, about 1 mg to about 30 mg, about 1 mg to about 25 mg, about 1 mg to about 20 mg, about 1 mg to about 15 mg, about 1 mg to about 10 mg, about 1 mg to about 5 mg, about 5 mg to about 200 mg, about 5 mg to about 195 mg, about 5 mg to about 190 mg, about 5 mg to about 185 mg, about 5

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DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 241

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

Claims:

1. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

administering to the subject a pharmaceutical formulation that comprises a live biotherapeutic such as a stem cell,

wherein the pharmaceutical formulation is released at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

2. The method of claim 1, wherein the pharmaceutical formulation is administered in an ingestible device.

3. The method of claim 1, wherein the pharmaceutical formulation is released from an ingestible device.

4. The method of claim 2 or 3, wherein the ingestible device comprises a housing, a reservoir containing the pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device,

wherein the reservoir is releasably or permanently attached to the exterior of the housing or internal to the housing.

5. The method of claim 2 or 3, wherein the ingestible device comprises a housing, a reservoir containing the pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device, wherein the reservoir is internal to the device.

6. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

administering to the subject an ingestible device comprising a housing, a reservoir containing a pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device;

wherein the reservoir is releasably or permanently attached to the exterior of the housing or internal to the housing;

wherein the pharmaceutical formulation comprises a live biotherapeutic such as a stem cell, and

the ingestible device releases the pharmaceutical formulation at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

7. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

administering to the subject an ingestible device comprising a housing, a reservoir containing a pharmaceutical formulation, and a release mechanism for releasing the pharmaceutical formulation from the device;

wherein the reservoir is internal to the device;

wherein the pharmaceutical formulation comprises a live biotherapeutic such as a stem cell, and

the ingestible device releases the pharmaceutical formulation at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

8. The method of any one of claims 4 to 7, wherein the housing is non-biodegradable in the GI tract.

9. The method of any one of claims 2 to 8, wherein the release of the formulation is triggered autonomously.

10. The method of any one of claims 2 to 9, wherein the device is programmed to release the formulation with one or more release profiles that may be the same or different at one or more locations in the GI tract.

11. The method of any one of claims 2 to 10, wherein the device is programmed to release the formulation at a location proximate to one or more sites of disease.

12. The method of claim 11, wherein the location of one or more sites of disease is predetermined.

13. The method of any one of claims 4 to 12, wherein the reservoir is made of a material that allows the formulation to leave the reservoir

14. The method of claim 13, wherein the material is a biodegradable material.

15. The method of any one of claims 2 to 14, wherein the release of the formulation is triggered by a pre-programmed algorithm.

16. The method of any one of claims 2 to 15, wherein the release of the formulation is triggered by data from a sensor or detector to identify the location of the device.

17. The method of claim 16, wherein the data is not based solely on a physiological parameter.

18. The method of any one of claims 2 to 17, wherein the device comprises a detector configured to detect light reflectance from an environment external to the housing.

19. The method of claim 18, wherein the release is triggered autonomously or based on the detected reflectance.

20. The method of any one of claims 2 to 19, wherein the device releases the formulation at substantially the same time as one or more sites of disease are detected.

21. The method of any one of claims 4 to 20, wherein the release mechanism is an actuation system.

22. The method of claim 21, wherein the actuation system is a chemical actuation system.

23. The method of claim 21, wherein the actuation system is a mechanical actuation system.

24. The method of claim 21, wherein the actuation system is an electrical actuation system.

25. The method of claim 21, wherein the actuation system comprises a pump and releasing the formulation comprises pumping the formulation out of the reservoir.

26. The method of claim 21, wherein the actuation system comprises a gas generating cell.

27. The method of any one of claims 2 to 26, wherein the device comprises an anchoring mechanism.

28. The method of any one of claims 1 to 27, wherein the formulation comprises a therapeutically effective amount of the live biotherapeutic such as a stem cell.

29. The method of any one of the preceding claims, wherein the formulation comprises a human equivalent dose (HED) of the live biotherapeutic such as a stem cell .

30. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic.

31. The method of claim 30, wherein the pharmaceutical composition is an ingestible device and the method comprises administering orally to the subject the pharmaceutical composition.

32. The method of claim 30 or 31, wherein the method does not comprise releasing more than 10% of the live biotherapeutic at a location that is not proximate to a site of disease.

33. The method of claim 30 or 31, wherein the method provides a concentration of the live biotherapeutic at a location that is a site of disease or proximate to a site of disease that is 2-100 times greater than at a location that is not proximate to a site of disease.

34. The method of any one of the preceding claims, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 3 μg/ml.

35. The method of claim 34, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 0.3 μg/ml.

36. The method of claim 35, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 0.01 μg/ml.

37. The method of any one of claims 30 to 33, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 3 μg/ml.

38. The method of claim 37, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 0.3 μg/ml.

39. The method of claim 38, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 0.01 μg/ml.

40. The method of any one of claims 30 to 39, wherein the method delivers a population of cells that comprises at least about 50% stem cells.

41. The method of any one of claims 30 to 39, wherein the method delivers a population of cells that comprises at least about 55% stem cells.

42. The method of any one of claims 30 to 39, wherein the method delivers a population of cells that comprises at least about 60% stem cells.

43. The method of any one of claims 30 to 39, wherein the method delivers a population of cells that comprises at least about 65% stem cells.

44. The method of any one of claims 30 to 39, wherein the method delivers a population of cells that comprises at least about 70% stem cells.

45. The method of any one of claims 31 to 44, wherein the stem cell is present in a pharmaceutical formulation within the device.

46. The method of claim 45, wherein the formulation is a solution of the live biotherapeutic in a liquid medium.

47. The method of claim 46, wherein the formulation is a suspension of the live biotherapeutic in a liquid medium.

48. The method of any one of claims 30 to 47, wherein the disease of the GI tract is an inflammatory bowel disease.

49. The method of any one of claims 30 to 47, wherein the disease of the GI tract is ulcerative colitis.

50. The method of any one of claims 30 to 47, wherein the disease of the GI tract is Crohn's disease.

51. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the large intestine of the subject.

52. The method of claim 51, wherein the location is in the proximal portion of the large intestine.

53. The method of claim 51, wherein the location is in the distal portion of the large intestine.

54. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the ascending colon of the subject.

55. The method of claim 54, wherein the location is in the proximal portion of the ascending colon.

56. The method of claim 54, wherein the location is in the distal portion of the ascending colon.

57. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the cecum of the subject.

58. The method of claim 57, wherein the location is in the proximal portion of the cecum.

59. The method of claim 57, wherein the location is in the distal portion of the cecum.

60. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the sigmoid colon of the subject.

61. The method of claim 60, wherein the location is in the proximal portion of the sigmoid colon.

62. The method of claim 60, wherein the location is in the distal portion of the sigmoid colon.

63. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the transverse colon of the subject.

64. The method of claim 63, wherein the location is in the proximal portion of the transverse colon.

65. The method of claim 63, wherein the location is in the distal portion of the transverse colon.

66. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the descending colon of the subject.

67. The method of claim 66, wherein the location is in the proximal portion of the descending colon.

68. The method of claim 66, wherein the location is in the distal portion of the descending colon.

69. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the small intestine of the subject.

70. The method of claim 69, wherein the location is in the proximal portion of the small intestine.

71. The method of claim 69, wherein the location is in the distal portion of the small intestine.

72. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the duodenum of the subject.

73. The method of claim 72, wherein the location is in the proximal portion of the duodenum.

74. The method of claim 72, wherein the location is in the distal portion of the duodenum.

75. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the jejunum of the subject.

76. The method of claim 75, wherein the location is in the proximal portion of the jejunum.

77. The method of claim 75, wherein the location is in the distal portion of the jejunum.

78. The method of any one of claims 30 to 50, wherein the live biotherapeutic is released at a location in the ileum of the subject.

79. The method of claim 78, wherein the location is in the proximal portion of the ileum.

80. The method of claim 78, wherein the location is in the distal portion of the ileum.

81. The method of any one of the preceding claims, wherein the location at which the live biotherapeutic is released is 10 cm or less from one or more sites of disease.

82. The method of any one of the preceding claims, wherein the location at which the live biotherapeutic is released is 5 cm or less from one or more sites of disease.

83. The method of any one of the preceding claims, wherein the location at which the live biotherapeutic is released is 2 cm or less from one or more sites of disease.

84. The method of any one of the preceding claims, wherein the live biotherapeutic is released by mucosal contact.

85. The method of any one of the preceding claims, wherein the live biotherapeutic is delivered to the location by a process that does not comprise systemic transport of the live biotherapeutic.

86. The method of any one of the preceding claims, further comprising identifying the one or more sites of disease by a method comprising imaging of the gastrointestinal tract.

87. The method of claim any one of the preceding claims, wherein the method comprises identifying the disease site prior to administering the pharmaceutical composition.

88. The method of claim 58, wherein the method comprises releasing the live biotherapeutic substantially at the same time as identifying the disease site.

89. The method of any one of the preceding claims, comprising (a) identifying a subject having a disease of the gastrointestinal tract and (b) evaluating the subject for suitability to treatment.

90. The method of any one of claims 30 or 32 to 44 or 46 to 89, wherein releasing the live biotherapeutic is triggered by one or more of: a pH in the jejunum from 6.1 to 7.2, a pH in the mid small bowel from 7.0 to 7.8, a pH in the ileum from 7.0 to 8.0, a pH in the right colon from 5.7 to 7.0, a pH in the mid colon from 5.7 to 7.4, a pH in the left colon from 6.3 to 7.7, such as 7.0.

91. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is not dependent on the pH at or in the vicinity of the location.

92. The method of any one of claims 30 or 32 to 44 or 46 to 89, wherein releasing the live biotherapeutic is triggered by degradation of a release component located in the device.

93. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is not triggered by degradation of a release component located in the device.

94. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is not dependent on enzymatic activity at or in the vicinity of the location.

95. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is not dependent on bacterial activity at or in the vicinity of the location.

96. The method of any one of claims 30 to 89, wherein the composition comprises a plurality of electrodes comprising a coating, and releasing the live biotherapeutic is triggered by an electric signal by the electrodes resulting from the interaction of the coating with the one or more sites of disease.

97. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is triggered by a remote electromagnetic signal.

98. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is triggered by generation in the composition of a gas in an amount sufficient to expel the live biotherapeutic.

99. The method of any one of claims 30 to 89, wherein releasing the live biotherapeutic is triggered by an electromagnetic signal generated within the device according to a pre-determined drug release profile.

100. The method of any one of claims 31 to 89, wherein the ingestible device comprises an ingestible housing, wherein a reservoir storing the live biotherapeutic is attached to the housing.

101. The method of claim 100, further comprising:

detecting when the ingestible housing is proximate to a respective disease site of the one of the one or more sites of disease,

wherein releasing the live biotherapeutic comprises releasing the therapeutically effective amount of the live biotherapeutic from the reservoir proximate the respective disease site in response to the detection.

102. The method of claim 101, wherein detecting comprises detecting via one or more sensors coupled to the ingestible housing.

103. The method of claim 102, wherein the one or more sensors comprise a plurality of coated electrodes and wherein detecting comprises receiving an electric signal by one or more of the coated electrodes responsive to the one or more electrode contacting the respective disease site.

104. The method of claim 101, wherein releasing comprises opening one or more valves in fluid communication with the reservoir.

105. The method of claim 104, wherein the one or more valves is communicably coupled to a processor positioned in the housing, the processor communicably coupled to one or more sensors configured to detect the one or more sites of disease.

106. The method of claim 101, wherein releasing comprises pumping the therapeutically effective amount of the live biotherapeutic from the reservoir via pump positioned in the ingestible housing.

107. The method of claim 106, wherein the pump is communicably coupled to a processor positioned in the housing, the processor communicably coupled to one or more sensors configured to detect the one or more sites of disease.

108. The method of claim 100, wherein the therapeutically effective amount of the live biotherapeutic is stored in the reservoir at a reservoir pressure higher than a pressure in the gastrointestinal tract of the subject.

109. The method of claim 100, further comprising anchoring the ingestible housing at a location proximate to the respective disease site in response to the detection.

110. The method of claim 109, wherein anchoring the ingestible housing comprises one or more legs to extend from the ingestible housing.

111. The method of any one of the preceding claims, wherein the amount of the live biotherapeutic that is administered is from about 1 mg to about 500 mg.

112. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic (MSC).

113. The method of any one of the preceding claims, wherein the live biotherapeutic is an endothelial progenitor cells.

114. The method of any one of claims 30 to 113, wherein the amount of the live biotherapeutic is less than an amount that is effective when live biotherapeutic is administered systemically.

115. The method of any one of the preceding claims, comprising administering (i) an amount of the live biotherapeutic that is an induction dose.

116. The method of claim 115, further comprising (ii) administering an amount of the live biotherapeutic that is a maintenance dose following the administration of the induction dose.

117. The method of claim 115 or 116, wherein the induction dose is administered once a day.

118. The method of claim 115 or 116, wherein the induction dose is administered once every three days.

119. The method of claim 115 or 116, wherein the induction dose is administered once a week.

120. The method of claim 116, wherein step (ii) is repeated one or more times.

121. The method of claim 116, wherein step (ii) is repeated once a day over a period of about 6-8 weeks.

122. The method of claim 116, wherein step (ii) is repeated once every three days over a period of about 6-8 weeks.

123. The method of claim 116, wherein step (ii) is repeated once a week over a period of about 6-8 weeks.

124. The method of claim 116, wherein the induction dose is equal to the maintenance dose.

125. The method of claim 116, wherein the induction dose is greater than the maintenance dose.

126. The method of claim 116, wherein the induction dose is 5 times greater than the maintenance dose.

127. The method of claim 116, wherein the induction dose is 2 times greater than the maintenance dose.

128. The method of any one of the preceding claims, wherein the method comprises releasing the live biotherapeutic at the location in the gastrointestinal tract as a single bolus.

129. The method of any one of claims 30 to 127, wherein the method comprises releasing the live biotherapeutic at the location in the gastrointestinal tract as more than one bolus.

130. The method of any one of claims 30 to 127, wherein the method comprises delivering the live biotherapeutic at the location in the gastrointestinal tract in a continuous manner.

131. The method of claim 130, wherein the method comprises delivering the live biotherapeutic at the location in the gastrointestinal tract over a time period of 20 or more minutes.

132. The method of any one of claims 30 to 131, wherein the method does not comprise delivering a live biotherapeutic rectally to the subject.

133. The method of any one of claims 30 to 131, wherein the method does not comprise delivering a live biotherapeutic via an enema to the subject.

134. The method of any one of claims 30 to 131, wherein the method does not comprise delivering a live biotherapeutic via suppository to the subject.

135. The method of any one of claims 30 to 131, wherein the method does not comprise delivering a live biotherapeutic via instillation to the rectum of the subject.

136. The method of any one of claims 30 to 131, wherein the method does not comprise surgical implantation.

137. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic obtained from bone marrow.

138. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic obtained from placenta.

139. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic obtained from amniotic fluid.

140. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic obtained from Wharton's jelly.

141. The method of any one of the preceding claims, wherein the live biotherapeutic is a mesenchymal live biotherapeutic obtained from amniotic membrane.

142. The method of any one of claims 30 to 96 or 98 to 141, wherein the composition is an autonomous device.

143. The method of any one of claims 30 to 142, wherein the composition comprises a mechanism capable of releasing the live biotherapeutic.

144. The method of any one of claims 30 to 143, wherein the composition comprises a tissue anchoring mechanism for anchoring the composition to the location.

145. The method of claim 144, wherein the tissue anchoring mechanism is capable of activation for anchoring to the location.

146. The method of claim 144 to 145, wherein the tissue anchoring mechanism comprises an osmotically-driven sucker.

147. The method of claim 144, 145, or 146, wherein the tissue anchoring mechanism comprises a connector operable to anchor the composition to the location.

148. The method of claim 147, wherein the connector is operable to anchor the composition to the location using an adhesive, negative pressure and/or fastener.

149. The method of claim 100, wherein the reservoir is an anchorable reservoir.

150. The method of any one of claims 30 to 89, wherein the pharmaceutical composition is an ingestible device, comprising:

a housing;

a reservoir located within the housing and containing the live biotherapeutic,

a mechanism for releasing the live biotherapeutic from the reservoir;

and;

an exit valve configured to allow the live biotherapeutic to be released out of the housing from the reservoir.

151. The method of claim 150, wherein the ingestible device further comprises:

an electronic component located within the housing; and

a gas generating cell located within the housing and adjacent to the electronic component,

wherein the electronic component is configured to activate the gas generating cell to generate gas.

152. The method of claim 150 or 151, wherein the ingestible device further comprises:

a safety device placed within or attached to the housing,

wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

153. The method of claim 30 to 89, wherein the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component,

wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing,

wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an exit valve located at the first end of the housing,

wherein the exit valve is configured to allow the dispensable substance to be released out of the first end of the housing from the reservoir; and

a safety device placed within or attached to the housing,

wherein the safety device is configured to relieve an internal pressure within the housing when the internal pressure exceeds a threshold level.

154. The method of claim 30 to 89, wherein the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an electronic component located within the housing,

a gas generating cell located within the housing and adjacent to the electronic component,

wherein the electronic component is configured to activate the gas generating cell to generate gas;

a reservoir located within the housing,

wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

an injection device located at the first end of the housing,

wherein the jet injection device is configured to inject the dispensable substance out of the housing from the reservoir; and

a safety device placed within or attached to the housing,

wherein the safety device is configured to relieve an internal pressure within the housing.

155. The method of claim 30 to 89, wherein the pharmaceutical composition is an ingestible device, comprising:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

an optical sensing unit located on a side of the housing, wherein the optical sensing unit is configured to detect a reflectance from an environment external to the housing;

an electronic component located within the housing;

a gas generating cell located within the housing and adjacent to the electronic component,

wherein the electronic component is configured to activate the gas generating cell to generate gas in response to identifying a location of the ingestible device based on the reflectance;

a reservoir located within the housing, wherein the reservoir stores a dispensable substance and a first end of the reservoir is attached to the first end of the housing;

a membrane in contact with the gas generating cell and configured to move or deform into the reservoir by a pressure generated by the gas generating cell; and

a dispensing outlet placed at the first end of the housing, wherein the dispensing outlet is configured to deliver the dispensable substance out of the housing from the reservoir.

156. The method of any one of claims 30 to 89, wherein the pharmaceutical composition is an ingestible device as disclosed in US Patent Application Ser. No. 62/385,553, incorporated by reference herein in its entirety.

157. The method of any one of claims 30 to 89, wherein the pharmaceutical composition is an ingestible device as disclosed in US Patent Application Ser. No. 62/478,955, incorporated by reference herein in its entirety.

158. The method of any one of claims 30 to 89, wherein the pharmaceutical composition is an ingestible device comprising a localization mechanism as disclosed in international patent application PCT/US2015/052500, incorporated by reference herein in its entirety.

159. A method of treating a disease of the large intestine of a subject, comprising:

releasing a live biotherapeutic at a location in the proximal portion of the large intestine of the subject that is proximate to one or more sites of disease,

wherein the method comprises administering endoscopically to the subject a therapeutically effective amount of the live biotherapeutic, wherein the method does not comprise releasing more than 20% of the live biotherapeutic at a location that is not proximate to a site of disease.

160. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic at a location in the proximal portion of the large intestine of the subject that is proximate to one or more sites of disease, wherein the method comprises administering endoscopically to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic, wherein the pharmaceutical composition is an ingestible device.

161. The method of claim 159 or 160, wherein the method does not comprise releasing more than 20% of the live biotherapeutic at a location that is not proximate to a site of disease

162. The method of claim 159, 160 or 161 wherein the method does not comprise releasing more than 10% of the live biotherapeutic at a location that is not proximate to a site of disease.

163. The method of any one of claims 159, 160 or 161, wherein the method provides a concentration of the live biotherapeutic at a location that is a site of disease or proximate to a site of disease that is 2-100 times greater than at a location that is not proximate to a site of disease.

164. The method of any one of claims 159 to 163, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 3 μg/ml.

165. The method of claim 164, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 0.3 μg/ml.

166. The method of claim 165, wherein the method provides a concentration of the live biotherapeutic in the plasma of the subject that is less than 0.01 μg/ml.

167. The method of any one of claims 159 to 163, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 3 μg/ml.

168. The method of any one of claims 159 to 163, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 0.3 μg/ml.

169. The method of any one of claims 159 to 163, wherein the method provides a C_{24} value of the live biotherapeutic in the plasma of the subject that is less than 0.01 μg/ml.

170. The method of any one of claims 159 to 163, wherein the composition does not comprise an enteric coating.

171. The method of any one of claims 159 to 170, wherein the live biotherapeutic is not a cyclic peptide.

172. The method of any one of claims 159 to 170, wherein the live biotherapeutic is present in a pharmaceutical formulation within the device.

173. The method of claim 172, wherein the formulation is a solution of the live biotherapeutic in a liquid medium.

174. The method of claim 172, wherein the formulation is a suspension of the live biotherapeutic in a liquid medium.

175. The method of any one of claims 159 to 174, wherein the disease of the large intestine is an inflammatory bowel disease.

176. The method of any one of claims 159 to 174, wherein the disease of the large intestine is ulcerative colitis.

177. The method of any one of claims 159 to 174, wherein the disease the large intestine is Crohn's disease.

178. The method of any one of claims 159 to 177, wherein the live biotherapeutic is released at a location in the proximal portion of the ascending colon.

179. The method of any one of claims 159 to 177, wherein the live biotherapeutic is released at a location in the proximal portion of the cecum.

180. The method of any one of claims 159 to 177, wherein the live biotherapeutic is released at a location in the proximal portion of the sigmoid colon.

181. The method of any one of claims 159 to 177, wherein the live biotherapeutic is released at a location in the proximal portion of the transverse colon.

182. The method of any one of claims 159 to 177, wherein the live biotherapeutic is released at a location in the proximal portion of the descending colon.

183. The method of any one of claims 159 to 177, wherein the method comprises administering to the subject a reservoir comprising the therapeutically effective amount of the live biotherapeutic, wherein the reservoir is connected to the endoscope.

184. The method of any one of the preceding claims, further comprising administering a second agent orally, intravenously or subcutaneously, wherein the second agent is the same live biotherapeutic; a different live biotherapeutic; or an agent having a different biological target from the live biotherapeutic, wherein the second agent is an agent suitable for treating an inflammatory bowel disease.

185. The method of claim 184, wherein the live biotherapeutic is administered prior to the second agent.

186. The method of claim 184, wherein the live biotherapeutic is administered after the second agent.

187. The method of claim 184, wherein the live biotherapeutic and the second agent are administered substantially at the same time.

188. The method of any one of claims 184, wherein the second agent is administered intravenously.

189. The method of any one of claims 184, wherein the second agent is administered subcutaneously.

190. The method of any one of claims 184 to 189, wherein the amount of the second agent is less than the amount of the second agent when the live biotherapeutic and the second agent are both administered systemically.

191. The method of claim 190, wherein the second agent is a stem cell.

192. The method of claim 190, wherein second agent is methotrexate.

193. The method of any one of claims 30 to 183, wherein the method does not comprise administering a second agent.

194. The method of any one of claims 148 to 193, wherein the method comprises identifying the disease site prior to endoscopic administration.

195. The method of any one of claims 148 to 193, wherein the method comprises identifying the disease site substantially at the same time as releasing the live biotherapeutic.

196. The method of any one of the preceding claims, wherein the method comprising monitoring the progress of the disease.

197. The method of claim 196, wherein monitoring the progress of the disease comprises measuring the weight of the subject over a period of about 1-14 weeks, such as about 6-8 weeks following administration of the live biotherapeutic.

198. The method of claim 196 or 197, wherein monitoring the progress of the disease comprises measuring the food intake of the subject over a period of about 1-14 weeks, such as about 6-8 weeks following administration of the live biotherapeutic.

199. The method of claim 196, 197 or 198, wherein monitoring the progress of the disease comprises measuring the level of blood in the feces of the subject over a period of about 1-14 weeks, such as about 6-8 weeks following administration of the live biotherapeutic.

200. The method of claim 196, 197 or 198, wherein monitoring the progress of the disease comprises measuring the level of abdominal pain of the subject over a period of about 1-14 weeks, such as about 6-8 weeks following administration of the live biotherapeutic.

201. The method of any one of claims 30 to 200, wherein the method does not comprise administering a live biotherapeutic with a spray catheter.

202. The method of any one of claims 30 to 201, wherein the method comprises administering a live biotherapeutic with a spray catheter.

203. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic the method comprising one or more of the following steps:

h) identifying a subject having a disease of the gastrointestinal tract;

i) determination of the severity of the disease;

j) determination of the location of the disease;

k) evaluating the subject for suitability to treatment;

l) administration of an induction dose of the live biotherapeutic;

m) monitoring the progress of the disease; and/or

n) optionally repeating steps e) and f) one or more times.

204. The method of claim 203, wherein the pharmaceutical composition is an ingestible device and the method comprises administering orally to the subject the pharmaceutical composition.

205. The method of claim 203 or 204, wherein the method comprises administering one or more maintenance doses following administration of the induction dose in step e).

206. The method of claim 205, wherein the induction dose is a dose of the live biotherapeutic administered in an ingestible device.

207. The method of claim 205 or 206, wherein the maintenance dose is a dose of the live biotherapeutic administered in an ingestible device as disclosed herein.

208. The method of claim 205 or 206, wherein the maintenance dose is a dose of the live biotherapeutic delivered systemically.

209. The method of claim 205, wherein the induction dose is a dose of the live biotherapeutic delivered systemically.

210. The method of claim 205 or 209, wherein the maintenance dose is a dose of the live biotherapeutic administered in an ingestible device.

211. The method of claim 205, wherein the induction dose is a dose of a second agent as delivered systemically.

212. The method of claim 205 or 209, wherein the maintenance dose is a dose of the live biotherapeutic administered in an ingestible device.

213. A live biotherapeutic delivery apparatus comprising:

an ingestible housing comprising a reservoir having a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutic stored therein;

a detector coupled to the ingestible housing, the detector configured to detect when the ingestible housing is proximate to a respective disease site of the one of the one or more sites of disease;

a valve system in fluid communication with the reservoir system; and

a controller communicably coupled to the valve system and the detector, the controller configured to cause the valve system to open in response to the detector detecting that the ingestible housing is proximate to the respective disease site so as to release the therapeutically effective amount of the live biotherapeutic at the respective disease site.

214. The live biotherapeutic delivery apparatus according to claim 213, further comprising a pump positioned in the ingestible housing, the pump configured to pump the therapeutically effective amount of the live biotherapeutic from the reservoir in response to activation of the pump by the controller responsive to detection by the detector of the ingestible housing being proximate to the respective disease site.

215. The live biotherapeutic delivery apparatus according to claim 214, wherein the controller is configured to cause the pump to pump the therapeutically effective amount of the live biotherapeutic from the reservoir according to the following protocol.

216. The live biotherapeutic delivery apparatus according to claim 213, wherein the valve system comprises a dissolvable coating.

217. The live biotherapeutic delivery apparatus according to claim 213, wherein the valve system comprises one or more doors configured for actuation by at least one of sliding, pivoting, and rotating.

218. The live biotherapeutic delivery apparatus according to claim 213, wherein the valve system comprises an electrostatic shield.

219. The live biotherapeutic delivery apparatus according to claim 213, wherein the reservoir comprises a pressurized cell.

220. The live biotherapeutic delivery apparatus according to claim 213, further comprising at least one actuatable anchor configured to retain the ingestible housing at the respective disease site upon actuation.

221. The live biotherapeutic delivery apparatus according to claim 213, wherein the actuatable anchor is retractable.

222. A composition comprising a therapeutically effective amount of the live biotherapeutic of any one of the preceding claims, wherein the composition is capable of releasing the live biotherapeutic at a location in the gastrointestinal tract of the subject.

223. The composition of claim 222, wherein the composition comprises a tissue anchoring mechanism for anchoring the composition to the location.

224. The composition of claim 223, wherein the tissue anchoring mechanism is capable of anchoring for anchoring to the location.

225. The composition of claim 223 or 224, wherein the tissue anchoring mechanism comprises an osmotically-driven sucker.

226. The composition of claim 223, 224 or 225, wherein the tissue anchoring mechanism comprises a connector operable to anchor the composition to the location.

227. The composition of claim 226, wherein the connector is operable to anchor the composition to the location using an adhesive, negative pressure and/or fastener.

228. A live biotherapeutic for use in a method of treating a disease of the gastrointestinal tract in a subject, wherein the method comprises orally administering to the subject an ingestible device loaded with the live biotherapeutic, wherein the live biotherapeutic is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

229. The live biotherapeutic for use of claim 228, wherein the live biotherapeutic is contained in a reservoir suitable for attachment to a device housing, and wherein the method comprises attaching the reservoir to the device housing to form the ingestible device, prior to orally administering the ingestible device to the subject.

230. An attachable reservoir containing a live biotherapeutic for use in a method of treating a disease of the gastrointestinal tract, wherein the method comprises attaching the reservoir to a device housing to form an ingestible device and orally administering the ingestible device to a subject, wherein the live biotherapeutic is released by device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

231. A composition comprising or consisting of an ingestible device loaded with a therapeutically effective amount of a live biotherapeutic, for use in a method of treatment, wherein the method comprises orally administering the composition to the subject, wherein the live biotherapeutic is released by the device at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

232. The live biotherapeutic for use according to claim 228 or 229, the attachable reservoir compartment for use according to claim 230, or the composition for use according to claim 231, wherein the sites of disease have been pre-determined.

233. The live biotherapeutic for use according to claim 228 or 229, the attachable reservoir compartment for use according to claim 230, or the composition for use according to claim 231, wherein the ingestible device further comprises an environmental sensor and the method further comprises using the environmental sensor to identify the location of one or more sites of disease.

234. The live biotherapeutic for use, the attachable reservoir compartment for use the composition for use, according to claim 233, wherein the environmental sensor is an imaging sensor and the method further comprising imaging the gastrointestinal tract to identify the location of one or more sites of disease.

235. The live biotherapeutic for use, the attachable reservoir compartment for use, or the composition for use, according to claim 234, wherein the imaging detects inflamed tissue and/or lesions associated with a disease of the gastrointestinal tract.

236. The live biotherapeutic for use, the attachable reservoir compartment for use or the composition for use, according to any one of claims 228 to 234, wherein the disease of the GI tract is one or more of an inflammatory bowel disease, ulcerative colitis and Crohn's disease.

237. An ingestible device loaded with a therapeutically effective amount of a live biotherapeutic, wherein the device is controllable to release the live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease.

238. The device of claim 237 for use in a method of treatment of the human or animal body.

239. The live biotherapeutic for use, the attachable reservoir compartment for use or the composition for use according to any one of claims 228 to 236, or the device according to claim 237 or claim 238, wherein the ingestible device comprises:

a housing defined by a first end, a second end substantially opposite from the first end, and a wall extending longitudinally from the first end to the second end;

a reservoir located within the housing and containing the live biotherapeutic wherein a first end of the reservoir is connected to the first end of the housing;

a mechanism for releasing the live biotherapeutic from the reservoir; and

an exit value configured to allow the live biotherapeutic to be released out of the housing from the reservoir.

240. The live biotherapeutic for use, the attachable reservoir compartment for use or the composition for use according to any one of claims 228 to 236, or the device according to claim 237 or claim 238, wherein the ingestible device comprises:

an ingestible housing comprising a reservoir compartment having a therapeutically effective amount of the live biotherapeutic stored therein;

a release mechanism having a closed state which retains the live biotherapeutic in the reservoir and an open state which releases the live biotherapeutic from the reservoir to the exterior of the device; and

an actuator which changes the state of the release mechanism from the closed to the open state.

241. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to claims 239 or 240, wherein the ingestible device further comprises an environmental sensor for detecting the location of the device in the gut and/or for detecting the presence of disease in the GI tract.

242. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to claim 241, wherein the ingestible device further comprises a communication system for transmitting data from the environmental sensor to an external receiver.

243. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to claim 241 or 242, wherein the ingestible device further comprises a processor or controller which is coupled to the environmental sensor and to the actuator and which triggers the actuator to cause the

release mechanism to transition from its closed state to its open state when it is determined that the device is in the presence of diseased tissue and/or is in a location in the gut that has been predetermined to be proximal to diseased tissue.

244. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to claim 242, wherein the communication system further comprises means for receiving a signal from an external transmitter, and wherein the actuator is adapted to be triggered in response to the signal.

245. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to any one of claims 239 to 244, wherein the ingestible device further comprises a communication system for transmitting localization data to an external receiver.

246. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to any one of claims 239 to 242, wherein the ingestible device further comprises a communication system for transmitting localization data to an external receiver and for receiving a signal from an external transmitter; wherein the actuator is adapted to be triggered in response to the signal.

247. The live biotherapeutic for use, the attachable reservoir compartment for use, the composition for use, or the device according to any one of claims 148 to 246, wherein the ingestible device further comprises a deployable anchoring system and an actuator for deploying the anchoring system, wherein the anchoring system is capable of anchoring or attaching the ingestible device to the subject's tissue.

248. The method of any one of claims 31 to 221, wherein the method comprises determining the level of the live biotherapeutics at the location of disease following administration of the device.

249. The method of any one of claims 31 to 221 or 248, wherein the method comprises determining that the level of live biotherapeutics at the location of disease at the time point following administration of the device is higher than the level of the live

biotherapeutics at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

250. The method of claim 248, wherein the method comprises determining the level of the live biotherapeutics in the GI tissue of the subject at a time point following administration of the device.

251. The method of claim of any one of claims 31 to 221 or 250, wherein the method comprises determining the level of the live biotherapeutics in one or more of the lumen/superficial mucosa, the lamina propria, the submucosa, and the tunica muscularis/serosa in the subject at a time point following administration of the device.

252. The method of any one of claims 31 to 221 or 250, wherein the method comprises determining that the level of the live biotherapeutics in the GI tissue at a time point following administration of the device is higher than the level of the live biotherapeutics in the GI tissue of a subject at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

253. The method of any one of claims 31 to 221 or 251, wherein the method comprises determining that the level of the live biotherapeutics in the lumen/superficial mucosa in the subject following administration of the device is elevated as compared to the level of live biotherapeutics in the lumen/superficial mucosa in a subject at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

254. The method of any one of claims 31 to 221 or 248 to 253, wherein the method comprises determining the level of the live biotherapeutics in the tissue of the subject within a time period of about 10 minutes to 10 hours following administration of the device.

255. The method of any one of claims 31 to 221 or 248 to 254, wherein the method comprises determining a level of a marker at the location of disease in the subject following administration of the device.

256. The method of claim 255, wherein the marker is a biomarker and the method comprises determining that the level of the biomarker at the location of disease in the subject at a time point following administration of the device is decreased as compared to a level of the biomarker in the subject prior to administration of the device or a level of the biomarker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

257. The method of claim 256, wherein the level of the biomarker in the subject at a time point following administration of the device is 1% decreased to 99% decreased as compared to the level of the biomarker in the subject prior to administration of the device or the level of the biomarker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

258. The method of claim 256 or 257, wherein the method comprises determining the level of the biomarker in the subject at a time point that is 10 minutes to 10 hours following administration of the device.

259. The method of claim 256, 257, or 258, wherein the level of the biomarker is one or more of: the level of interferon-γ in GI tissue, the level of IL-1β in GI tissue, the level of IL-6 in GI tissue, the level of IL-22 in GI tissue, the level of IL-17A in the GI tissue, the level of TNFα in GI tissue, the level of IL-2 in GI tissue.

260. The method of claim 226, wherein the method comprises determining that the level of the marker at the time point following administration of the device is decreased relative to the level of the marker in the subject prior to administration of the device or the level of the marker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

261. The method of claim 260, wherein the level of the marker in the subject at the time point following administration of the device is 1% decreased to 99% decreased as compared to the level of the marker in the subject prior to administration of the device or the level of the marker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

262. The method of claim 260 or 261, wherein the method comprises determining the level of the marker in the subject within a time period of about 10 minutes to about 10 hours following administration of the device.

263. The method of claim 260, 261 or 262, wherein the level of the marker is an endoscopy score in the subject.

264. The method of claim 238, wherein the method comprises determining that the level of the marker in the subject at the time point following administration of the device is elevated as compared to the level of the marker in the subject prior to administration of the device or the level of the marker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

265. The method of claim 247, wherein the level of the marker in the subject following administration of the device is 1% increased to 400% increased as compared to the level of the marker in the subject prior to administration of the device or the level of the marker in a subject at the same location of disease at substantially the same time point following systemic administration of an equal amount of the live biotherapeutics.

266. The method of claim 264 or 265, wherein the method comprises determining the level of the marker in the subject within a time period of about 10 minutes to about 10 hours of administration of the device.

267. The method of claim 264, 265 or 266 wherein the level of the marker is one or both of subject weight and stool consistency.

268. The method of any one of claims 31 to 221 or 248 to 267, wherein the method comprises determining the time period of onset of treatment following administration of the device.

269. A method for treating colitis in a subject, wherein the colitis is associated with treatment of the subject with one or more immuno-oncology agents, the method comprising releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutics.

270. The method of claim 269, wherein the pharmaceutical composition is an ingestible device and the method comprises administering orally to the subject the pharmaceutical composition.

271. The method of claim 269 or 270, wherein at least one of the one or more immuno-oncology agents is a chemotherapeutic agent.

272. The method of claim 271, wherein the chemotherapeutic agent is a chemotherapeutic immunomodulator.

273. The method of claim 272, wherein the chemotherapeutic immunomodulatory is an immune checkpoint inhibitor.

274. The method of claim 273, wherein the immune checkpoint inhibitor targets or decreases an activity of an immune checkpoint protein selected from the group consisting of: CTLA-4, PD-1, PD-L1, PD-1 – PD-L1, PD-1 – PD-L2, interleukin 2 (IL 2), indoleamine 2,3-dioxygenase (IDO), IL 10, transforming growth factor-β (TGFβ), T cell immunoglobulin and mucin 3 (TIM3 or HAVCR2), Galectin 9 – TIM3, Phosphatidylserine – TIM3, lymphocyte activation gene 3 protein (LAG3), MHC class II – LAG3, 4 1BB–4 1BB ligand, OX40–OX40 ligand, GITR, GITR ligand – GITR, CD27, CD70-CD27, TNFRSF25, TNFRSF25–TL1A, CD40L, CD40–CD40 ligand, HVEM–LIGHT–LTA, HVEM, HVEM – BTLA, HVEM – CD160, HVEM – LIGHT, HVEM–

BTLA–CD160, CD80, CD80 – PDL-1, PDL2 – CD80, CD244, CD48 – CD244, CD244, ICOS, ICOS–ICOS ligand, B7 H3, B7 H4, VISTA, TMIGD2, HHLA2–TMIGD2, Butyrophilins, including BTNL2, Siglec family, TIGIT and PVR family members, KIRs, ILTs and LIRs, NKG2D and NKG2A, MICA and MICB, CD244, CD28, CD86 – CD28, CD86 – CTLA, CD80 – CD28, CD39, CD73 Adenosine–CD39–CD73, CXCR4–CXCL12, Phosphatidylserine, TIM3, Phosphatidylserine – TIM3, SIRPA–CD47, VEGF, Neuropilin, CD160, CD30, and CD155.

275. The method of claim 273, wherein the immune checkpoint inhibitor is selected from the group consisting of: Urelumab, PF 05082566, MEDI6469, TRX518, Varlilumab, CP 870893, Pembrolizumab (PD1), Nivolumab (PD1), Atezolizumab (formerly MPDL3280A) (PDL1), MEDI4736 (PD-L1), Avelumab (PD-L1), PDR001 (PD1), BMS 986016, MGA271, Lirilumab, IPH2201, Emactuzumab, INCB024360, Galunisertib, Ulocuplumab, BKT140, Bavituximab, CC 90002, Bevacizumab, and MNRP1685A, and MGA271.

276. The method of claim 273, wherein the immune checkpoint inhibitor targets CTLA-4.

277. The method of claim 273, wherein the immune checkpoint inhibitor is an antibody.

278. The method of claim 277, wherein the antibody is ipilimumab or tremelimumab.

279. The method of claim 273, wherein the immune checkpoint inhibitor targets PD1 or PD-L1.

280. The method of claim 273, wherein the immune checkpoint inhibitor is selected from the group of: nivolumab, lambroizumab, and BMS-936559.

281. The method of claim 269, wherein at least one of the one or more immuno-oncology agents is a T-cell that expresses a chimeric antigen receptor (a CAR-T cell).

282. The method of any one of claims 269 to 281, wherein the treatment of the subject with one or more immuno-oncology agents further includes treatment of the patient with an immunosuppressant.

283. The method of claim 269, wherein at least one of the one or more immuno-oncology agents is a PI-3 kinase inhibitor.

284. A method for treating colitis in a subject comprising:

determining that the subject has colitis associated with treatment of the subject with one or more immuno-oncology agents; and

releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of colitis, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutics. In some embodiments, the pharmaceutical composition is an ingestible device. In some embodiments, the pharmaceutical composition is an ingestible device and the method comprises administering orally to the subject the pharmaceutical composition.

285. A method for treating colitis, comprising releasing a live biotherapeutic at a location in the gastrointestinal tract of a subject who has been determined to have colitis associated with treatment of the subject with one or more immuno-oncology agents, wherein the location is proximate to one or more sites of colitis, wherein the method comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of the live biotherapeutics.

286. The method of claim 264 or 285, wherein the pharmaceutical composition is an ingestible device and the method comprises administering orally to the subject the pharmaceutical composition.

287. An ingestible device, comprising:

a live biotherapeutic;

one or more processing devices; and

one more machine readable hardware storage devices storing instructions that are executable by the one or more processing devices to determine a location of the ingestible device in a portion of a GI tract of a subject to an accuracy of at least 85%.

288. The ingestible device of claim 287, wherein the accuracy is at least 90%.

289. The ingestible device of claim 287, wherein the accuracy is at least 95%.

290. The ingestible device of claim 287, wherein the accuracy is at least 97%.

291. The ingestible device of claim 287, wherein the accuracy is at least 98%

292. The ingestible device of claim 287, wherein the accuracy is at least 99%.

293. The ingestible device of claim 287, wherein the accuracy is 100%.

294. The ingestible device of claim 287, wherein the portion of the portion of the GI tract of the subject comprises the duodenum.

295. The ingestible device of claim 287, wherein the portion of the portion of the GI tract of the subject comprises the jejunum.

296. The ingestible device of claim 287, wherein the portion of the portion of the GI tract of the subject comprises the terminal ileum, cecum and colon.

297. The ingestible device of any of claims 287-296, further comprising first and second light sources, wherein the first light source is configured to emit light at a first wavelength, and the second light source is configured to emit light at a second wavelength different from the first wavelength.

298. The ingestible device of claim 297, further comprising first and second detectors, wherein the first detector is configured to detect light at the first wavelength, and the second detector is configured to detect light at the second wavelength.

299. An ingestible device, comprising:

a live biotherapeutic;

one or more processing devices; and

one more machine readable hardware storage devices storing instructions that are executable by the one or more processing devices to determine that the ingestible device is in the cecum of a subject to an accuracy of at least 70%.

300. The ingestible device of claim 299, wherein the accuracy is at least 75%.

301. The ingestible device of claim 299, wherein the accuracy is at least 80%.

302. The ingestible device of claim 299, wherein the accuracy is at least 85%.

303. The ingestible device of claim 299, wherein the accuracy is at least 88%

304. The ingestible device of claim 299, wherein the accuracy is at least 89%.

305. An ingestible device, comprising:

a live biotherapeutic;

one or more processing devices; and

one more machine readable hardware storage devices storing instructions that are executable by the one or more processing devices to transmit data to a device capable of implementing the data to determine a location of the medical device in a portion of a GI tract of a subject to an accuracy of at least 85%.

306. The ingestible device of claim 305, wherein the accuracy is at least 90%.

307. The ingestible device of claim 305, wherein the accuracy is at least 95%.

308. The ingestible device of claim 305, wherein the accuracy is at least 97%.

309. The ingestible device of claim 305, wherein the accuracy is at least 98%

310. The ingestible device of claim 305, wherein the accuracy is at least 99%.

311. The ingestible device of claim 305, wherein the accuracy is 100%.

312. The ingestible device of claim 305, wherein the portion of the portion of the GI tract of the subject comprises the duodenum.

313. The ingestible device of claim 305, wherein the portion of the portion of the GI tract of the subject comprises the jejunum.

314. The ingestible device of claim 305, wherein the portion of the portion of the GI tract of the subject comprises the terminal ileum, cecum and colon.

315. The ingestible device of any of claims 305 to 314, further comprising first and second light sources, wherein the first light source is configured to emit light at a first wavelength, and the second light source is configured to emit light at a second wavelength different from the first wavelength.

316. The ingestible device of claim 315, further comprising first and second detectors, wherein the first detector is configured to detect light at the first wavelength, and the second detector is configured to detect light at the second wavelength.

317. The ingestible device of any of claims 305 to 315, wherein the data comprise intensity data for at least two different wavelengths of light.

318. An ingestible device, comprising:

a live biotherapeutic;

one or more processing devices; and

one more machine readable hardware storage devices storing instructions that are executable by the one or more processing devices to transmit data to an external device capable of implementing the data to determine that the ingestible device is in the cecum of subject to an accuracy of at least 70%.

319. The ingestible device of claim 318, wherein the accuracy is at least 75%.

320. The ingestible device of claim 318, wherein the accuracy is at least 80%.

321. The ingestible device of claim 318, wherein the accuracy is at least 85%.

322. The ingestible device of claim 318, wherein the accuracy is at least 88%

323. The ingestible device of claim 318, wherein the accuracy is at least 89%.

324. The device of any one of claims 287 to 317, wherein the live biotherapeutics are present in a therapeutically effective amount.

325. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering orally to the subject the ingestible device of any one of claims 287 to 324,

the method further comprising determining a location of the ingestible medical device in a portion of a GI tract of a subject to an accuracy of at least 85%.

326. The method of claim 325, wherein the accuracy is at least 90%.

327. The method of claim 325, wherein the accuracy is at least 95%.

328. The method of claim 325, wherein the accuracy is at least 97%.

329. The method of claim 325, wherein the accuracy is at least 98%

330. The method of claim 325, wherein the accuracy is at least 99%.

331. The method of claim 325, wherein the accuracy is 100%.

332. The method of claim 325, wherein the portion of the portion of the GI tract of the subject comprises the duodenum.

333. The method of claim 325, wherein the portion of the portion of the GI tract of the subject comprises the jejunum.

334. The method of claim 325, wherein the portion of the portion of the GI tract of the subject comprises the terminal ileum, cecum and colon.

335. The method of claim 325, wherein determining the location of the ingestible device within the GI tract of a subject comprises determining reflected light signals within the GI tract, wherein the reflected signals comprise light of at least two different wavelengths.

336. The method of claim 335, wherein the reflected signals comprise light of at least three different wavelengths.

337. The method of claim 335 or 336, wherein:
the reflected light comprises first and second wavelengths;
the first wavelength is between 495–600 nm; and
the second wavelength is between 400–495 nm.

338. The method of claim 337, wherein the first and second wavelengths are separated by at least 50 nm.

339. A method of treating a disease of the gastrointestinal tract in a subject, comprising:

releasing a live biotherapeutic at a location in the gastrointestinal tract of the subject that is proximate to one or more sites of disease, wherein the method comprises administering orally to the subject the ingestible device of any one of claims 287 to 324,

the method further comprising determining a location of an ingestible medical device within the GI tract of a subject based on measured reflected light signals within the GI tract,

wherein the reflected signals comprise light of at least two different wavelengths.

340. The method of claim 339, wherein the reflected signals comprise light of at least three different wavelengths.

341. The method of claim 339, wherein:

the at least two different wavelengths comprise first and second wavelengths;

the first wavelength is between 495–600 nm; and

the second wavelength is between 400–495 nm.

342. The method of claim 341, wherein the first and second wavelengths are separated by at least 50 nm.

343. The method of any one of claims 325 to 342, wherein the live biotherapeutics are present in a therapeutically effective amount

344. An ingestible device, comprising:

a housing;

a gas generating cell located within the housing; and

a storage reservoir located within the housing,

wherein:

the storage reservoir stores a live biotherapeutic; and

the ingestible device is configured so that, when the gas generating cell generates a gas, the live biotherapeutic exits the ingestible device via an opening in the ingestible device.

345. The ingestible device of claim 344, further comprising an injection device configured so that, when the gas generating cell generates the gas, the gas moves the injection device to force the live biotherapeutic out of the ingestible device via the opening.

346. The ingestible device of claim 345, wherein the injection device comprises a syringe.

347. The ingestible device of claim 345 or 346, further comprising a component configured to position the injection device at an epithelial layer and spread the epithelial layer prior to a delivery of the live biotherapeutic.

348. The ingestible device of any one of claims 344 to 347, further comprising a membrane configured so that, when the gas generating cell generates the gas, the gas moves the membrane to force the live biotherapeutic out of the ingestible device via the opening.

349. The ingestible device of claim 348, wherein the membrane comprises a piston configured so that, when the gas generating cell generates the gas, the gas moves the membrane to force the live biotherapeutic out of the ingestible device via the opening.

350. The ingestible device of any one of claims 344 to 349, further comprising an optical sensing unit supported by the housing, wherein the optical sensing unit is configured to detect a reflectance from an environment external to the housing.

351. The ingestible device of claim 350, wherein the ingestible device is configured to determine a location of the ingestible device based on the reflectance detected by the optical sensing unit.

352. The ingestible device of claim 350 or claim 351, wherein the gas generating cell generates the gas based on the reflectance detected by the optical sensing unit.

353. The ingestible device of any one of claims 344 to 352, further comprising an electronic component within the housing, wherein the electronic component is configured to active the gas generating cell.

354. The ingestible device of claim 353, wherein the gas generating cell is adjacent the electronic component.

355. The ingestible device of any one of claims 344 to 354, further comprising a safety device configured to relieve an internal pressure within the housing.

356. The ingestible device of any one of claims 344 to 355, wherein:

the housing has a first end, a second end and a wall extending between the first and second ends; and

the storage reservoir is adjacent to the first end.

357. The ingestible device of any one of claims 344 to 356, wherein the storage reservoir stores a therapeutically effective amount of the live biotherapeutic.

358. A reservoir configured for use in an ingestible device, wherein the reservoir comprises a therapeutic agent.

359. The reservoir of claim 358, wherein the reservoir comprises a housing and the housing comprises a plastic.

360. The reservoir of claim 358 or 359, wherein the plastic comprises at least one material selected from the group consisting of PVC, silicone and polycarbonate.

361. The reservoir of any of claims 358 to 360, wherein the ingestible device when fully assembled and packaged satisfies the regulatory requirements for marketing a medical device in the United States of America.

362. The reservoir of claim 30, wherein the therapeutic agent comprises a live biotherapeutic.

363. The reservoir of any one of claims 358 to 362, wherein the reservoir is configured to partially fit within the housing of the ingestible device.

364. The reservoir of any one of claims 358 to 363, wherein the reservoir is configured to entirely fit within the housing of the ingestible device

365. The reservoir of any of claims 358 to 362, wherein the reservoir is configured to attach to the housing of the ingestible device.

366. The reservoir of any one of claims 358 to 365, wherein the reservoir is configured to friction fit with the ingestible device.

367. The reservoir of any one of claims 358 to 366, wherein the reservoir is configured to be held to the ingestible device via a biasing mechanism.

368. The reservoir of claim 367, wherein the biasing mechanism comprises at least one member selected from the group consisting of a spring, a latch, a hook, a magnet, and electromagnetic radiation.

369. The reservoir of any one of claims 358 to 368, wherein the reservoir is configured to fit into a groove or a track in the housing of the ingestible device.

370. The reservoir of any one of claims 358 to 369, wherein the reservoir is configured to snap fit to the ingestible device.

371. The reservoir of any one of claims 358 to 370, wherein the reservoir is configured to be pierced.

372. The reservoir of any one of claims 358 to 371, wherein the reservoir comprises a plastic.

373. The reservoir of any one of claims 358 to 372, wherein the reservoir comprises at least one material selected from the group consisting of PVC, polycarbonate and silicone.

374. The reservoir of any one of claims 358 to 373, wherein the reservoir comprises a metal or an alloy.

375. The reservoir of claim 374, wherein the reservoir comprises stainless steel.

376. The reservoir of any one of claims 358 to 375, wherein the reservoir is configured to carry electronic components.

377. A kit, comprising:

an ingestible device; and

a reservoir configured for use in an ingestible device, wherein the reservoir comprises a therapeutic agent.

378. The ingestible device of any one of claims 287 to 298, further comprising one or more elements of a device as recited in any one of claims 100, 151, 152, 233, or 239 to 247.

379. The ingestible device of any one of claims 299 to 304, further comprising one or more elements of a device as recited in any one of claims 100, 151, 152, 233, or 239 to 247.

380. The ingestible device of any one of claims 305 to 317, further comprising one or more elements of a device as recited in any one of claims 100, 151, 152, 233, or 239 to 247.

381. The ingestible device of any one of claims 318 to 324, further comprising one or more elements of a device as recited in any one of claims 100, 151, 152, 233, or 239 to 247.

382. The ingestible device of any one of claims 344 to 357, further comprising one or more elements of a device as recited in any one of claims 100, 151, 152, 233, or 239 to 247.

383. The reservoir of any one of claims 358 to 376, wherein the reservoir is configured for use in a device of any one of claims 287 to 324, 344 to 357, or 378 to 382.

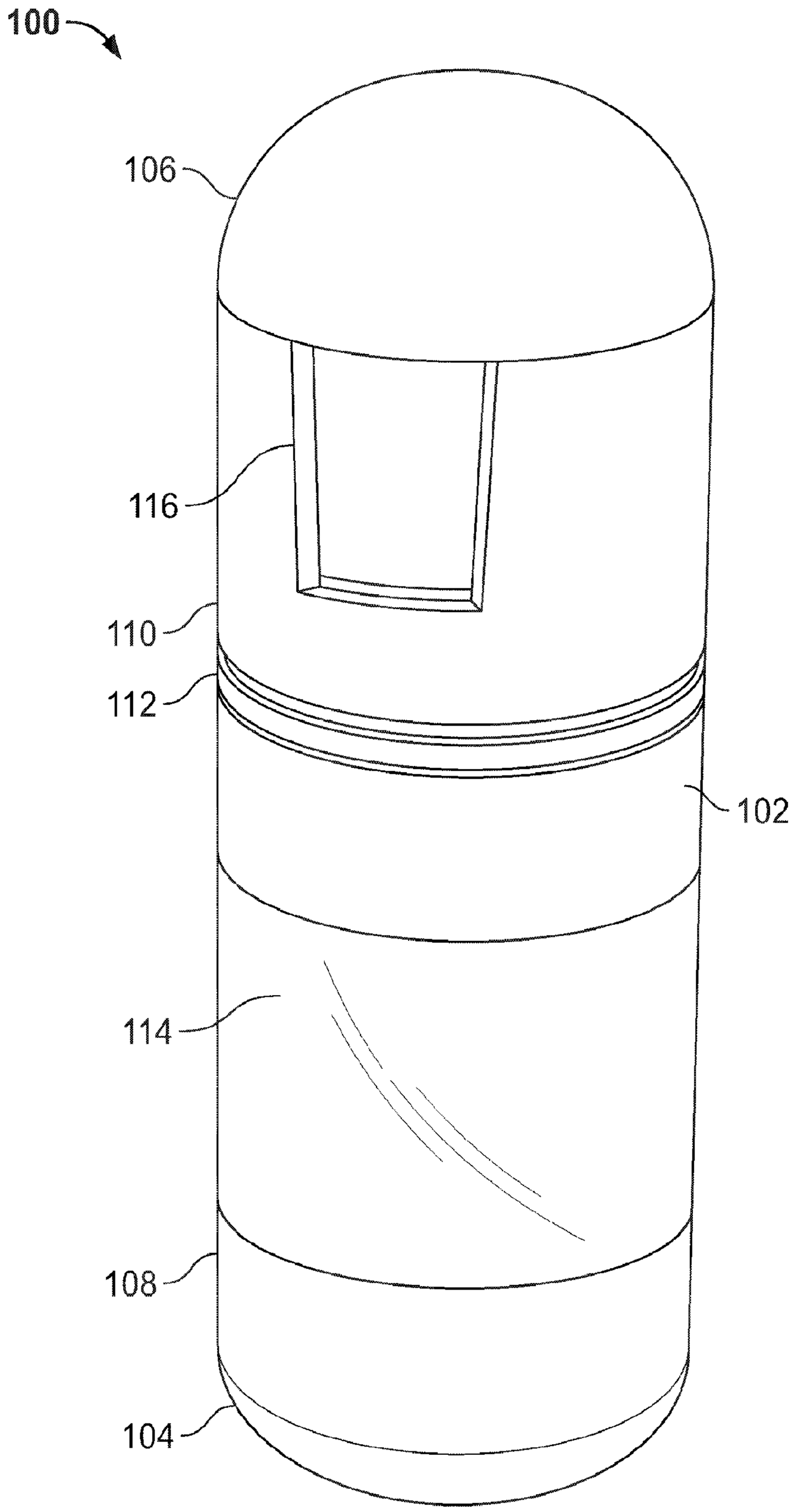


FIG. 1

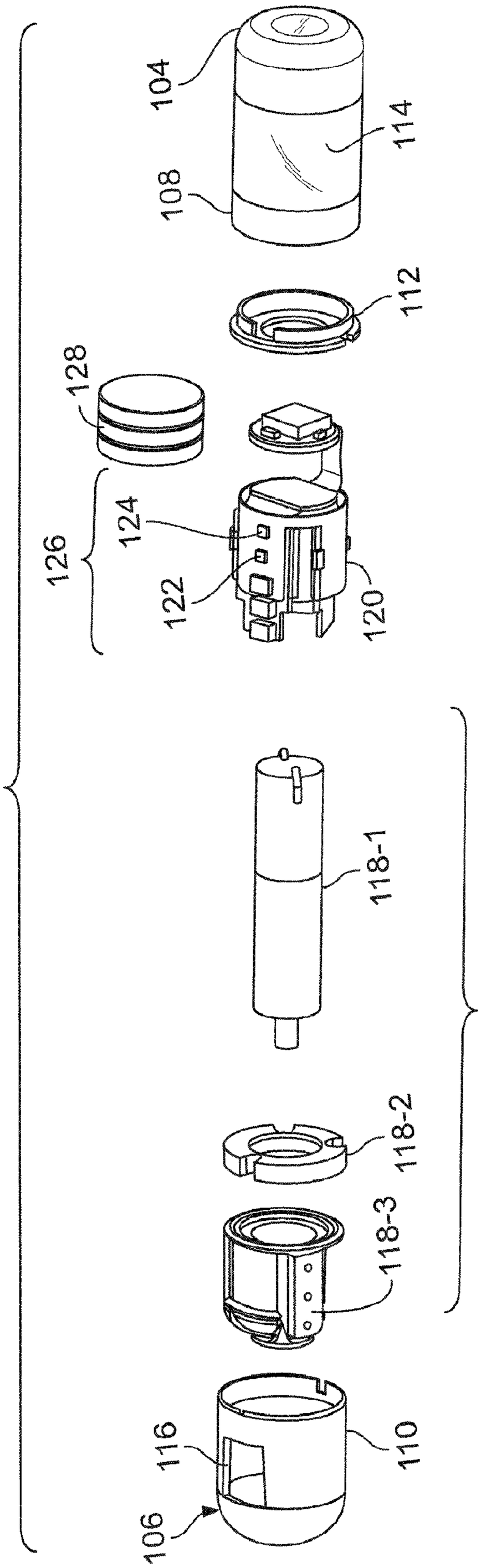

100
104
114
108
112
128
126
124
122
120
118
118-1
118-2
118-3
116
110
106

FIG. 2

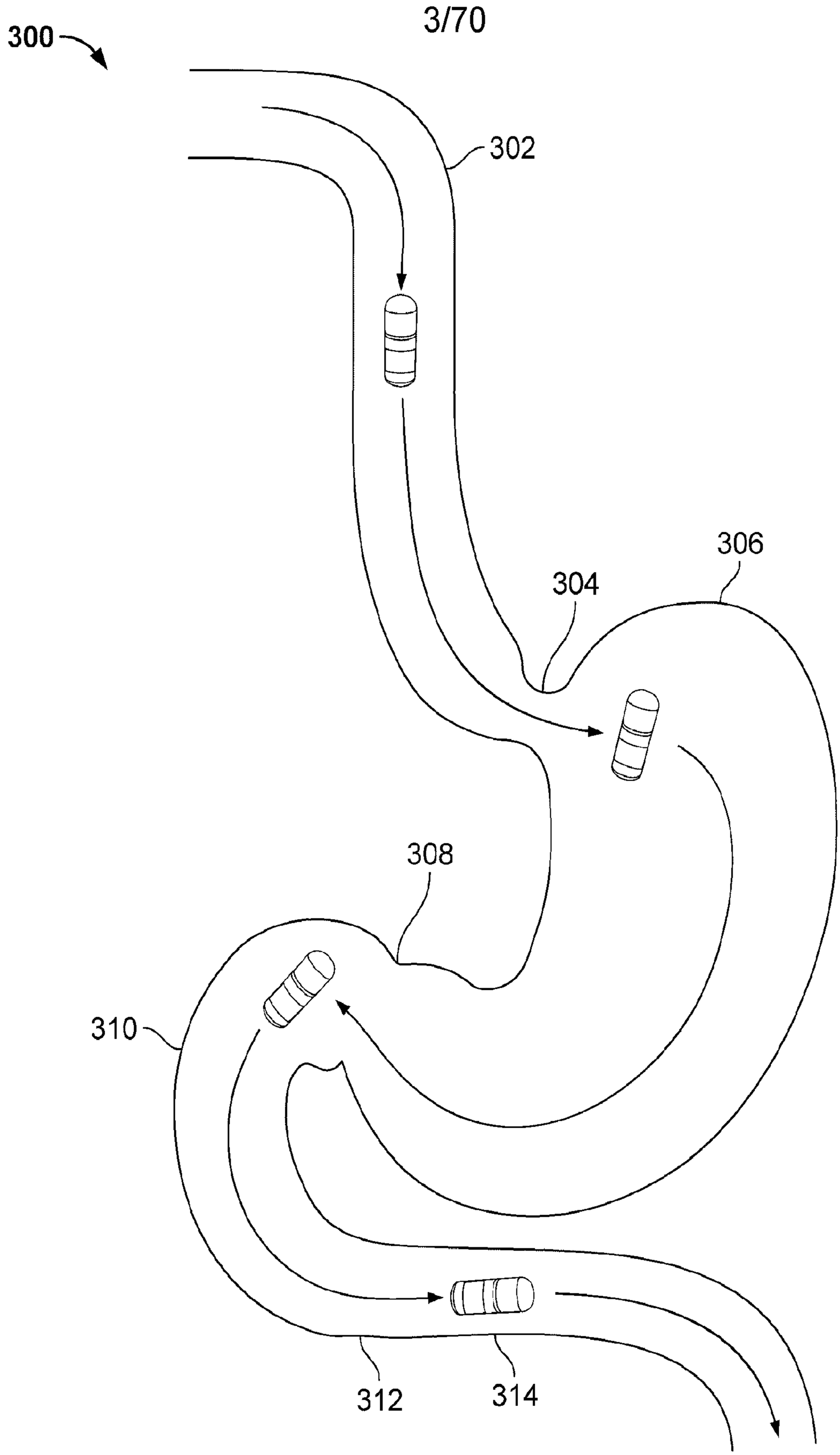


FIG. 3

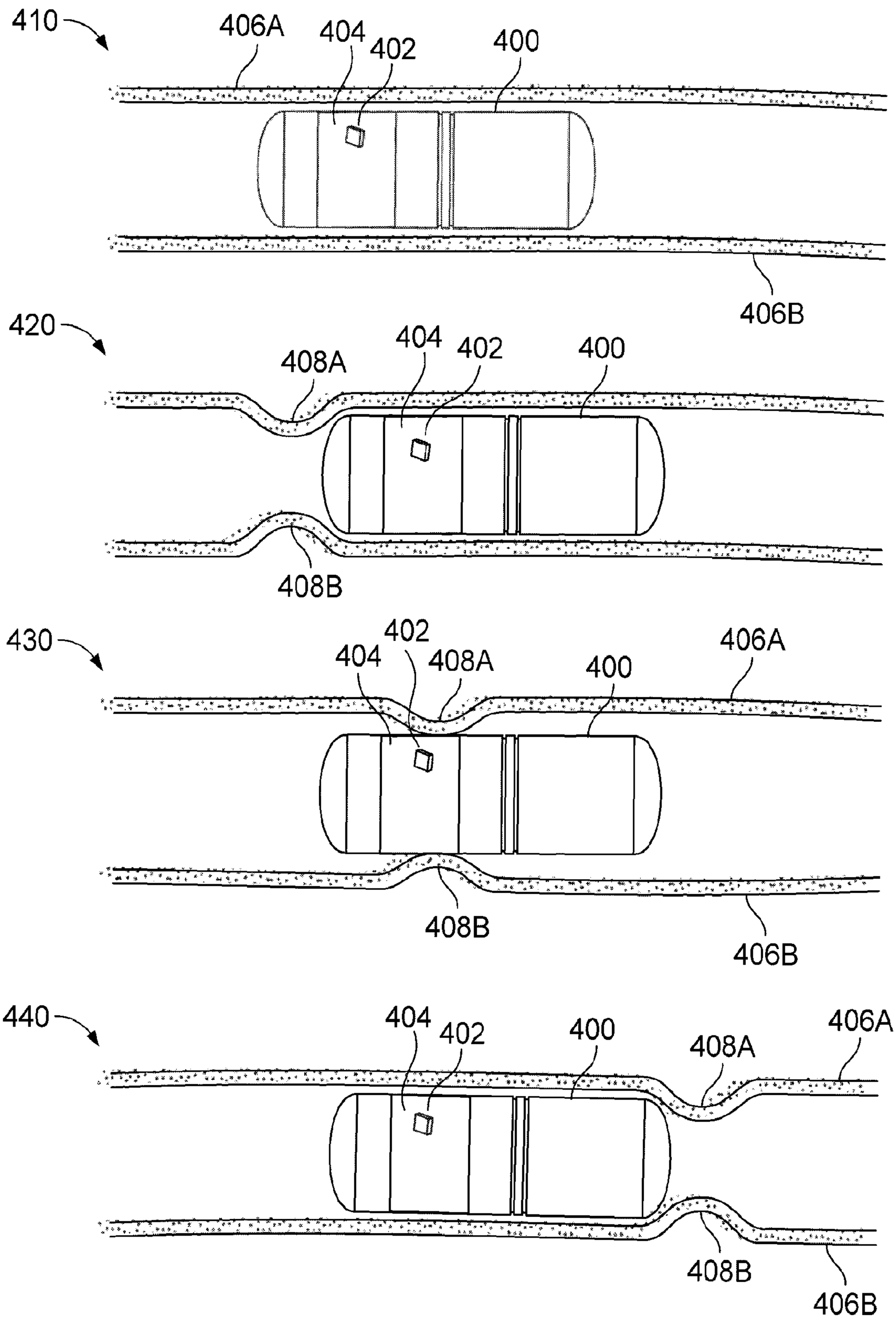

410
406A
404
402
400
406B
420
404
402
400
408A
408B
430
402
404
408A
400
406A
408B
406B
440
404
402
400
408A
406A
408B
406B

FIG. 4

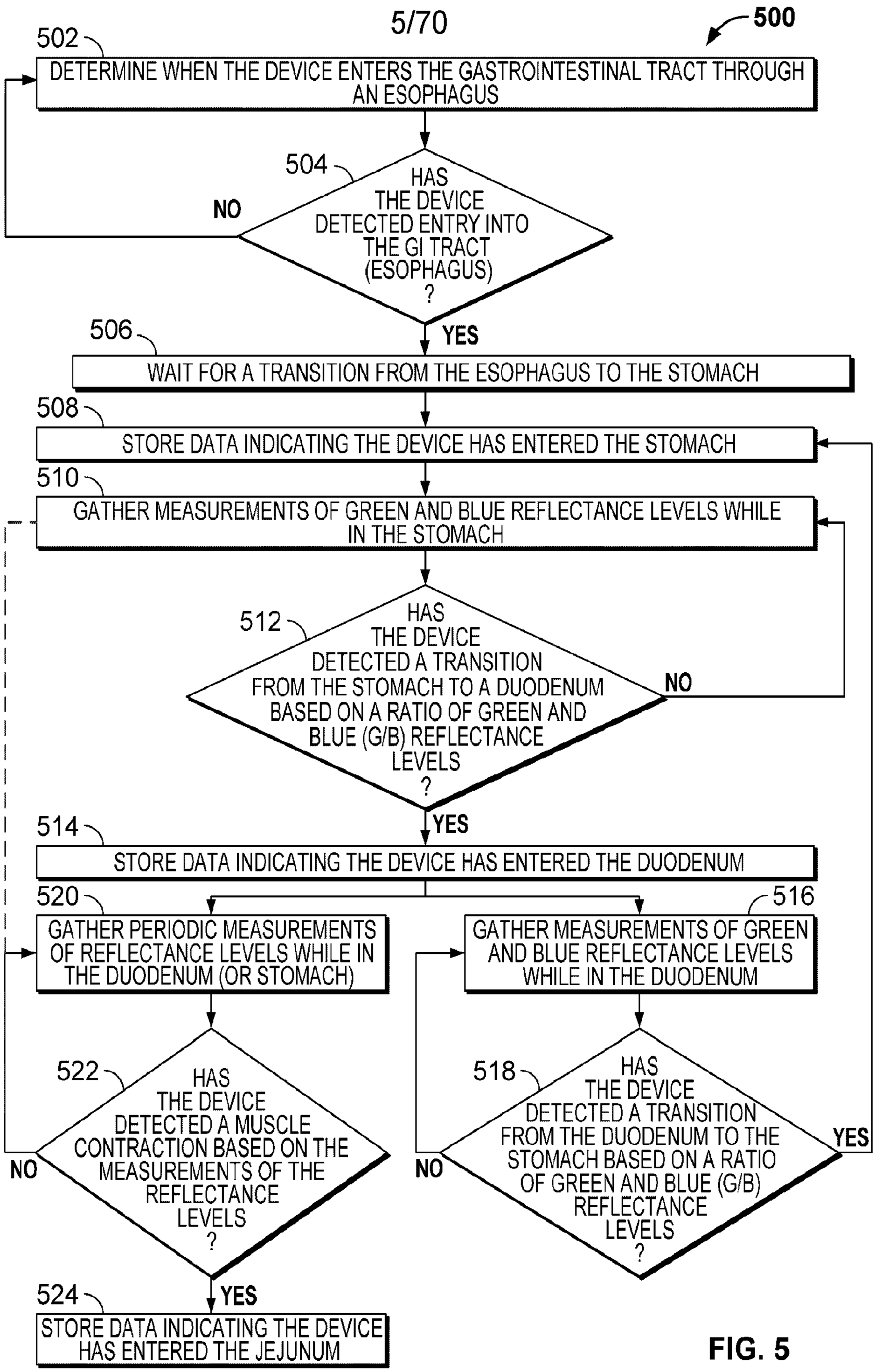

500
502
DETERMINE WHEN THE DEVICE ENTERS THE GASTROINTESTINAL TRACT THROUGH AN ESOPHAGUS
504
HAS THE DEVICE DETECTED ENTRY INTO THE GI TRACT (ESOPHAGUS) ?
NO
YES
506
WAIT FOR A TRANSITION FROM THE ESOPHAGUS TO THE STOMACH
508
STORE DATA INDICATING THE DEVICE HAS ENTERED THE STOMACH
510
GATHER MEASUREMENTS OF GREEN AND BLUE REFLECTANCE LEVELS WHILE IN THE STOMACH
512
HAS THE DEVICE DETECTED A TRANSITION FROM THE STOMACH TO A DUODENUM BASED ON A RATIO OF GREEN AND BLUE (G/B) REFLECTANCE LEVELS ?
NO
YES
514
STORE DATA INDICATING THE DEVICE HAS ENTERED THE DUODENUM
520
GATHER PERIODIC MEASUREMENTS OF REFLECTANCE LEVELS WHILE IN THE DUODENUM (OR STOMACH)
516
GATHER MEASUREMENTS OF GREEN AND BLUE REFLECTANCE LEVELS WHILE IN THE DUODENUM
522
HAS THE DEVICE DETECTED A MUSCLE CONTRACTION BASED ON THE MEASUREMENTS OF THE REFLECTANCE LEVELS ?
NO
YES
518
HAS THE DEVICE DETECTED A TRANSITION FROM THE DUODENUM TO THE STOMACH BASED ON A RATIO OF GREEN AND BLUE (G/B) REFLECTANCE LEVELS ?
NO
YES
524
STORE DATA INDICATING THE DEVICE HAS ENTERED THE JEJUNUM

FIG. 5

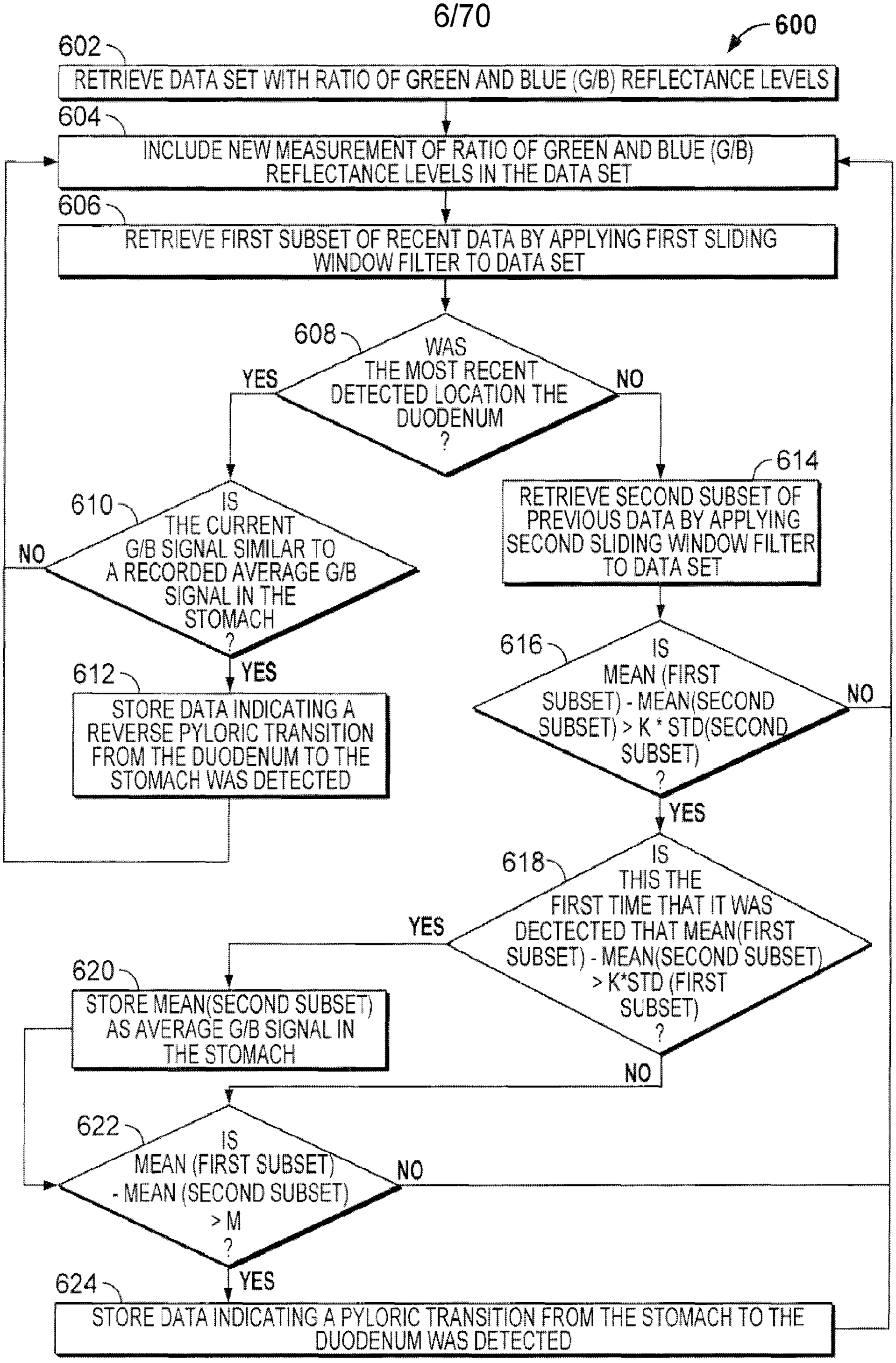

600
602
RETRIEVE DATA SET WITH RATIO OF GREEN AND BLUE (G/B) REFLECTANCE LEVELS
604
INCLUDE NEW MEASUREMENT OF RATIO OF GREEN AND BLUE (G/B) REFLECTANCE LEVELS IN THE DATA SET
606
RETRIEVE FIRST SUBSET OF RECENT DATA BY APPLYING FIRST SLIDING WINDOW FILTER TO DATA SET
608
WAS THE MOST RECENT DETECTED LOCATION THE DUODENUM ?
YES
NO
610
IS THE CURRENT G/B SIGNAL SIMILAR TO A RECORDED AVERAGE G/B SIGNAL IN THE STOMACH ?
NO
YES
612
STORE DATA INDICATING A REVERSE PYLORIC TRANSITION FROM THE DUODENUM TO THE STOMACH WAS DETECTED
614
RETRIEVE SECOND SUBSET OF PREVIOUS DATA BY APPLYING SECOND SLIDING WINDOW FILTER TO DATA SET
616
IS MEAN (FIRST SUBSET) - MEAN(SECOND SUBSET) > K * STD(SECOND SUBSET) ?
NO
YES
618
IS THIS THE FIRST TIME THAT IT WAS DECTECTED THAT MEAN(FIRST SUBSET) - MEAN(SECOND SUBSET) > K*STD (FIRST SUBSET) ?
YES
NO
620
STORE MEAN(SECOND SUBSET) AS AVERAGE G/B SIGNAL IN THE STOMACH
622
IS MEAN (FIRST SUBSET) - MEAN (SECOND SUBSET) > M ?
NO
YES
624
STORE DATA INDICATING A PYLORIC TRANSITION FROM THE STOMACH TO THE DUODENUM WAS DETECTED

FIG. 6

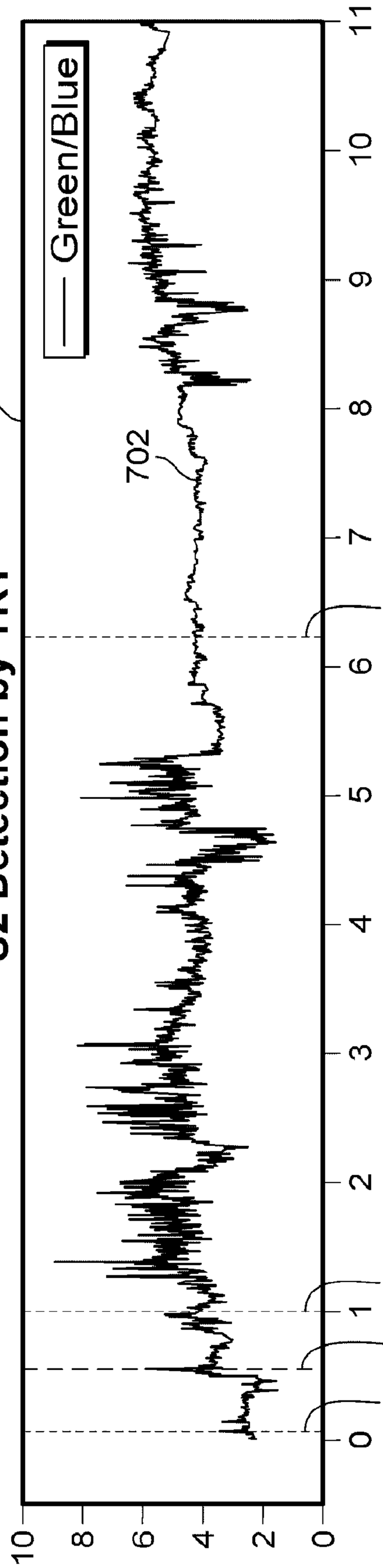

Green/Blue
700
702
710
S2 Detection by TRT
Time (hour)
708
706
704
Green Blue Ratio
11
10
9
8
7
6
5
4
3
2
1
0
10
8
6
4
2
0

FIG. 7

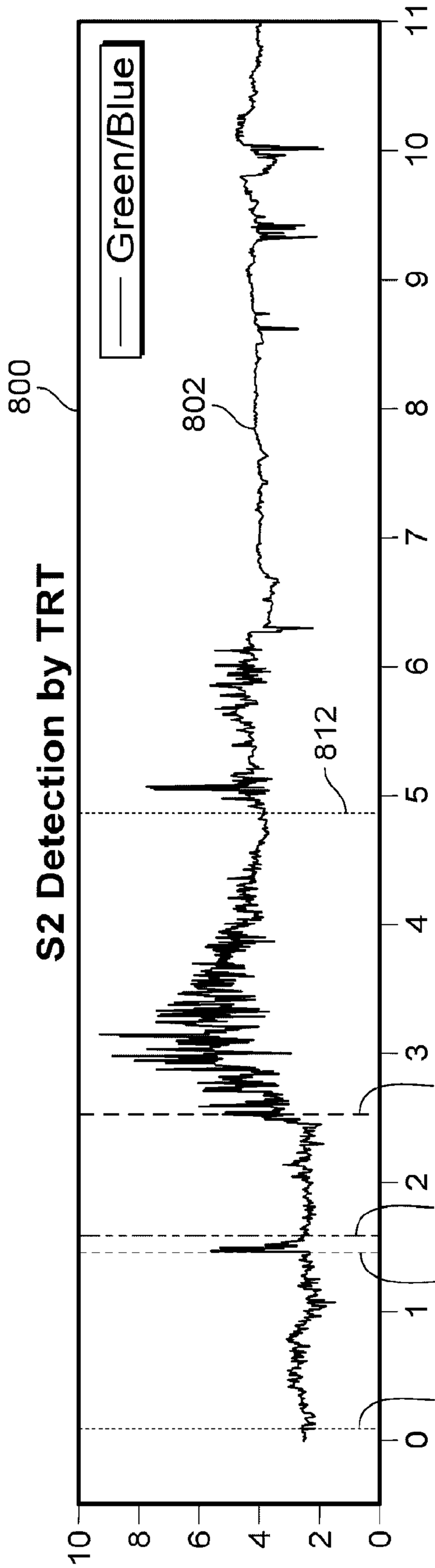

Green/Blue
800
802
812
S2 Detection by TRT
Time (hour)
810
808
806
804
Green Blue Ratio
11
10
9
8
7
6
5
4
3
2
1
0
10
8
6
4
2
0

FIG. 8

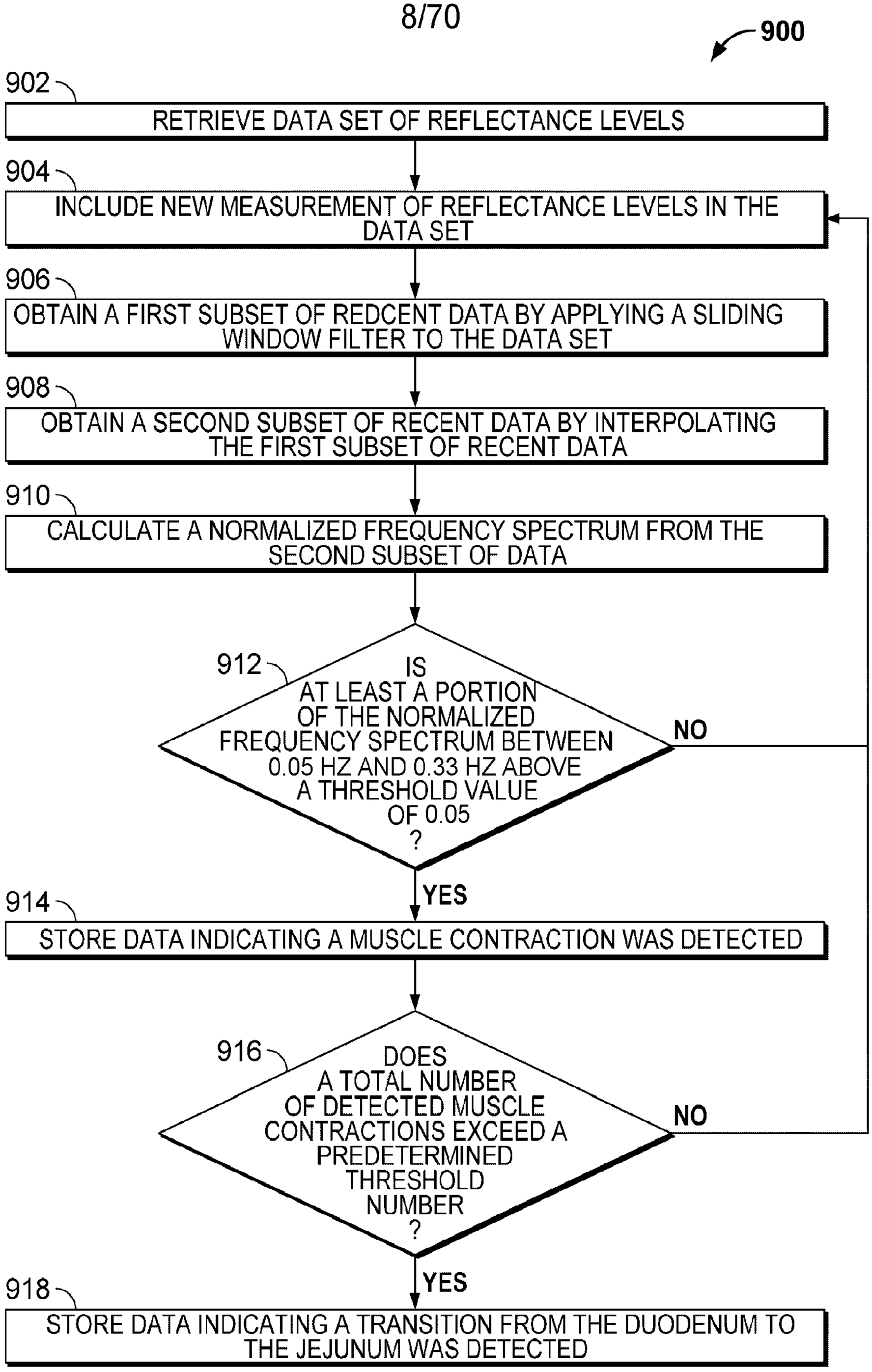

900
902
RETRIEVE DATA SET OF REFLECTANCE LEVELS
904
INCLUDE NEW MEASUREMENT OF REFLECTANCE LEVELS IN THE DATA SET
906
OBTAIN A FIRST SUBSET OF REDCENT DATA BY APPLYING A SLIDING WINDOW FILTER TO THE DATA SET
908
OBTAIN A SECOND SUBSET OF RECENT DATA BY INTERPOLATING THE FIRST SUBSET OF RECENT DATA
910
CALCULATE A NORMALIZED FREQUENCY SPECTRUM FROM THE SECOND SUBSET OF DATA
912
IS AT LEAST A PORTION OF THE NORMALIZED FREQUENCY SPECTRUM BETWEEN 0.05 HZ AND 0.33 HZ ABOVE A THRESHOLD VALUE OF 0.05 ?
NO
YES
914
STORE DATA INDICATING A MUSCLE CONTRACTION WAS DETECTED
916
DOES A TOTAL NUMBER OF DETECTED MUSCLE CONTRACTIONS EXCEED A PREDETERMINED THRESHOLD NUMBER ?
NO
YES
918
STORE DATA INDICATING A TRANSITION FROM THE DUODENUM TO THE JEJUNUM WAS DETECTED

FIG. 9

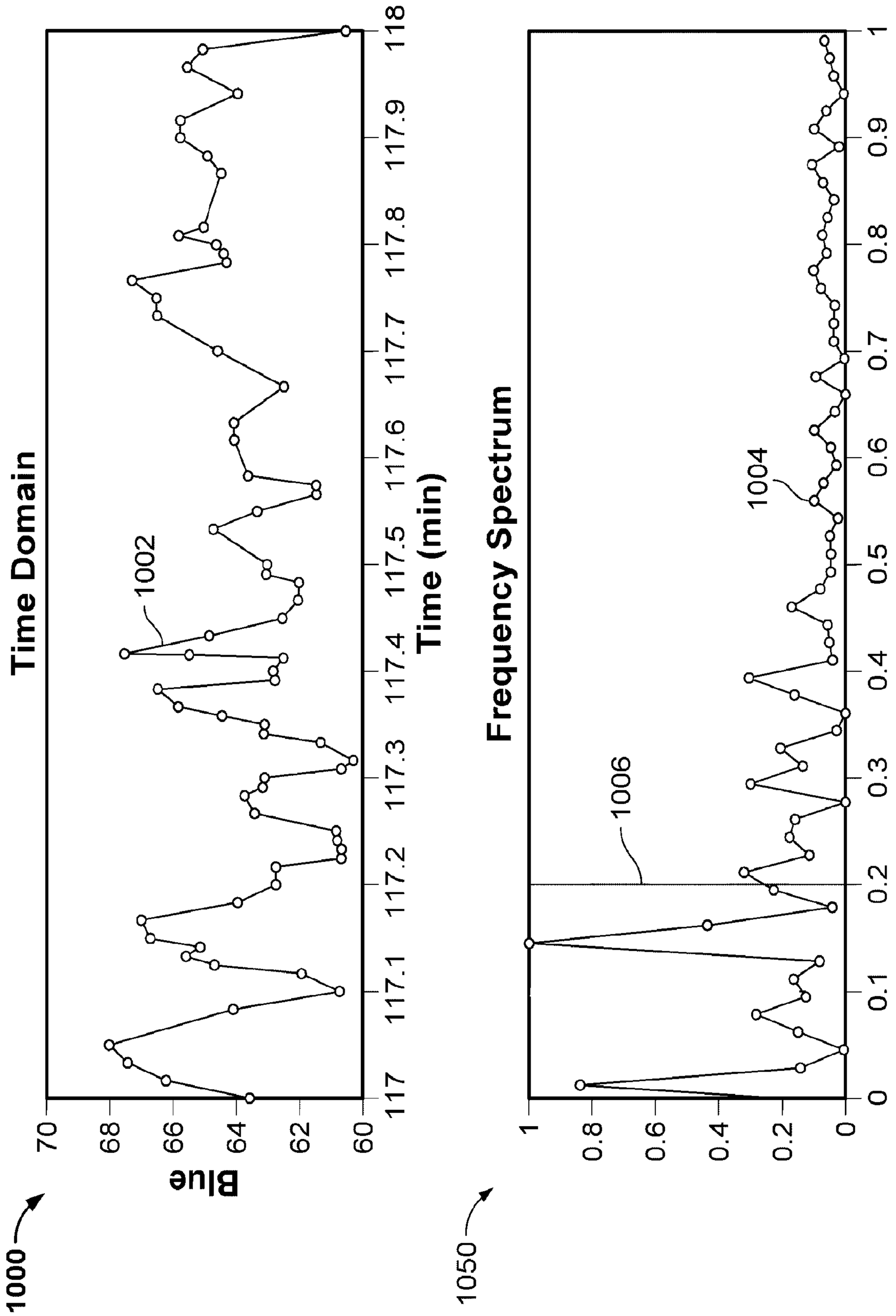

Time Domain
1002
Blue
70
68
66
64
62
60
118
117.9
117.8
117.7
117.6
117.5
117.4
Time (min)
117.3
117.2
117.1
117
1000
Frequency Spectrum
1004
1006
Frequency (Hz)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1050

FIG. 10

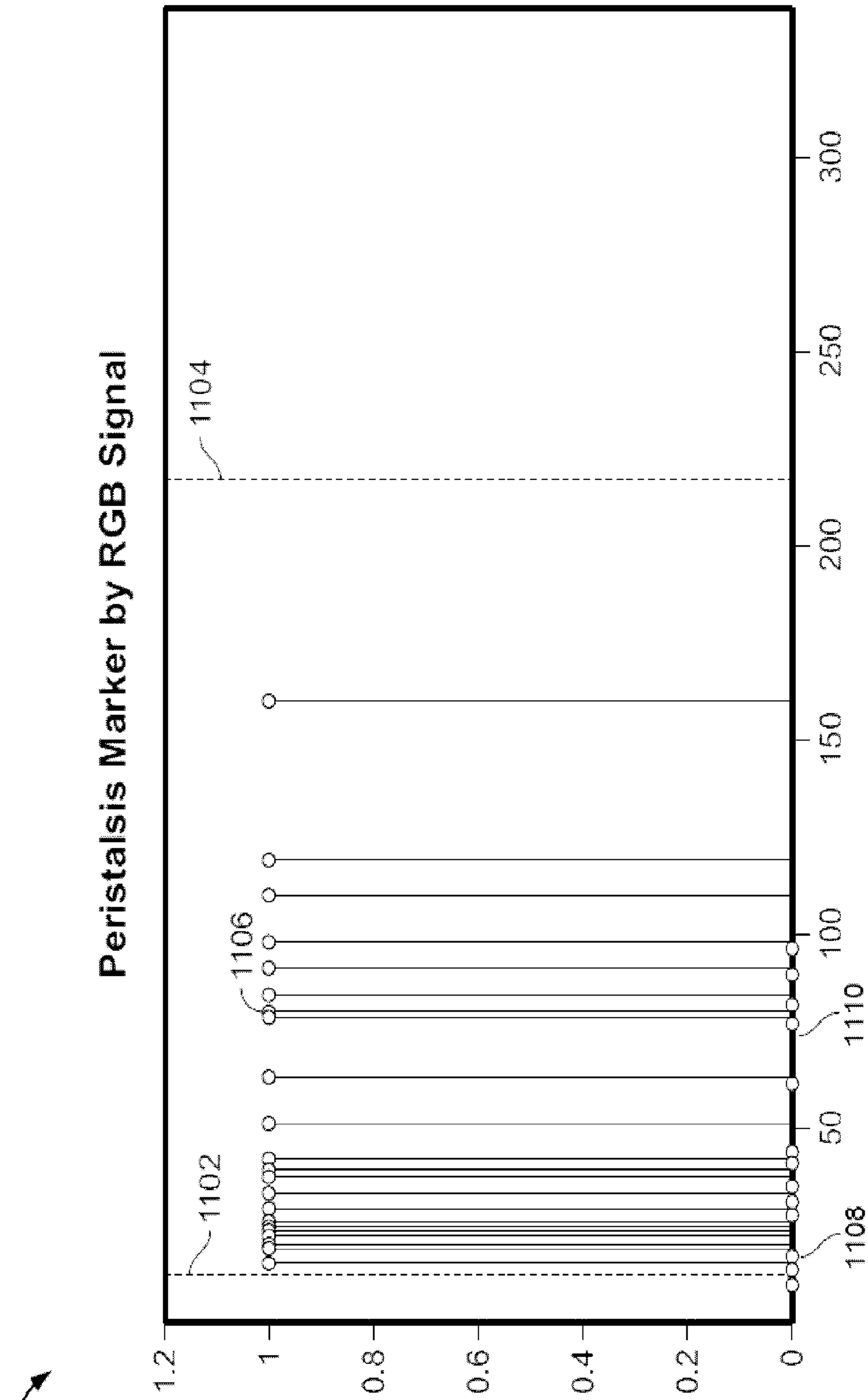

Peristalsis Marker by RGB Signal
1100
1102
1104
1106
1108
1110
1.2
1
0.8
0.6
0.4
0.2
0
50
100
150
200
250
300
Time (min)

FIG. 11

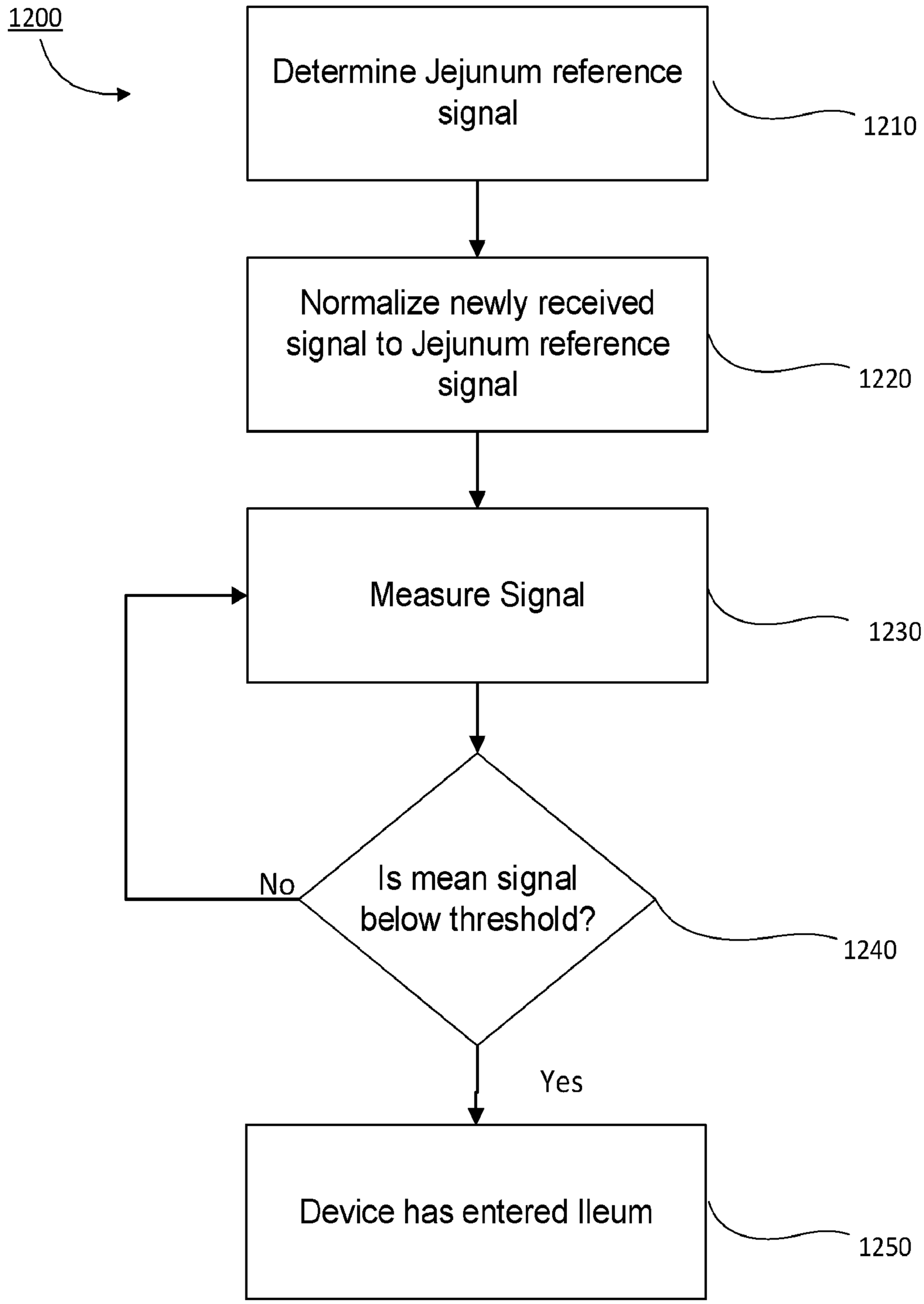

1200
Determine Jejunum reference signal
1210
Normalize newly received signal to Jejunum reference signal
1220
Measure Signal
1230
Is mean signal below threshold?
No
1240
Yes
Device has entered Ileum
1250

FIG. 12

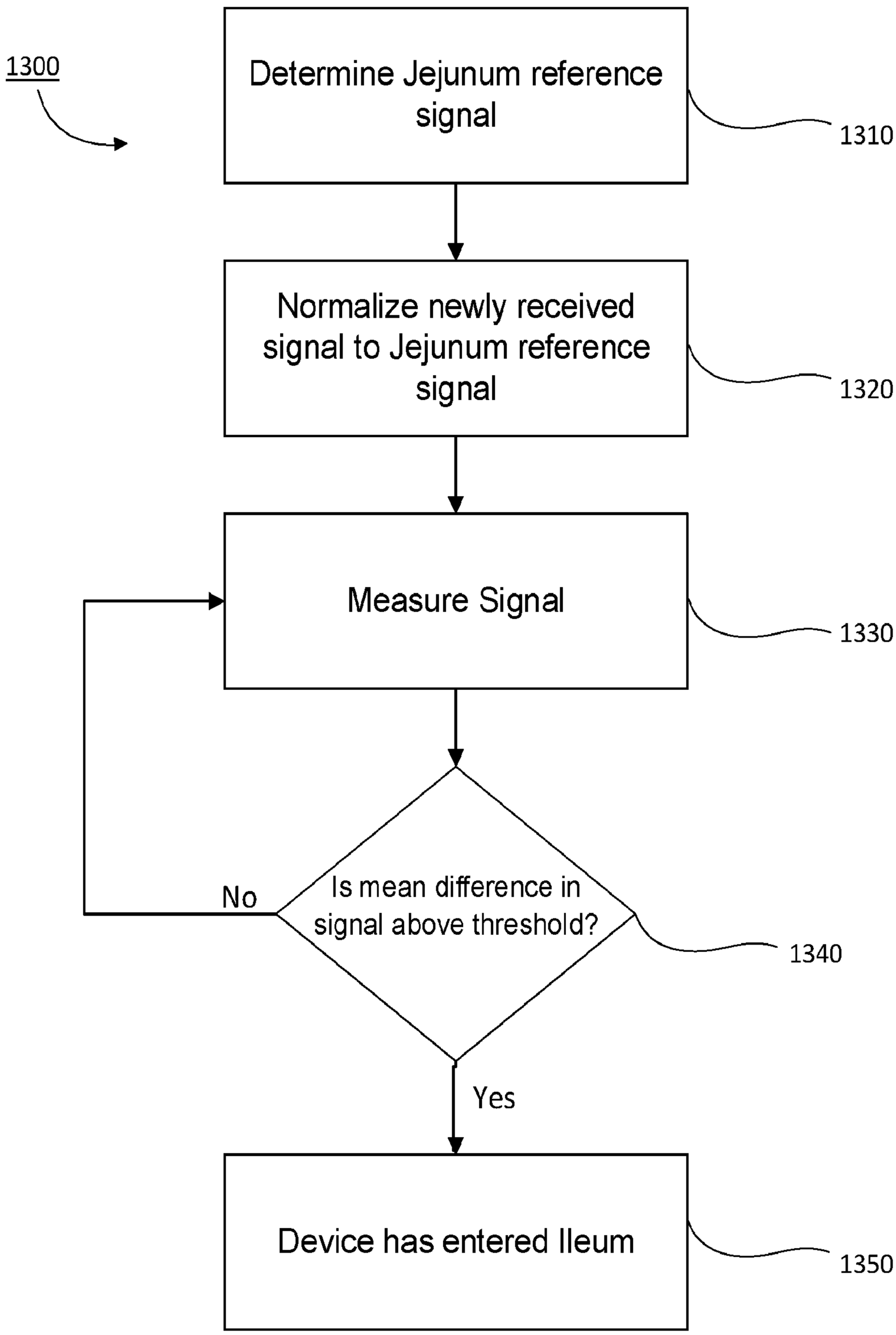

1300
Determine Jejunum reference signal
1310
Normalize newly received signal to Jejunum reference signal
1320
Measure Signal
1330
Is mean difference in signal above threshold?
No
1340
Yes
Device has entered Ileum
1350

FIG. 13

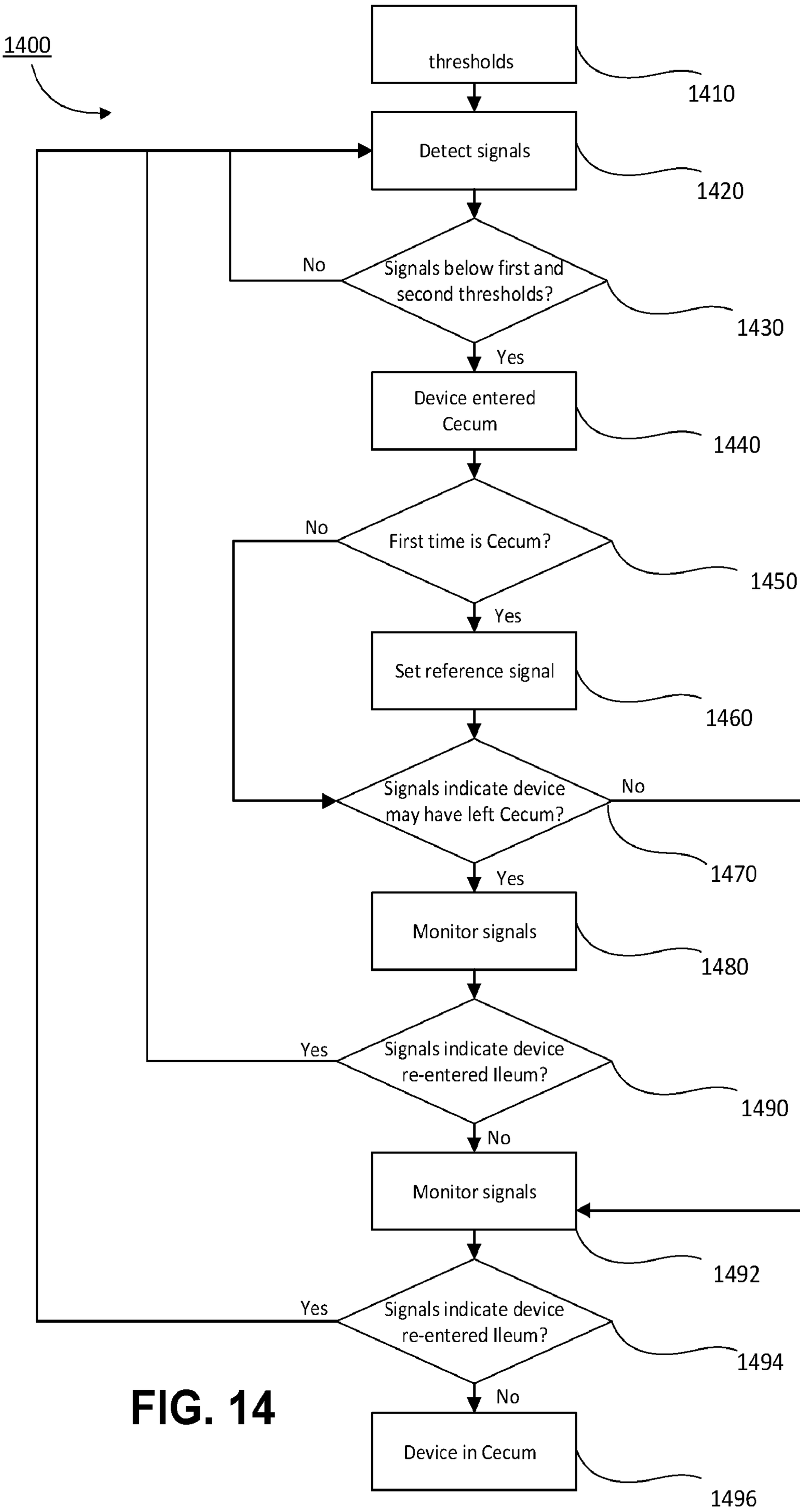

1400
thresholds
1410
Detect signals
1420
No
Signals below first and second thresholds?
1430
Yes
Device entered Cecum
1440
No
First time is Cecum?
1450
Yes
Set reference signal
1460
Signals indicate device may have left Cecum?
No
1470
Yes
Monitor signals
1480
Yes
Signals indicate device re-entered Ileum?
1490
No
Monitor signals
1492
Yes
Signals indicate device re-entered Ileum?
1494
No
Device in Cecum
1496

FIG. 14

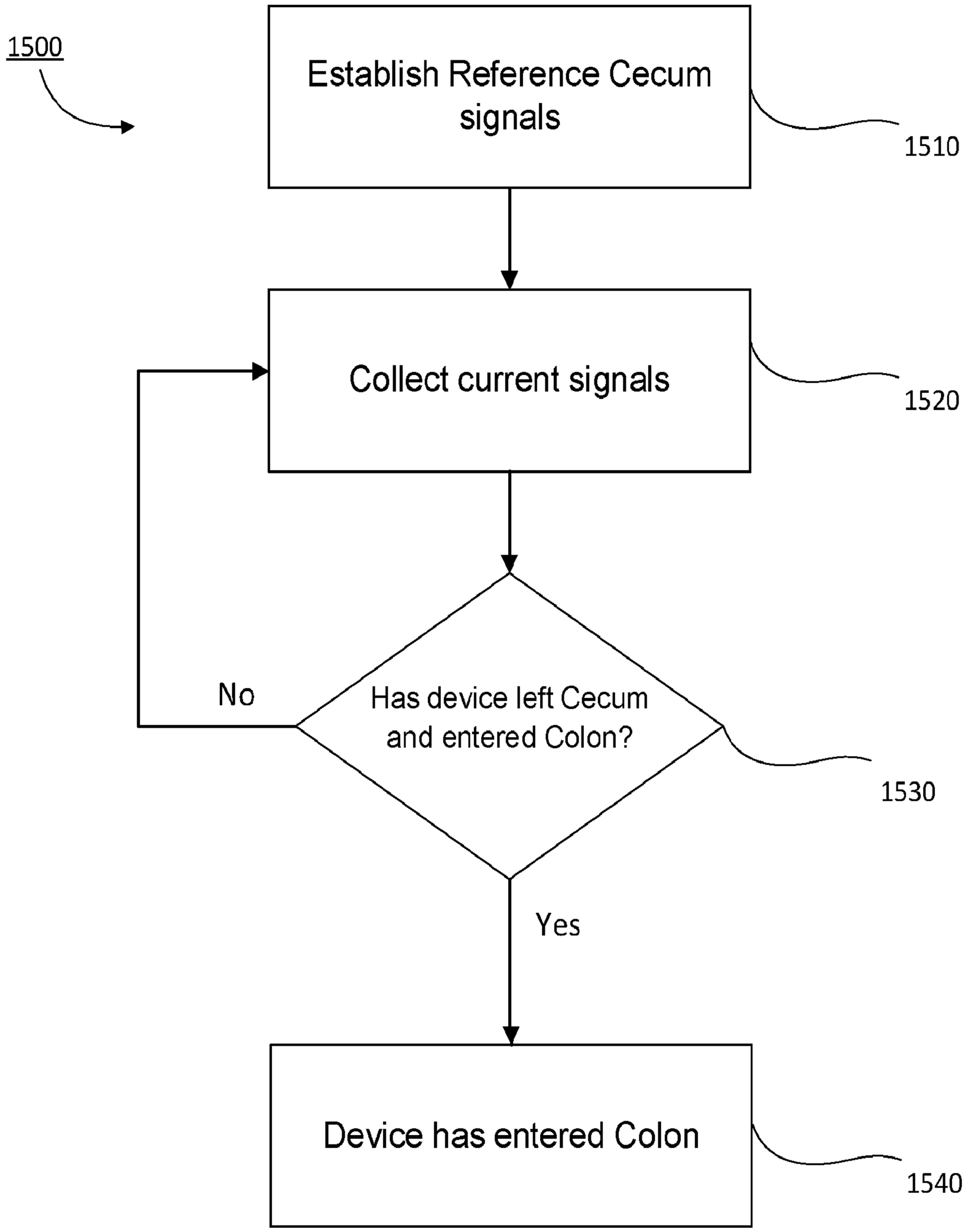

1500
Establish Reference Cecum signals
1510
Collect current signals
1520
No
Has device left Cecum and entered Colon?
1530
Yes
Device has entered Colon
1540

FIG. 15

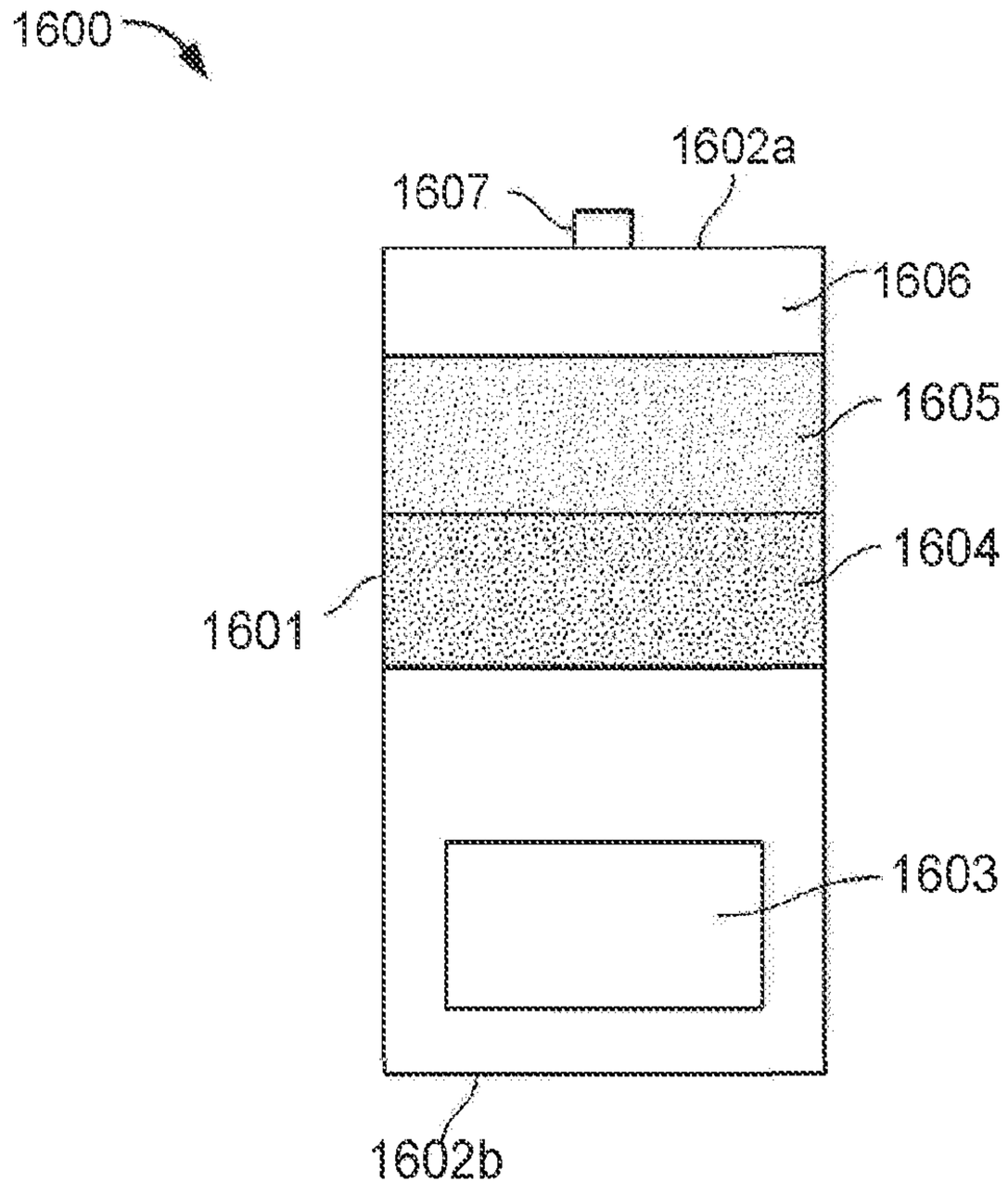

1600
1602a
1607
1606
1605
1604
1601
1603
1602b

FIG. 16

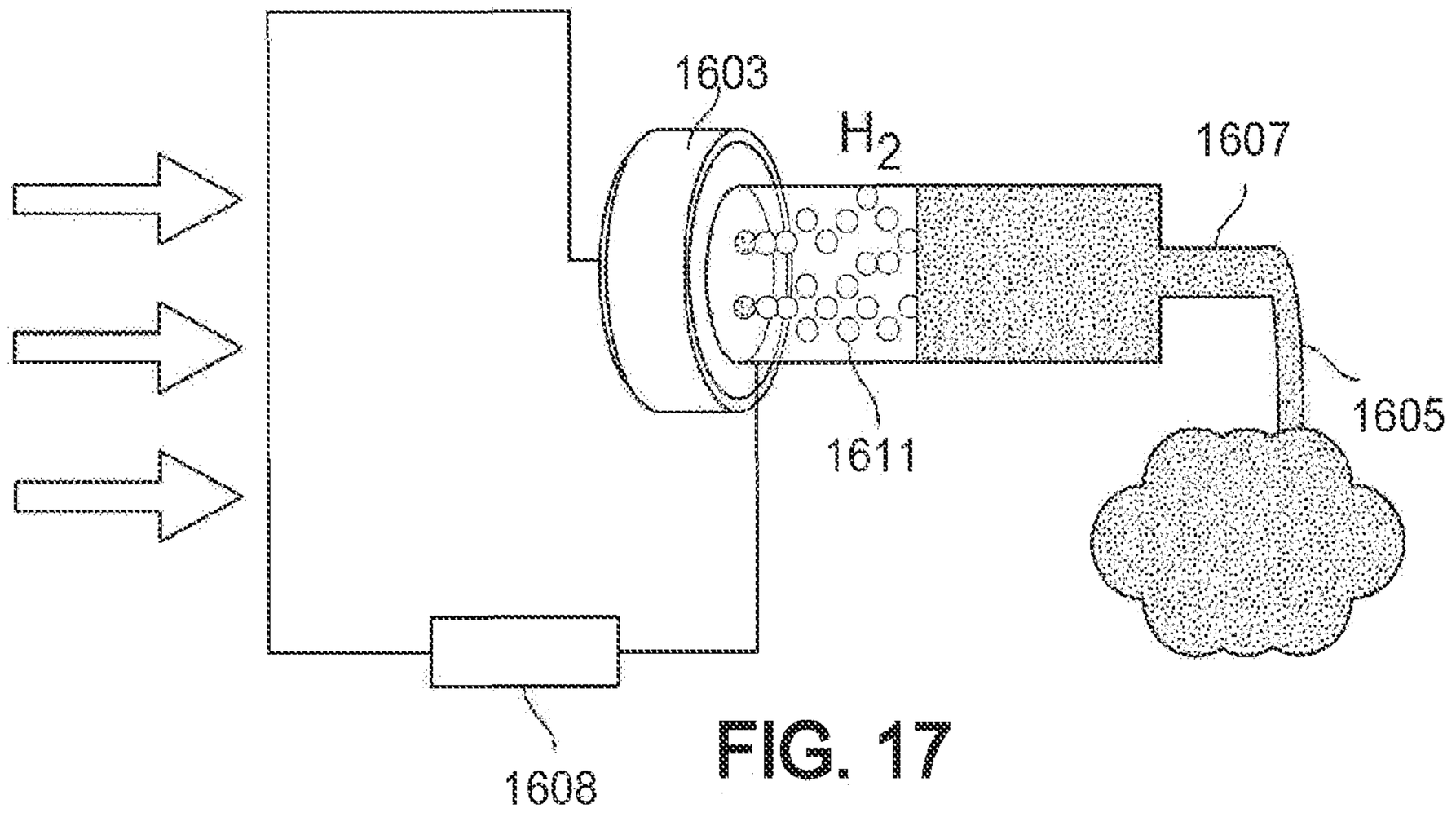

1603
H_2
1607
1605
1611
1608

FIG. 17

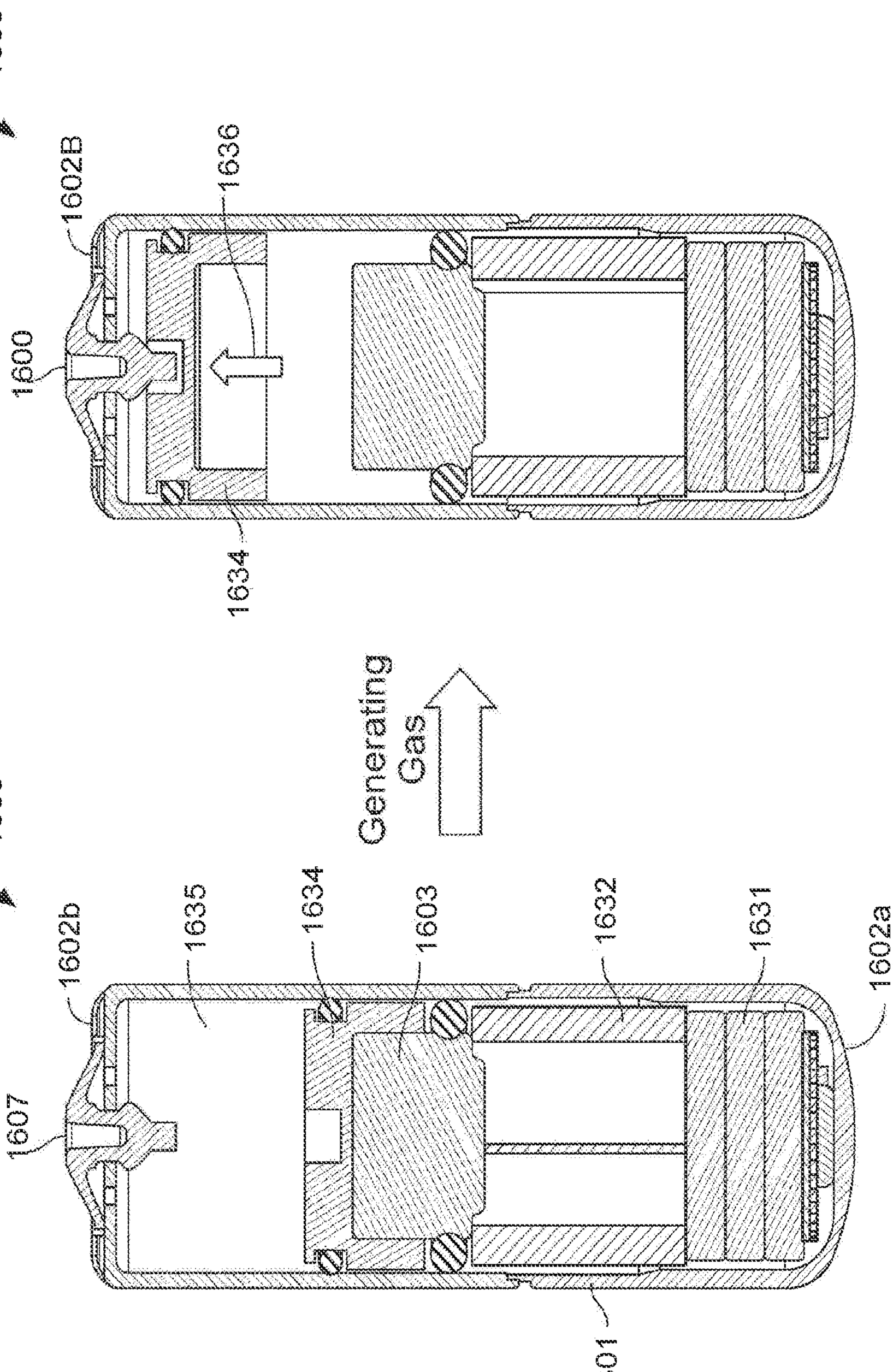

1600
1602b
1635
1634
1603
1632
1631
1602a
1607
1601
Generating Gas
1600
1636
1634
1600

FIG. 18

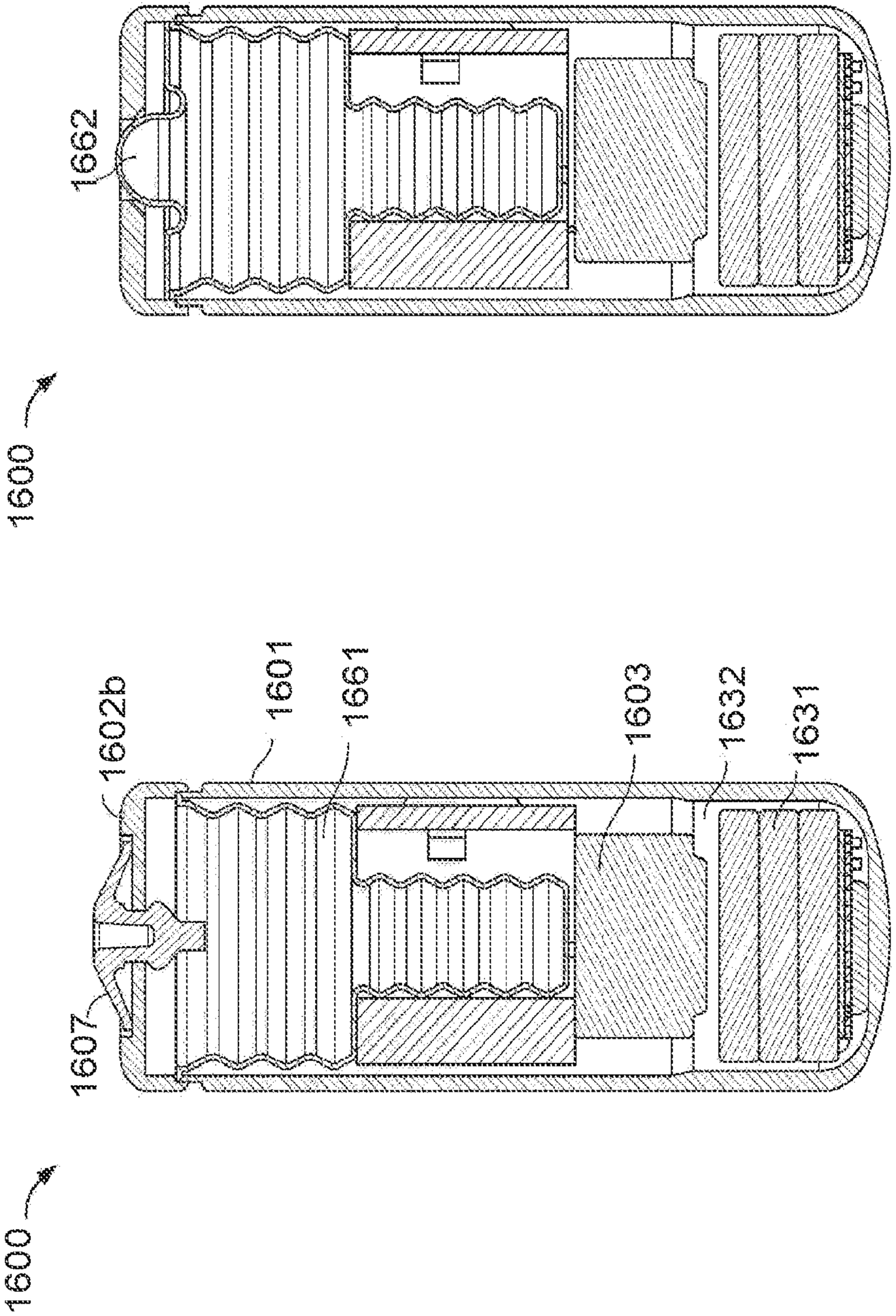


FIG. 19

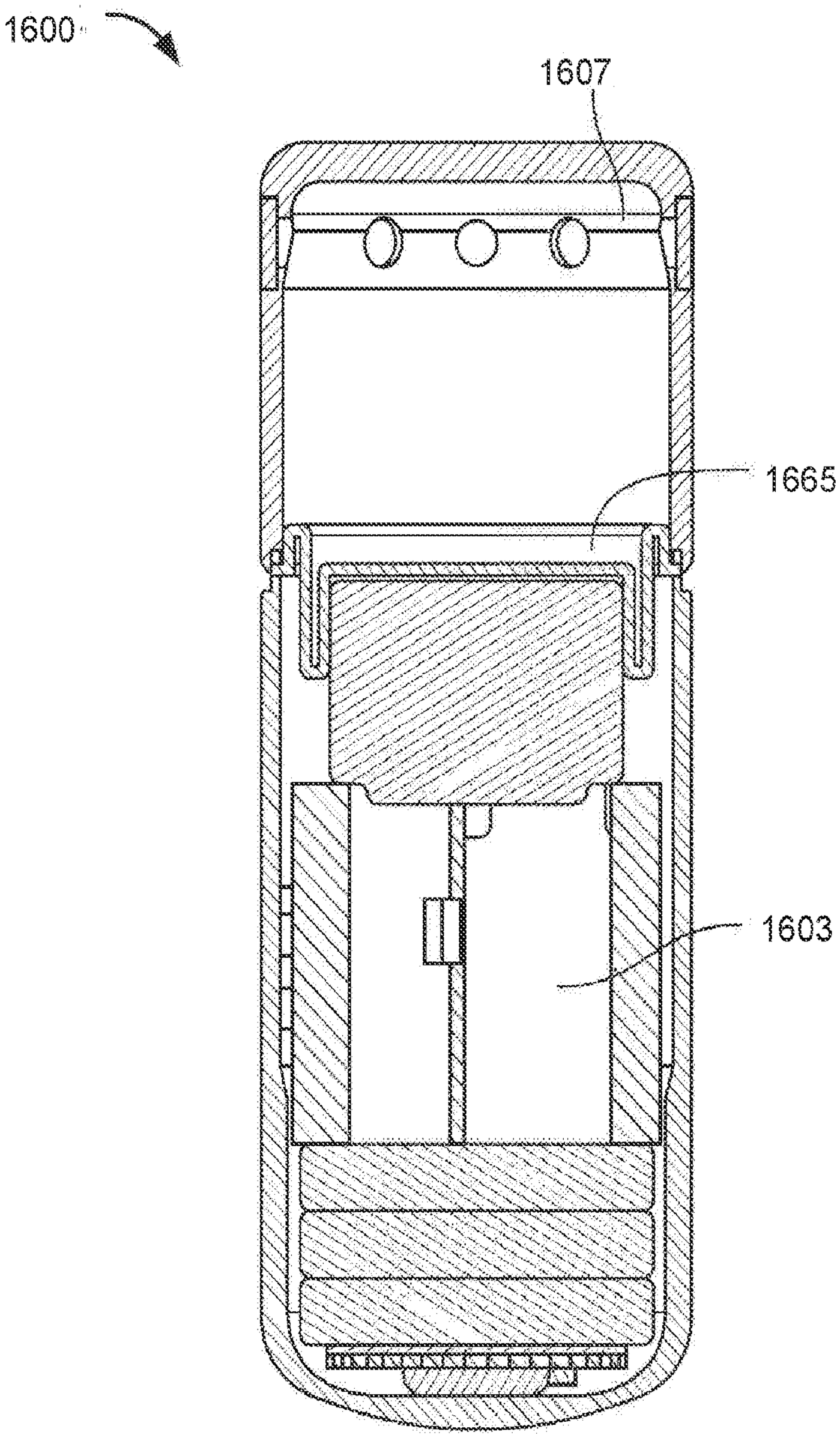


FIG. 20

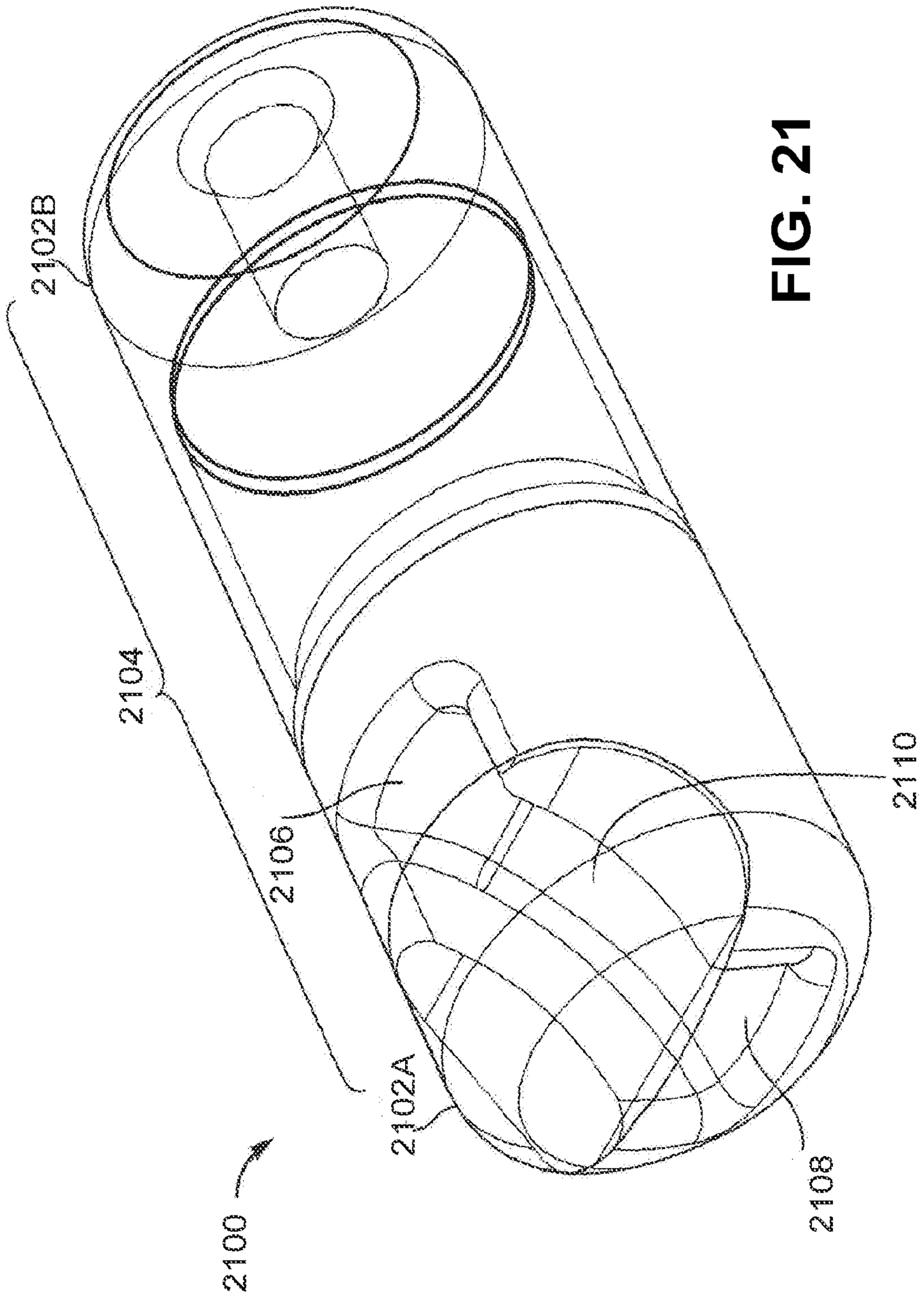


FIG. 21

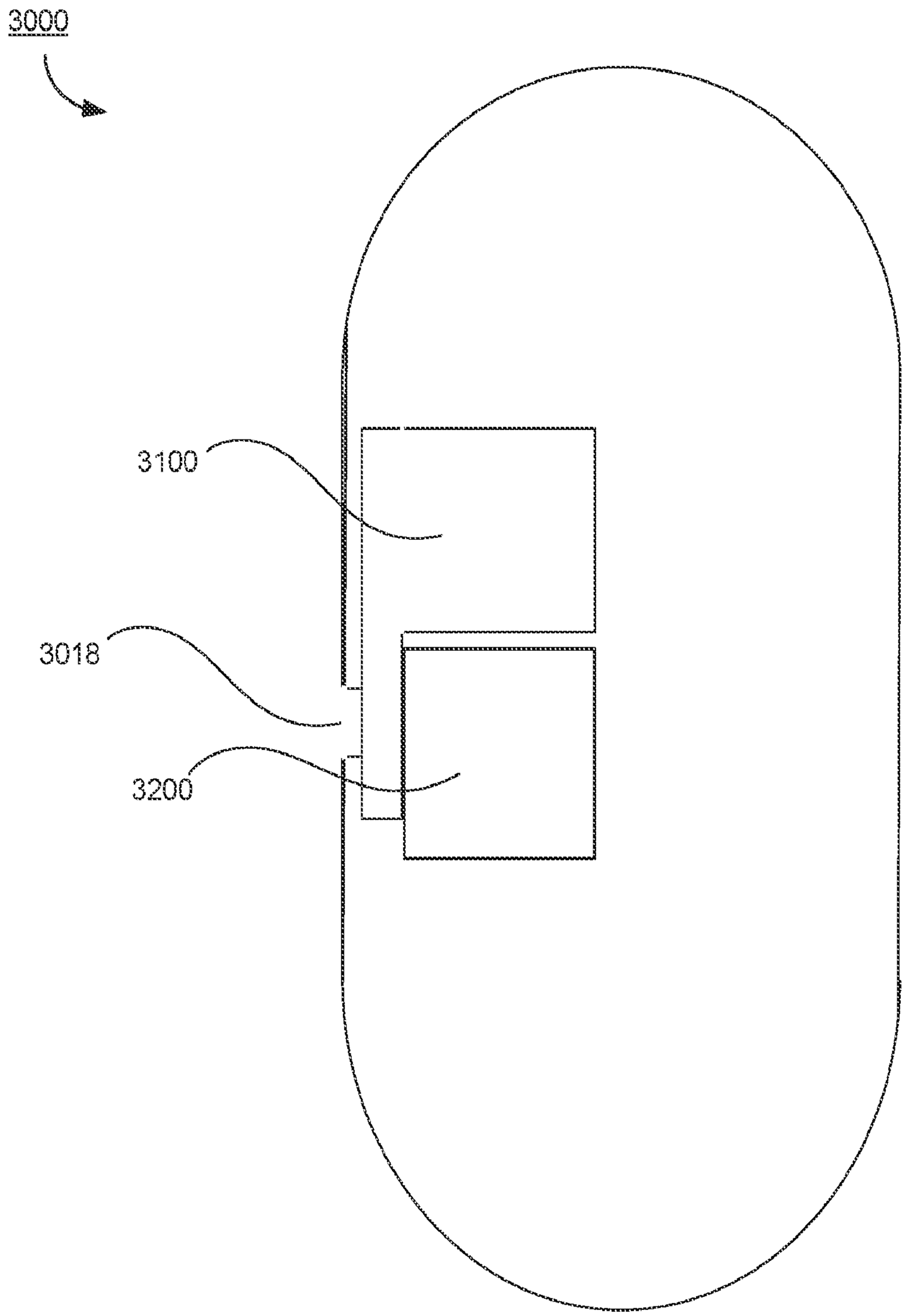


FIG. 22

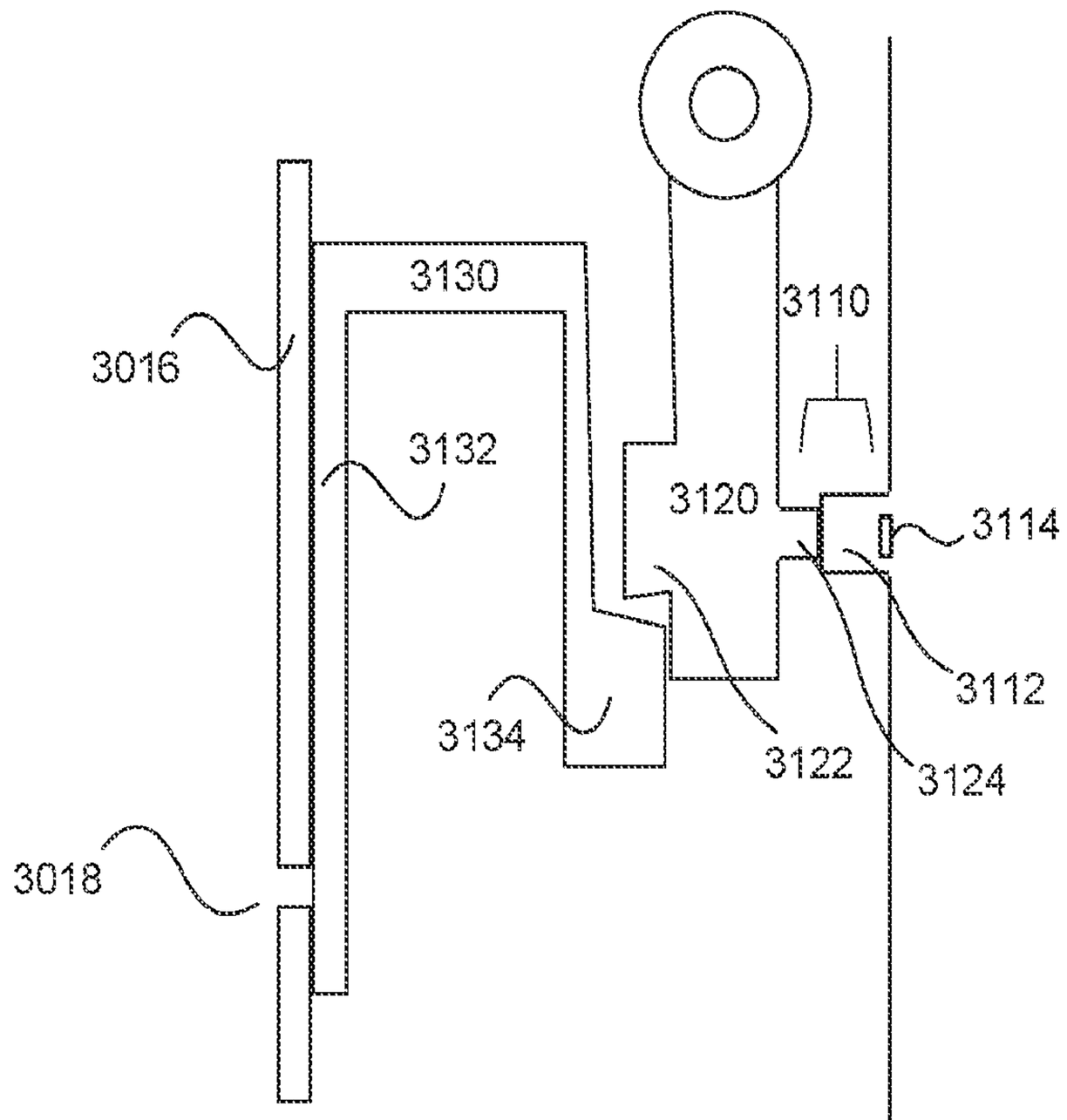


FIG. 23

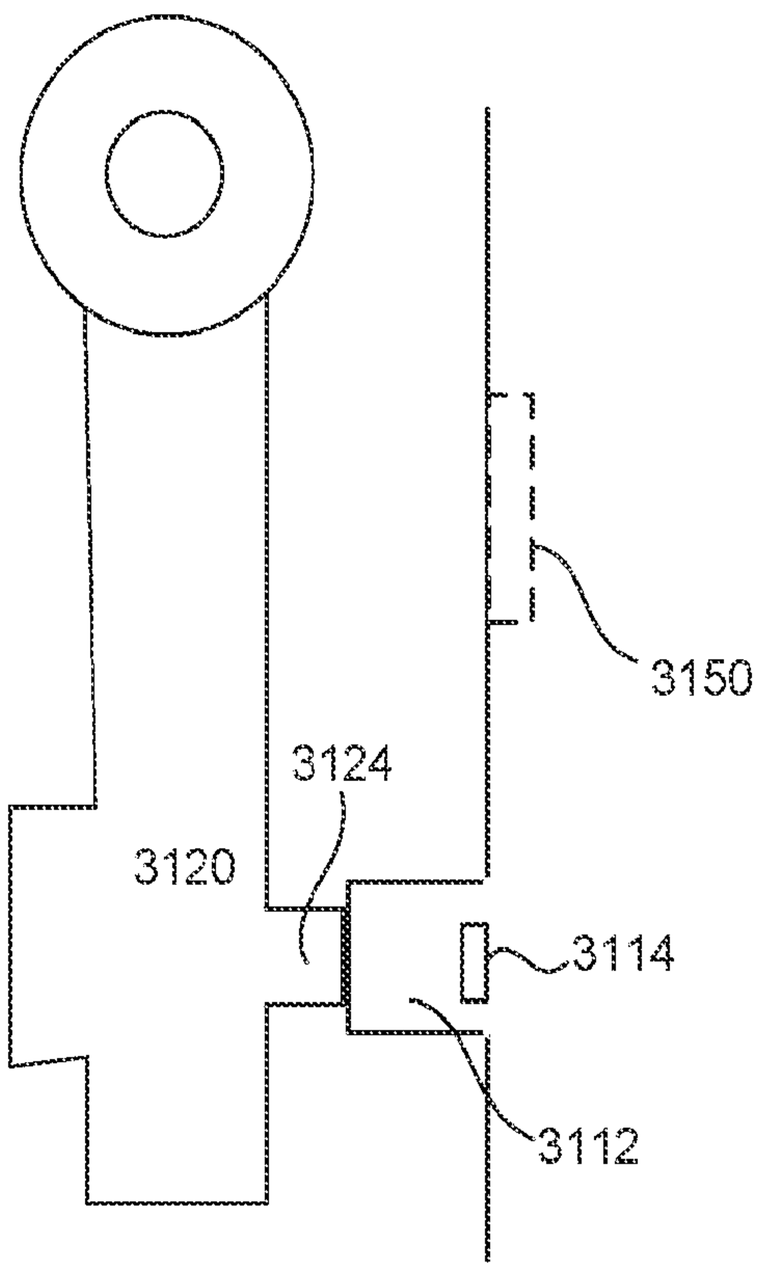


FIG. 24A

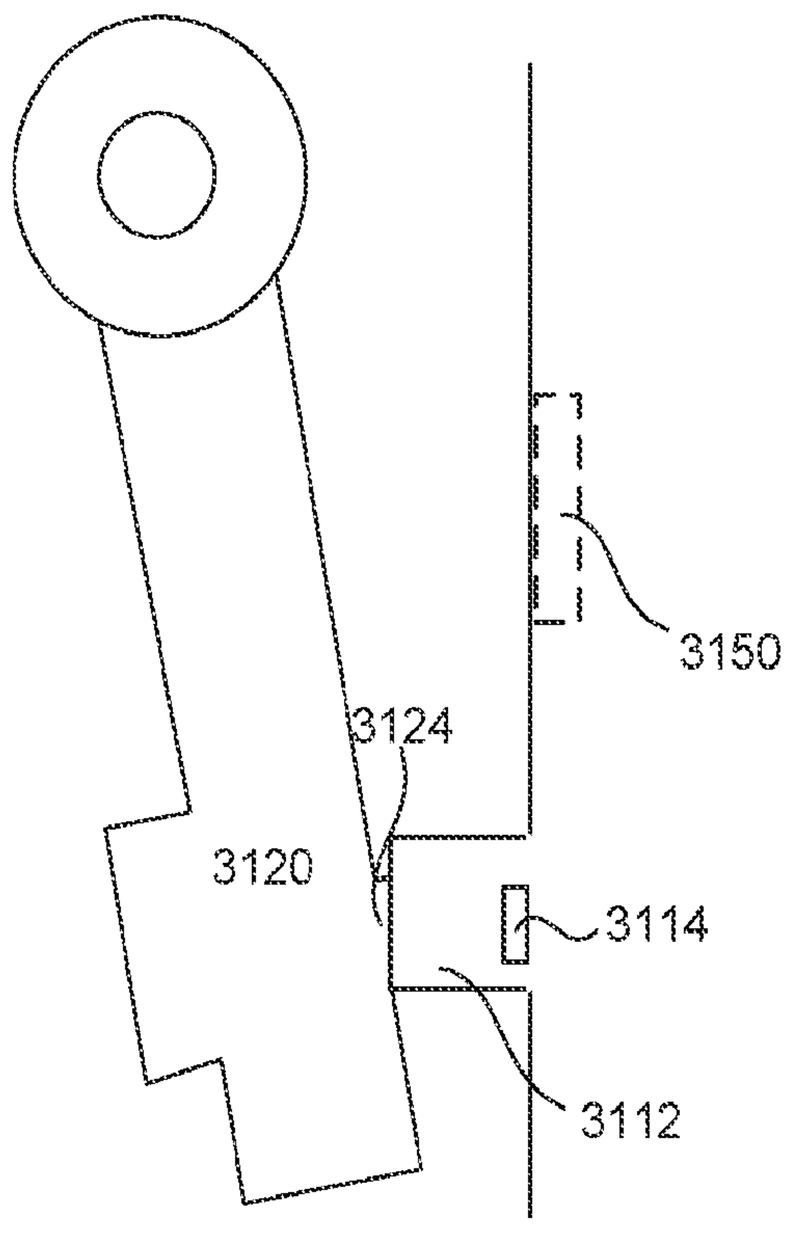


FIG. 24B

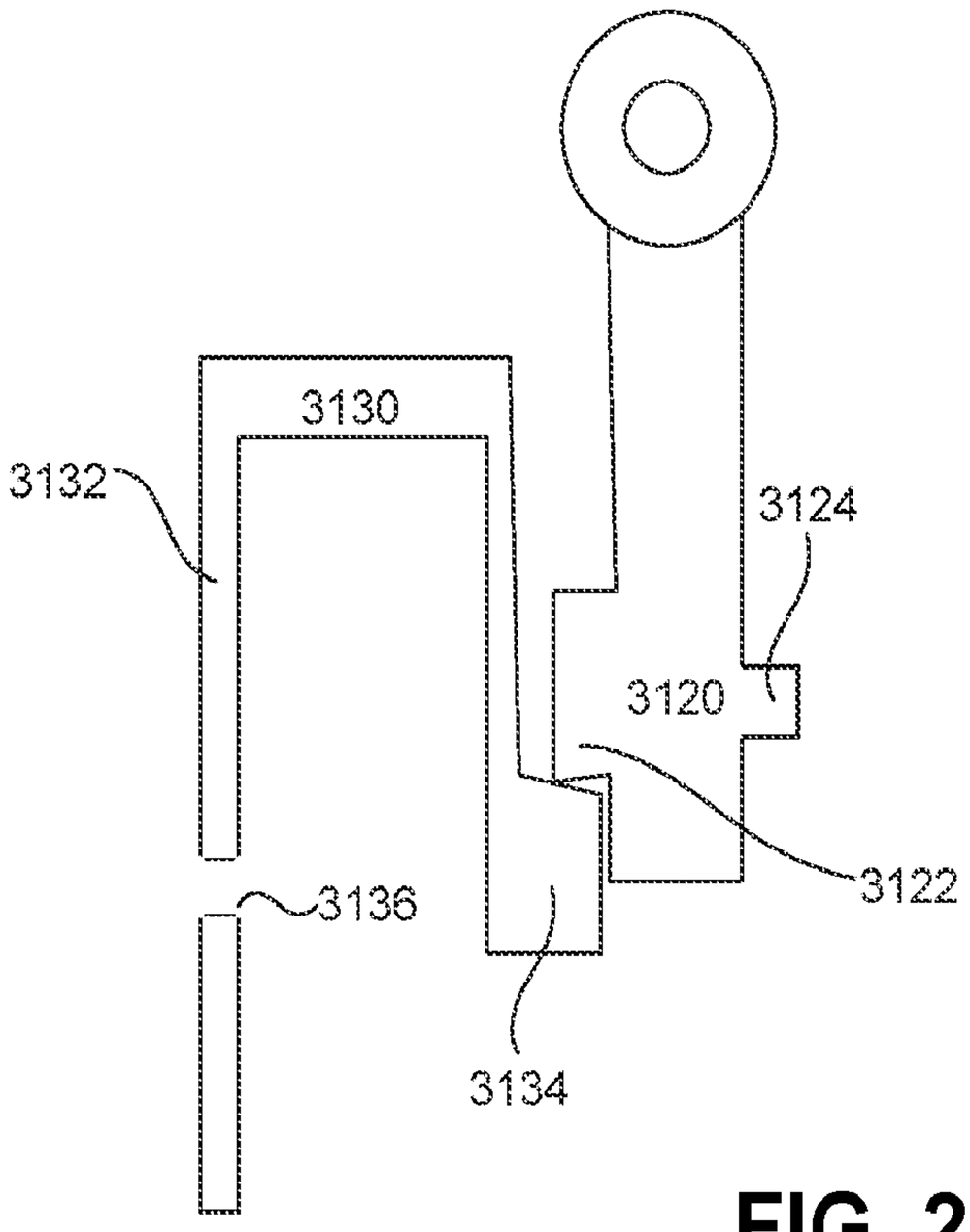


FIG. 25A

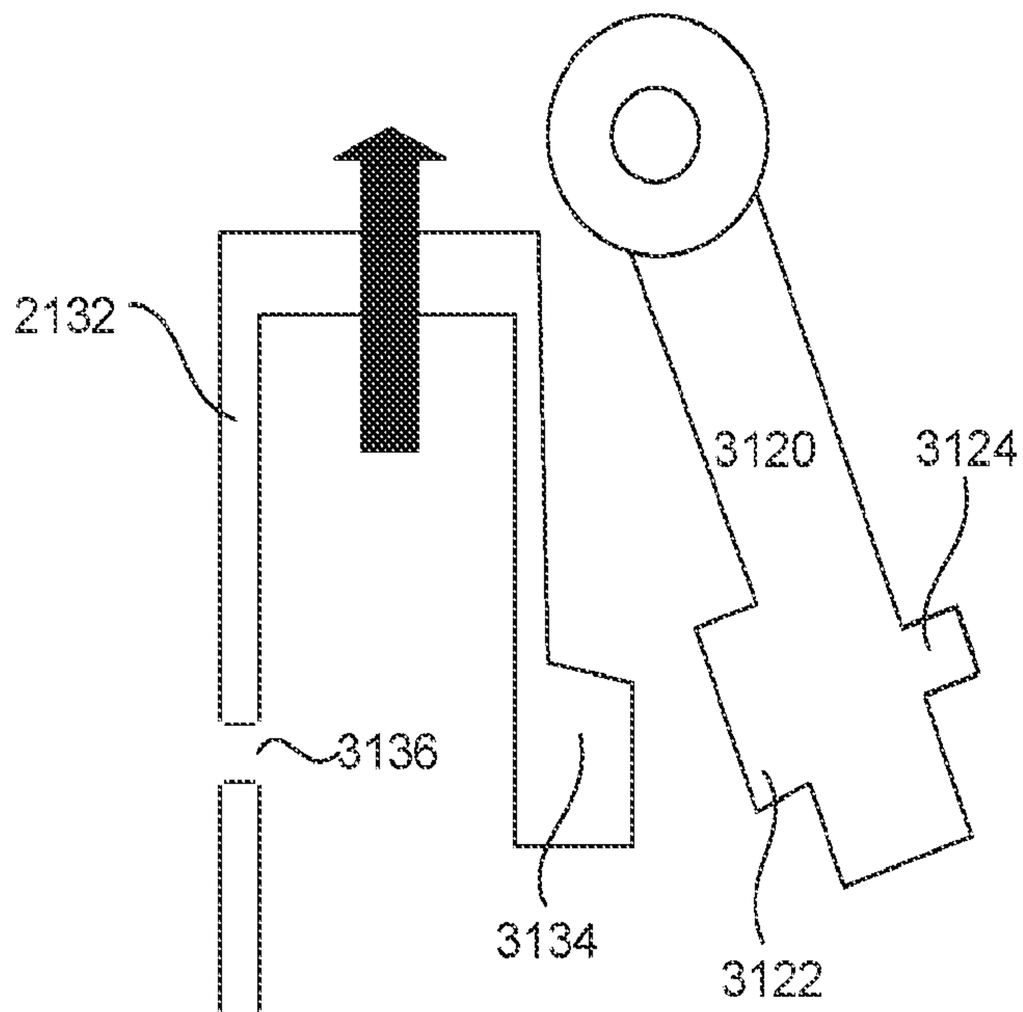


FIG. 25A

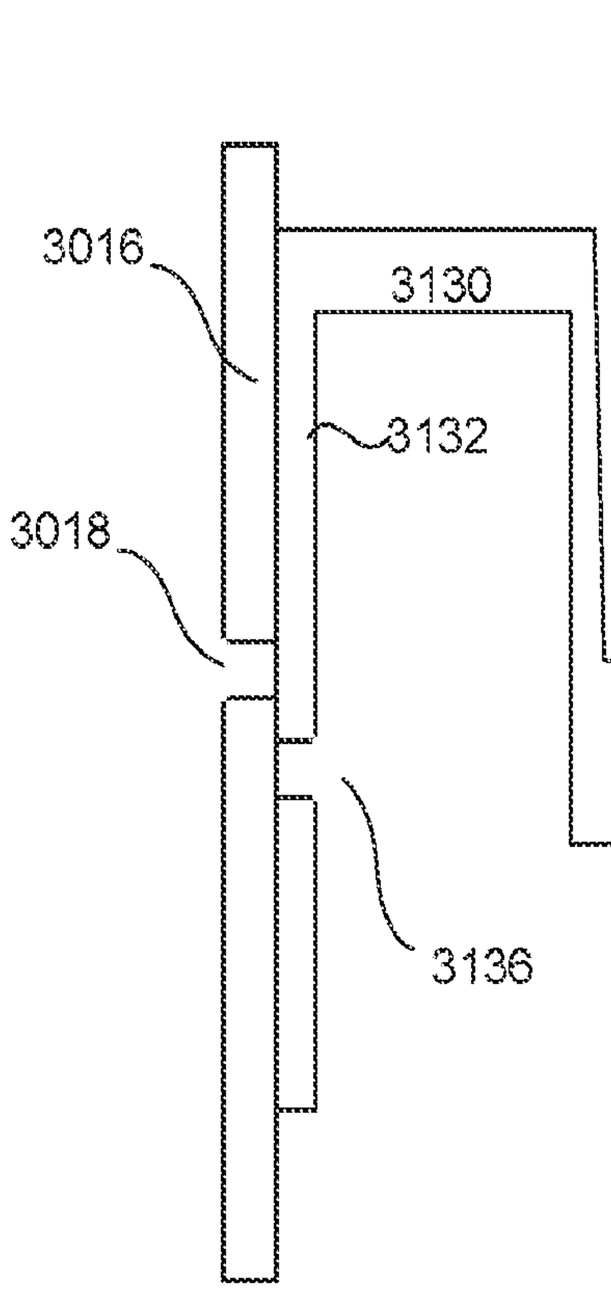


FIG. 26A

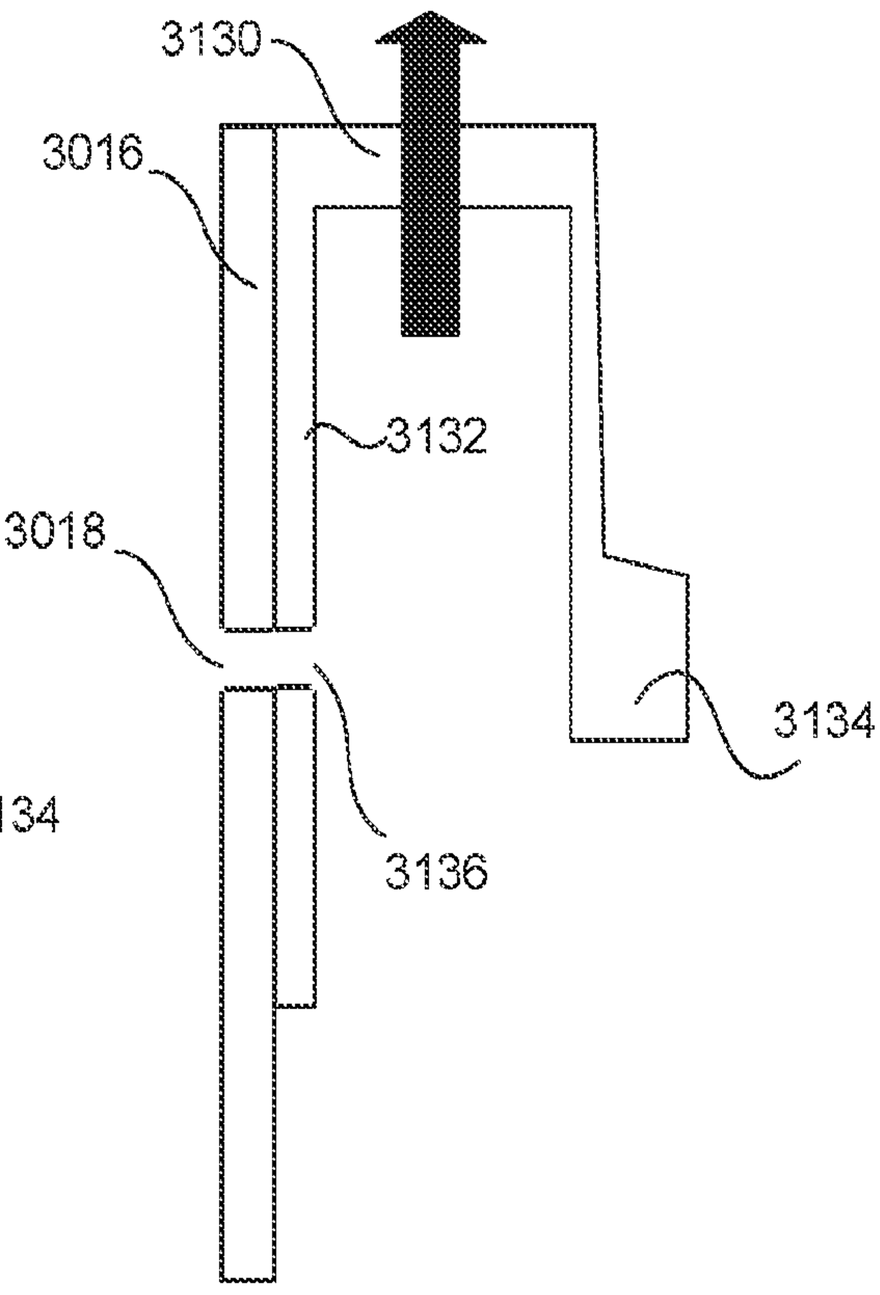


FIG. 26B

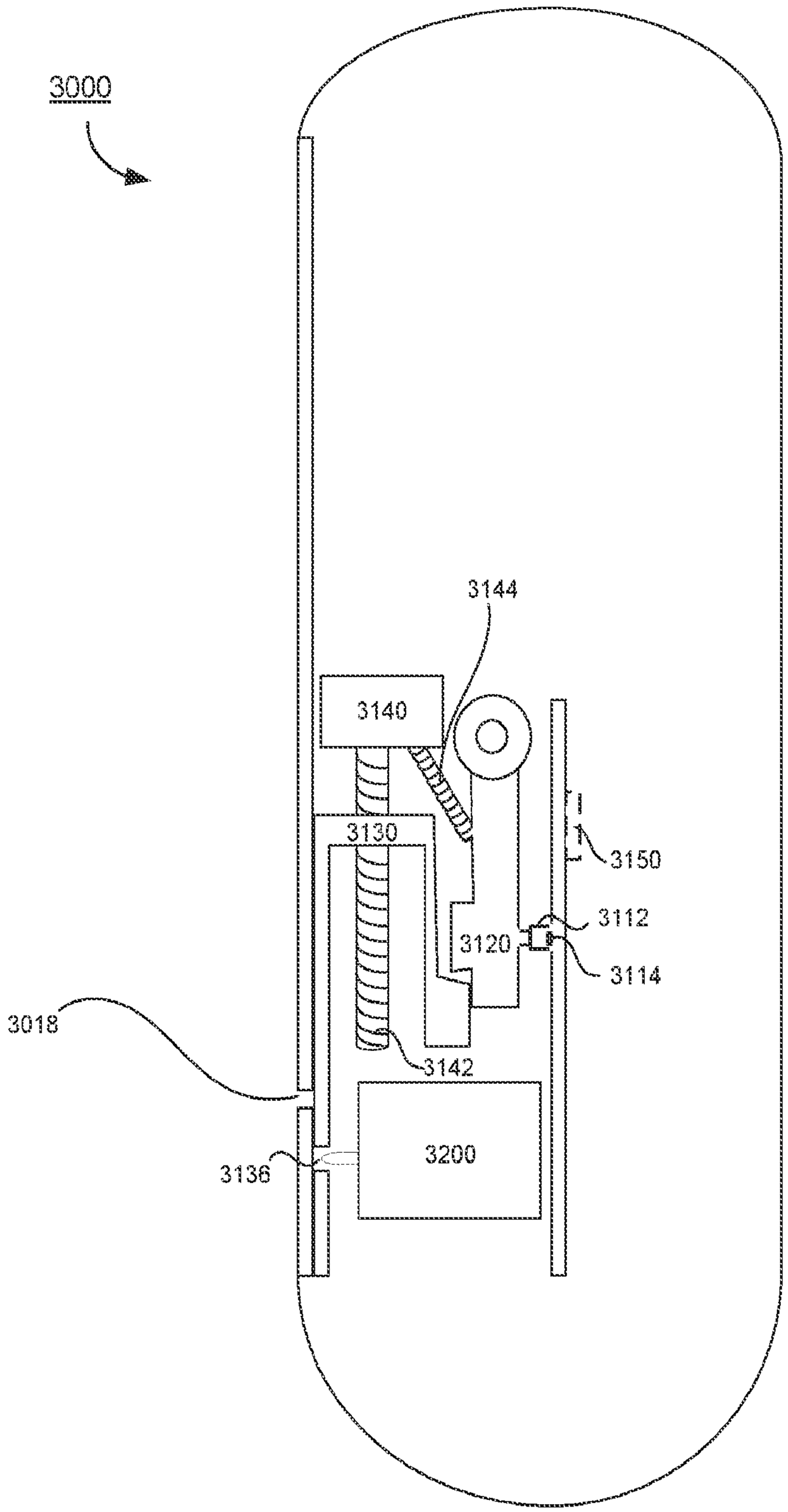


FIG. 27

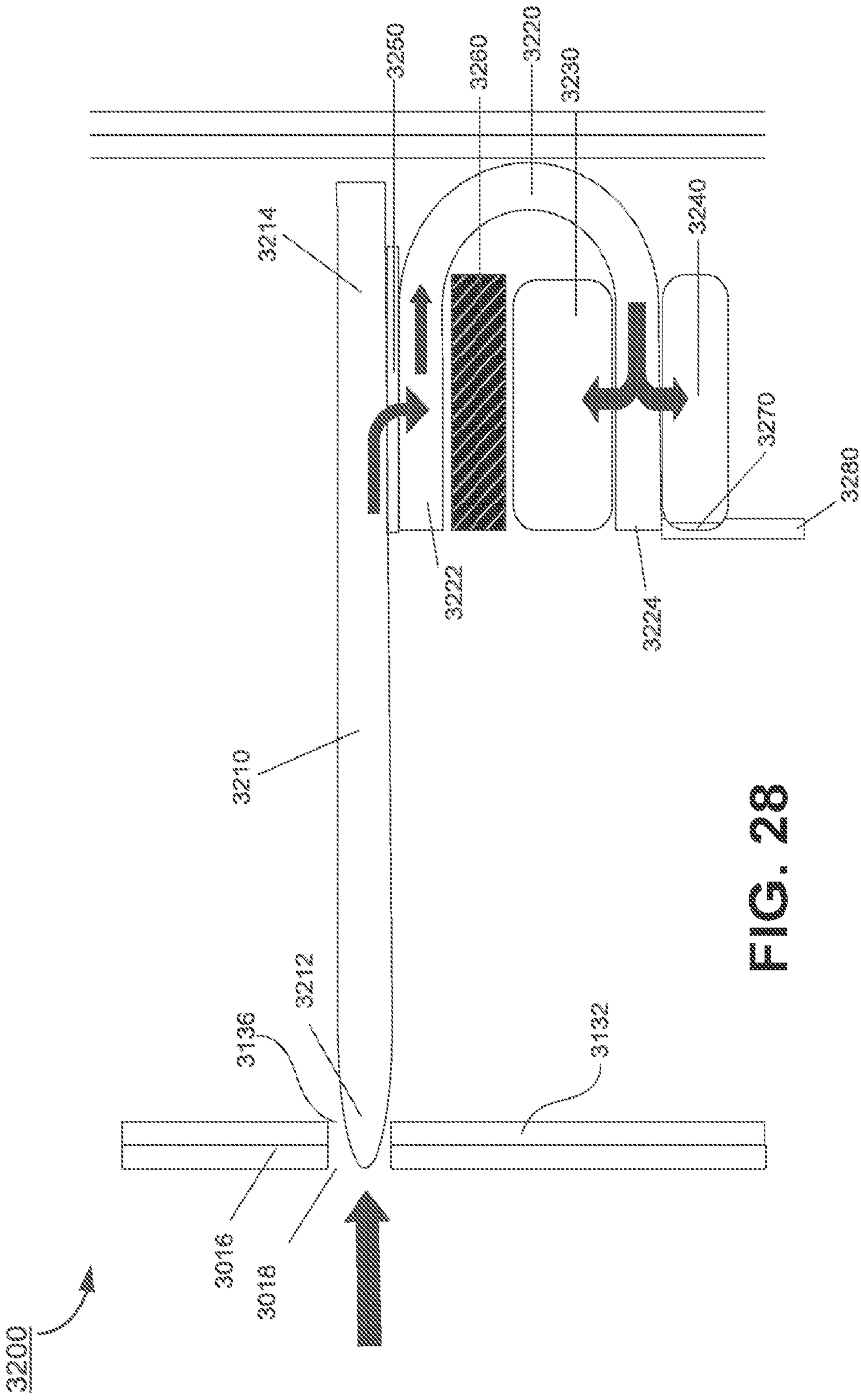

3250
3260
3220
3230
3214
3240
3270
3280
3222
3224
3210
3212
3136
3132
3016
3018
3200

FIG. 28

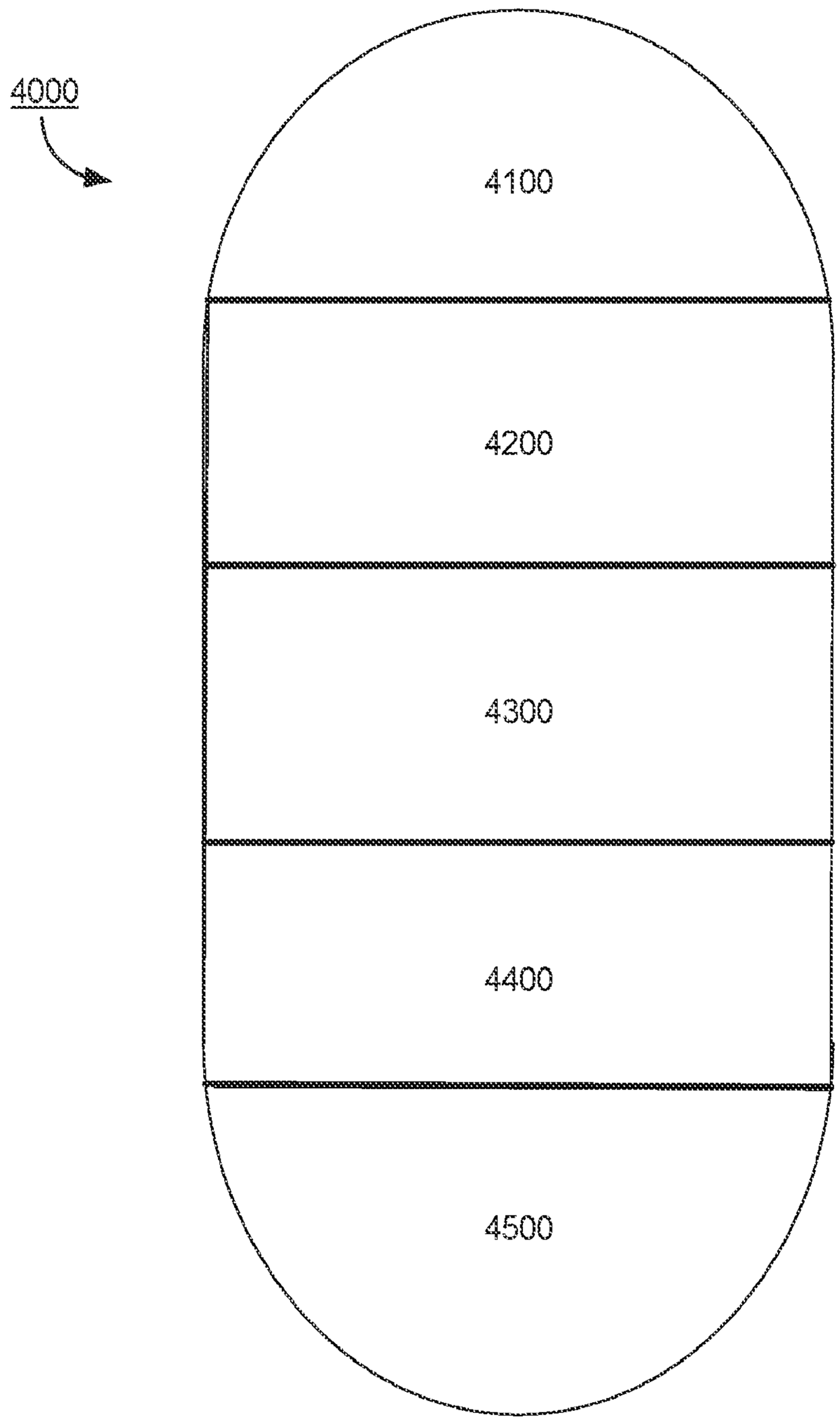


FIG. 29

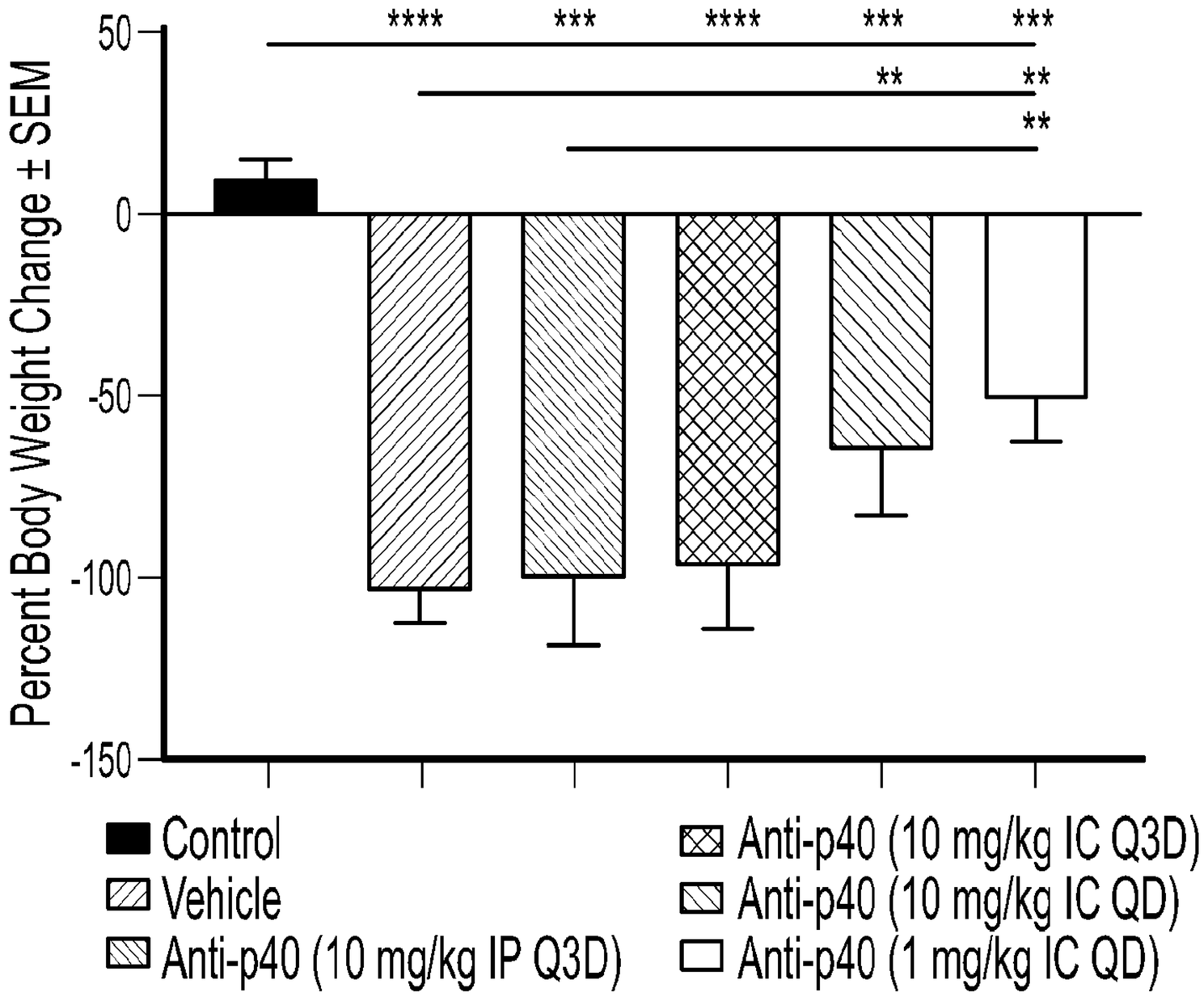

****
***
****
***
***
**
**
**
50
0
-50
-100
-150
Percent Body Weight Change ± SEM
Control
Vehicle
Anti-p40 (10 mg/kg IP Q3D)
Anti-p40 (10 mg/kg IC Q3D)
Anti-p40 (10 mg/kg IC QD)
Anti-p40 (1 mg/kg IC QD)

FIG. 30

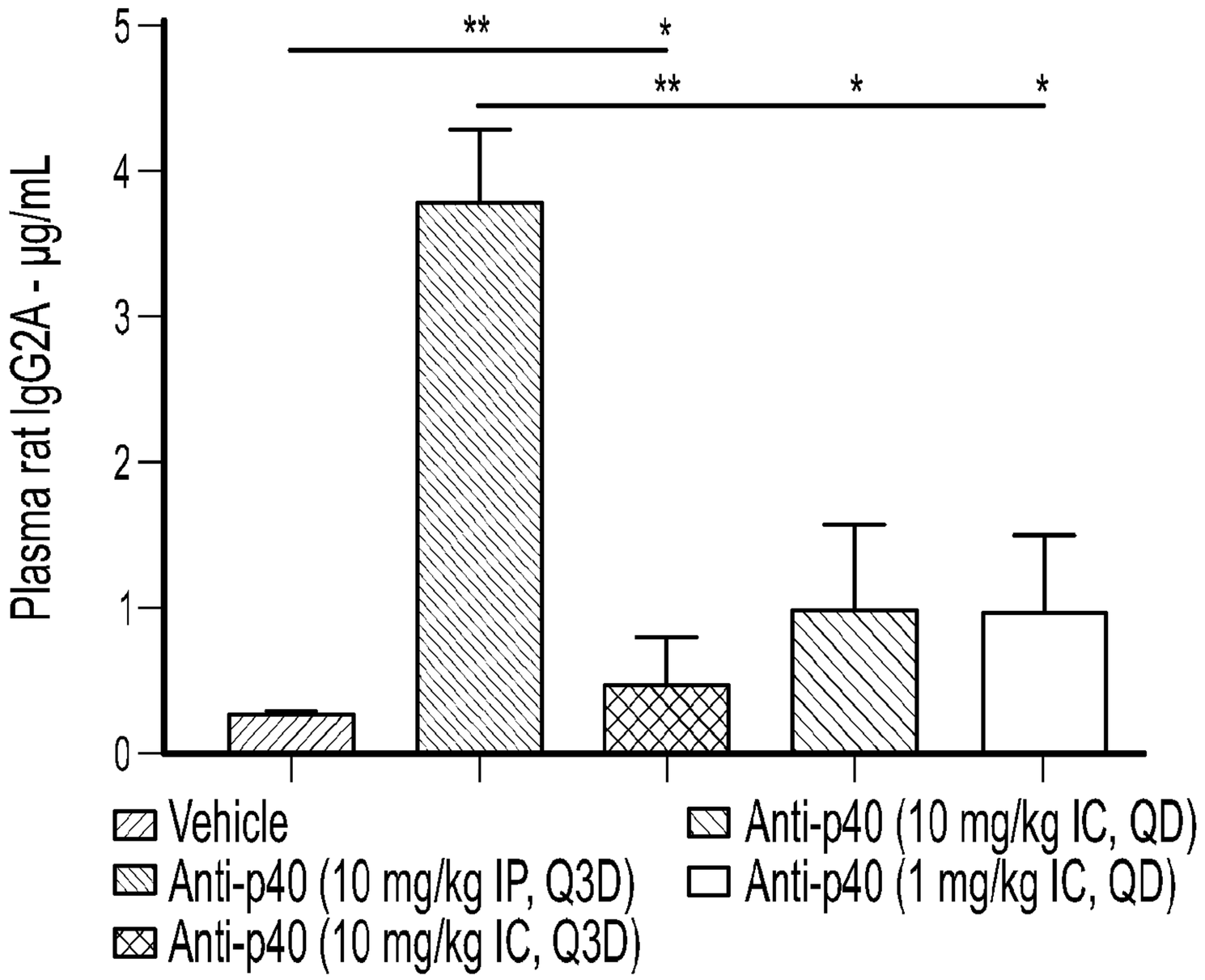

**
*
**
*
*
5
4
3
2
1
0
Plasma rat IgG2A - µg/mL
Vehicle
Anti-p40 (10 mg/kg IP, Q3D)
Anti-p40 (10 mg/kg IC, Q3D)
Anti-p40 (10 mg/kg IC, QD)
Anti-p40 (1 mg/kg IC, QD)

FIG. 31

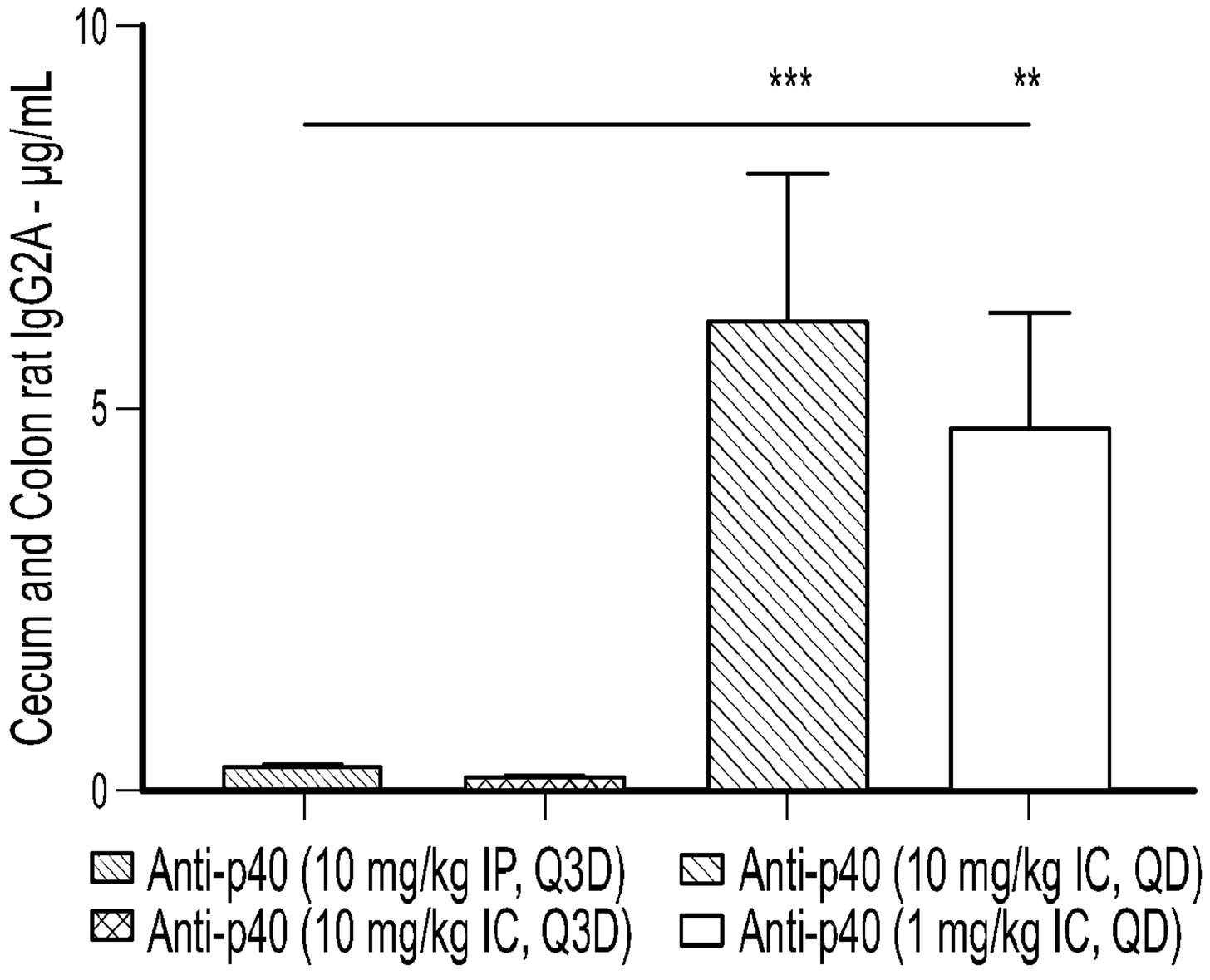

10
5
0
Cecum and Colon rat IgG2A - µg/mL
***
**
Anti-p40 (10 mg/kg IP, Q3D)
Anti-p40 (10 mg/kg IC, Q3D)
Anti-p40 (10 mg/kg IC, QD)
Anti-p40 (1 mg/kg IC, QD)

FIG. 32

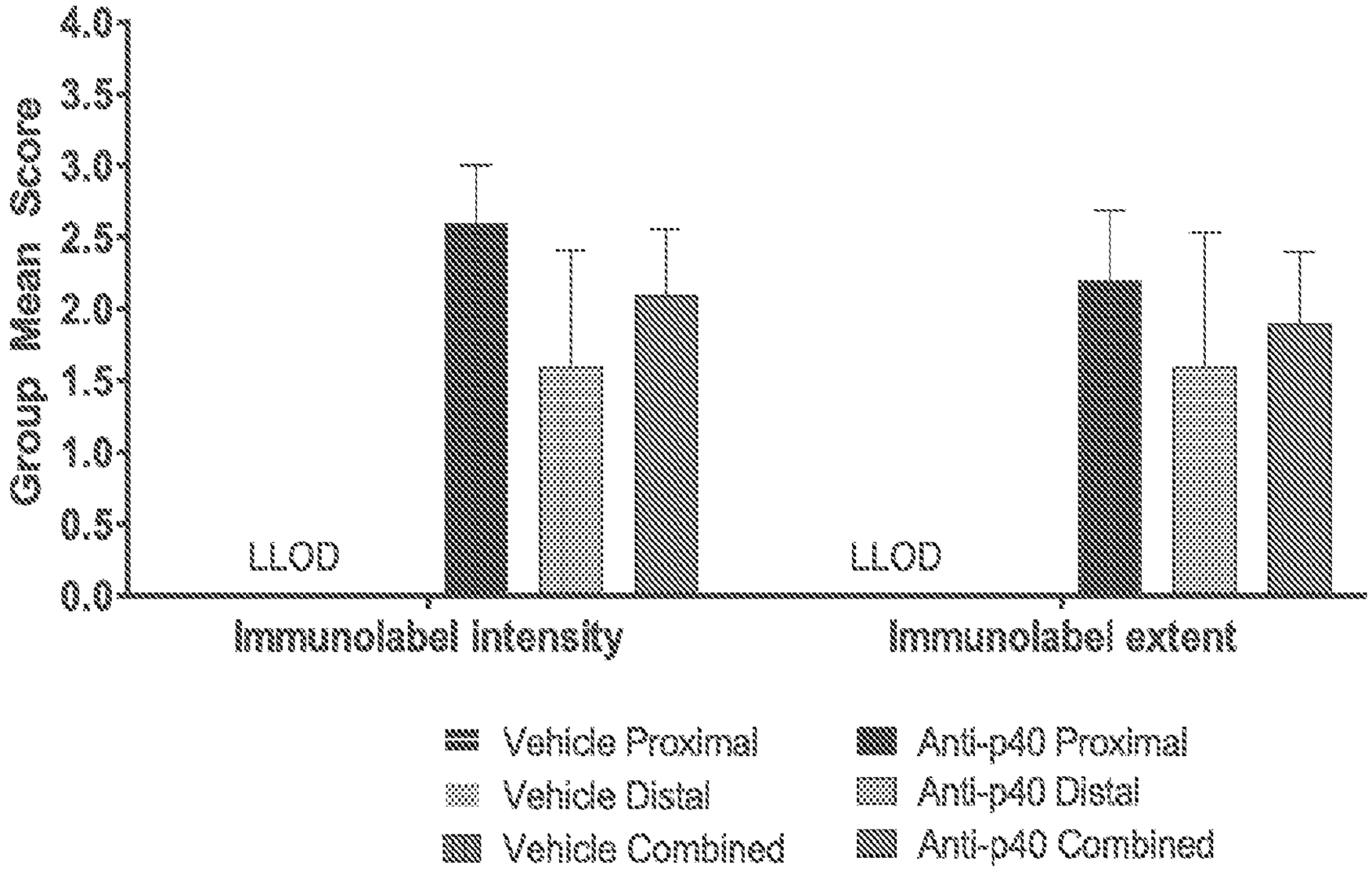

Group Mean Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
LLOD
LLOD
Immunolabel Intensity
Immunolabel extent
Vehicle Proximal
Vehicle Distal
Vehicle Combined
Anti-p40 Proximal
Anti-p40 Distal
Anti-p40 Combined

FIG. 33

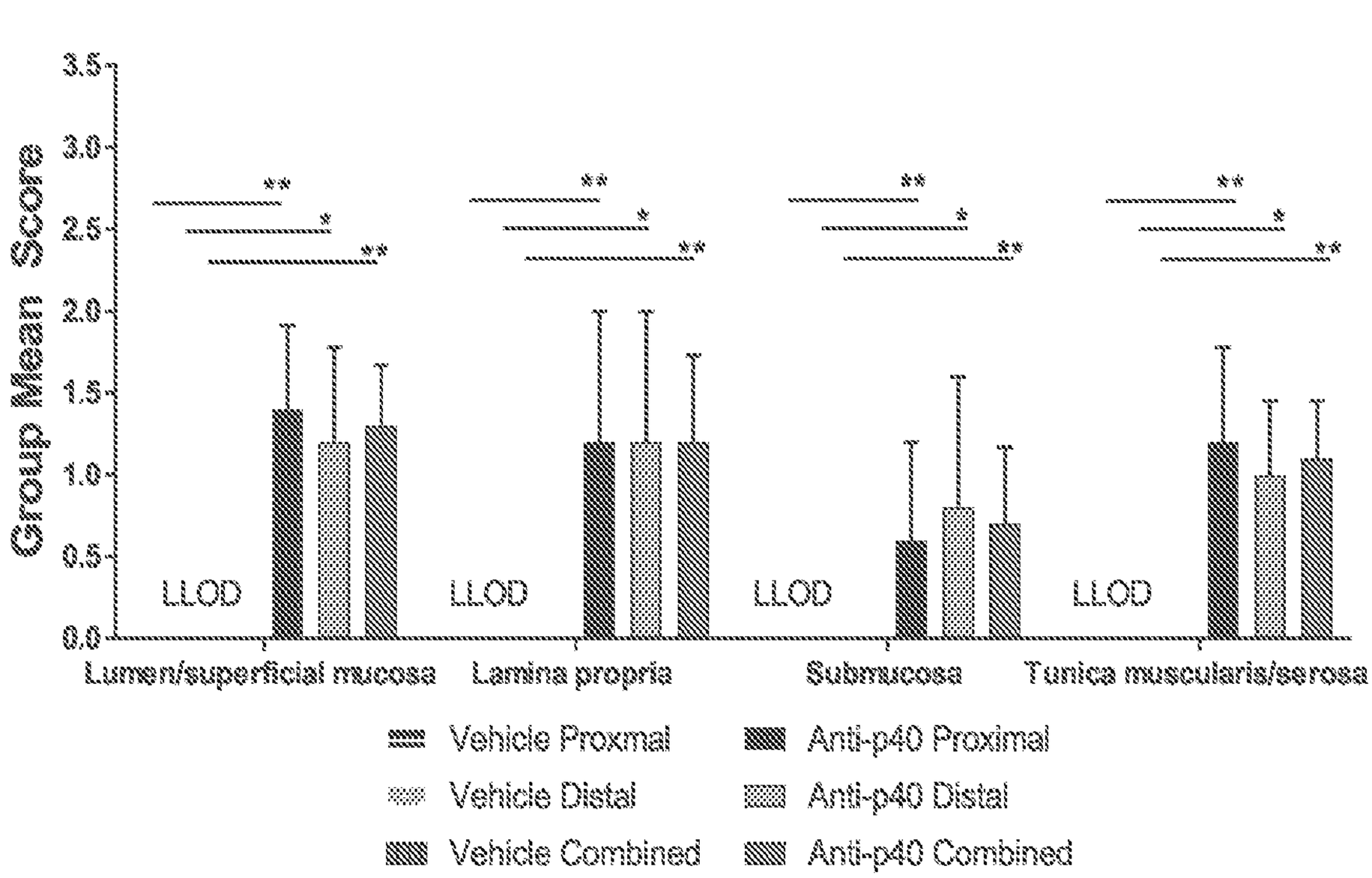
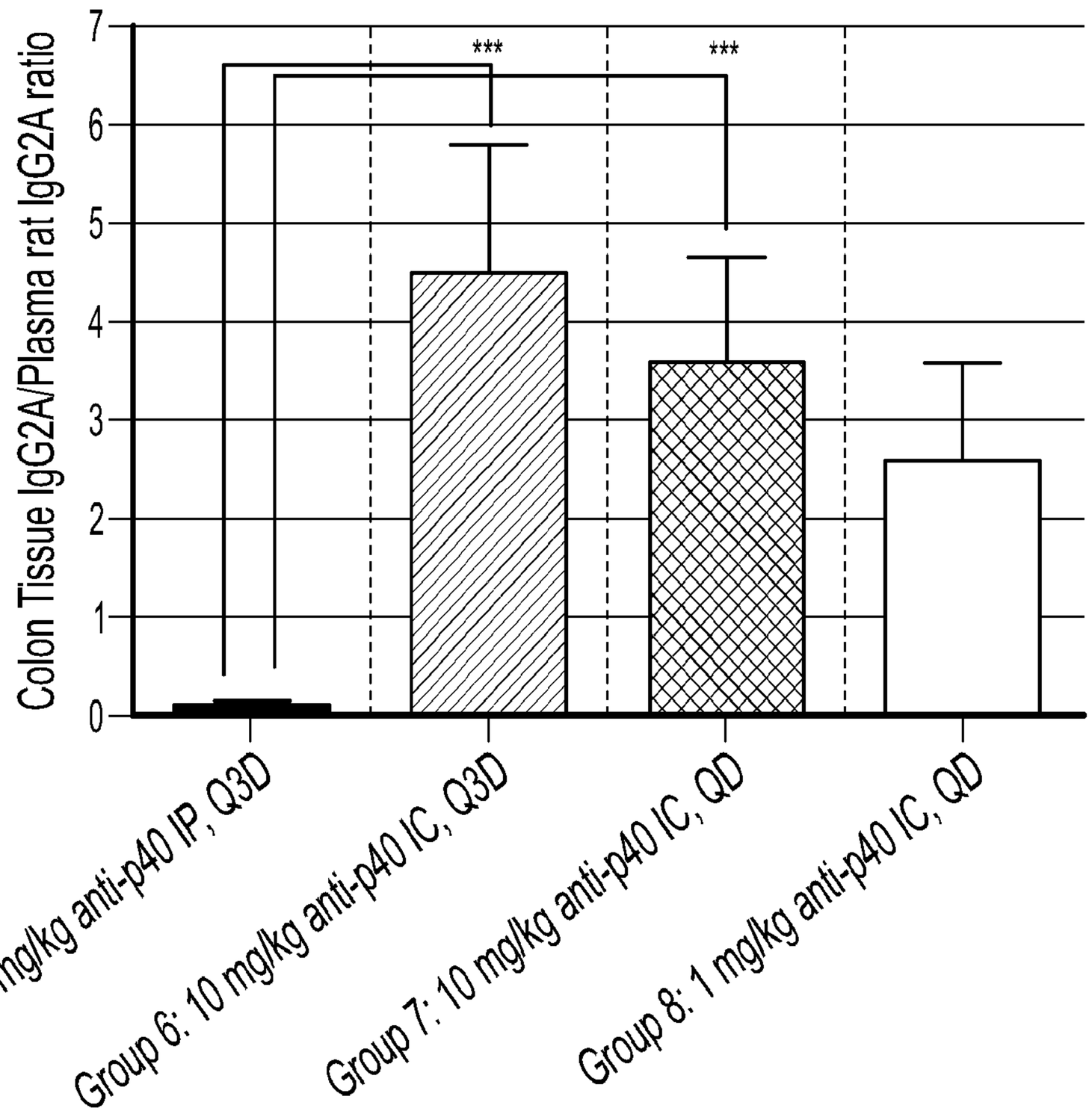

Group Mean Score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
**
LLOD
LLOD
LLOD
LLOD
Lumen/superficial mucosa
Lamina propria
Submucosa
Tunica muscularis/serosa
Vehicle Proxmal
Vehicle Distal
Vehicle Combined
Anti-p40 Proximal
Anti-p40 Distal
Anti-p40 Combined

FIG. 34



^Outlier removed from Group 5

FIG. 35

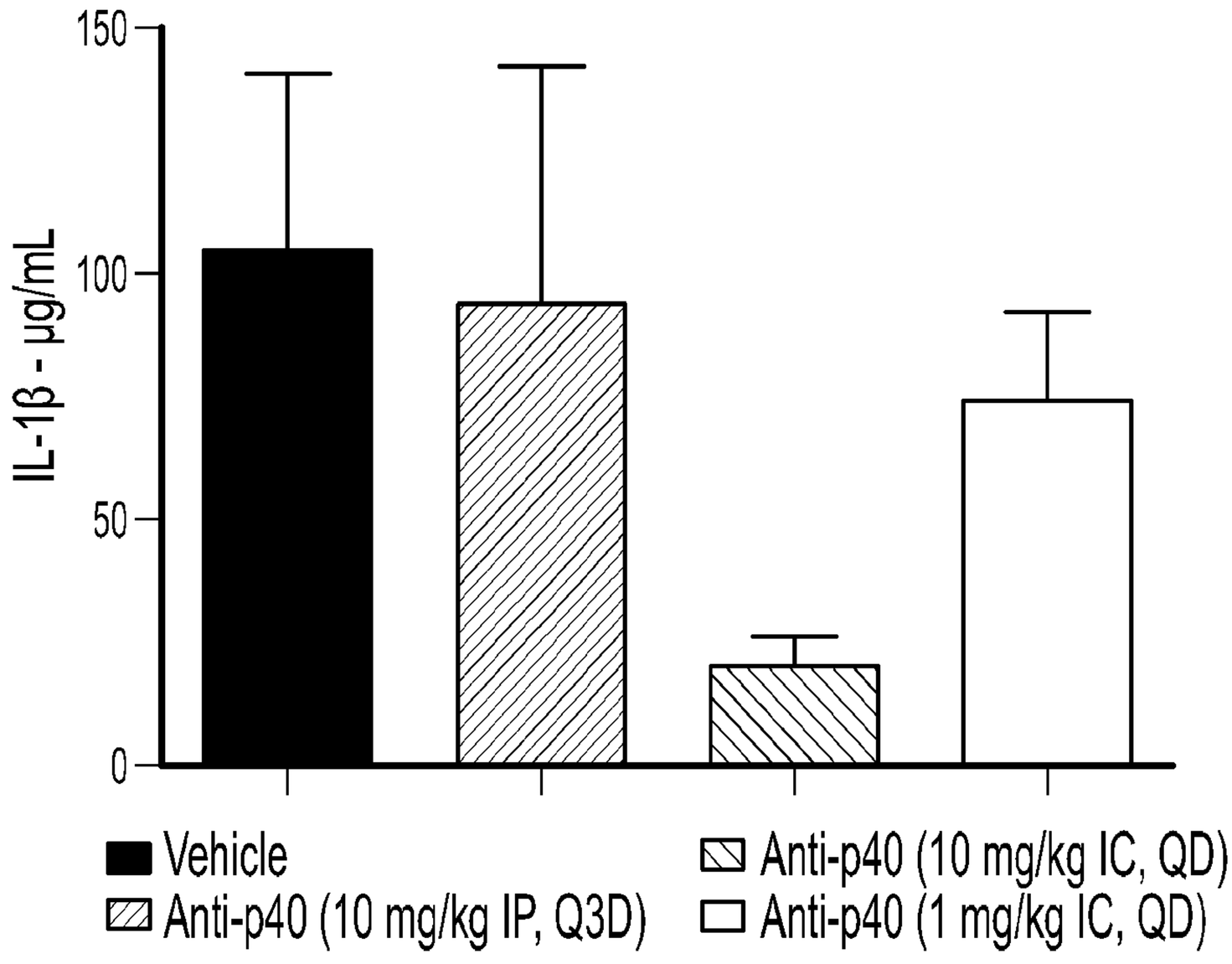

150
100
50
0
IL-1β - µg/mL
Vehicle
Anti-p40 (10 mg/kg IP, Q3D)
Anti-p40 (10 mg/kg IC, QD)
Anti-p40 (1 mg/kg IC, QD)

FIG. 36

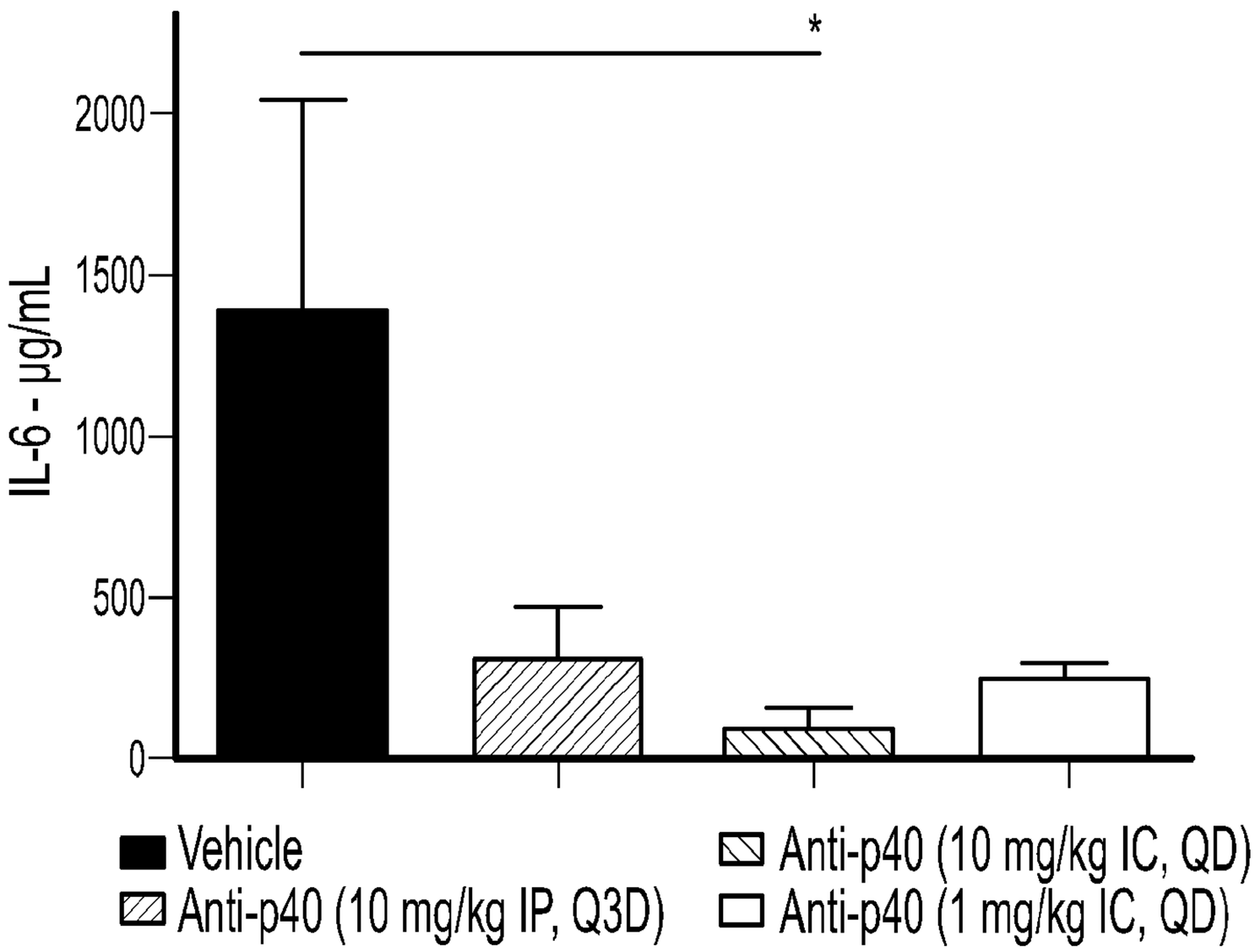

*
2000
1500
1000
500
0
IL-6 - µg/mL
Vehicle
Anti-p40 (10 mg/kg IP, Q3D)
Anti-p40 (10 mg/kg IC, QD)
Anti-p40 (1 mg/kg IC, QD)

FIG. 37

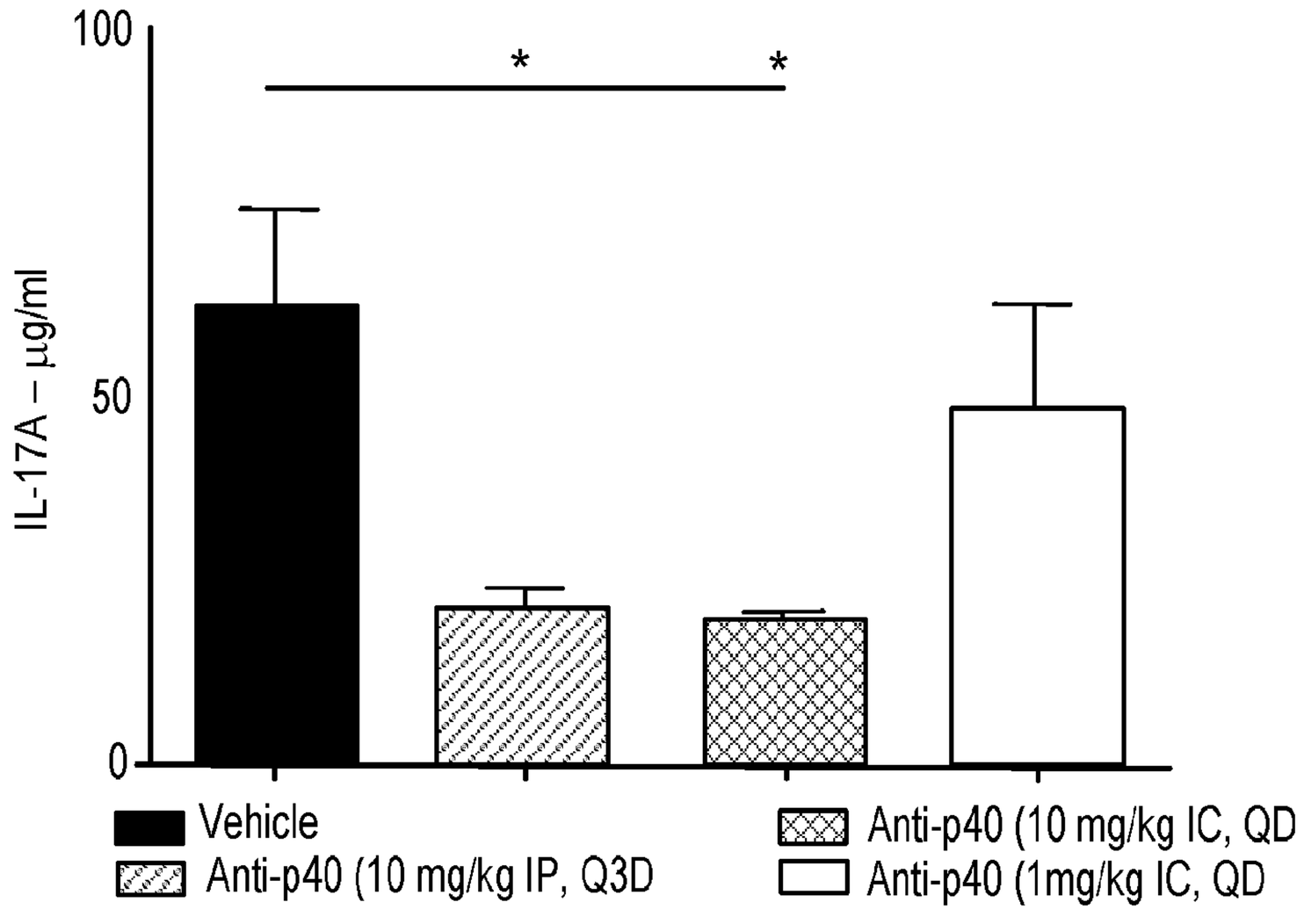

100
50
0
IL-17A – µg/ml
*
*
Vehicle
Anti-p40 (10 mg/kg IP, Q3D
Anti-p40 (10 mg/kg IC, QD
Anti-p40 (1mg/kg IC, QD

FIG. 38

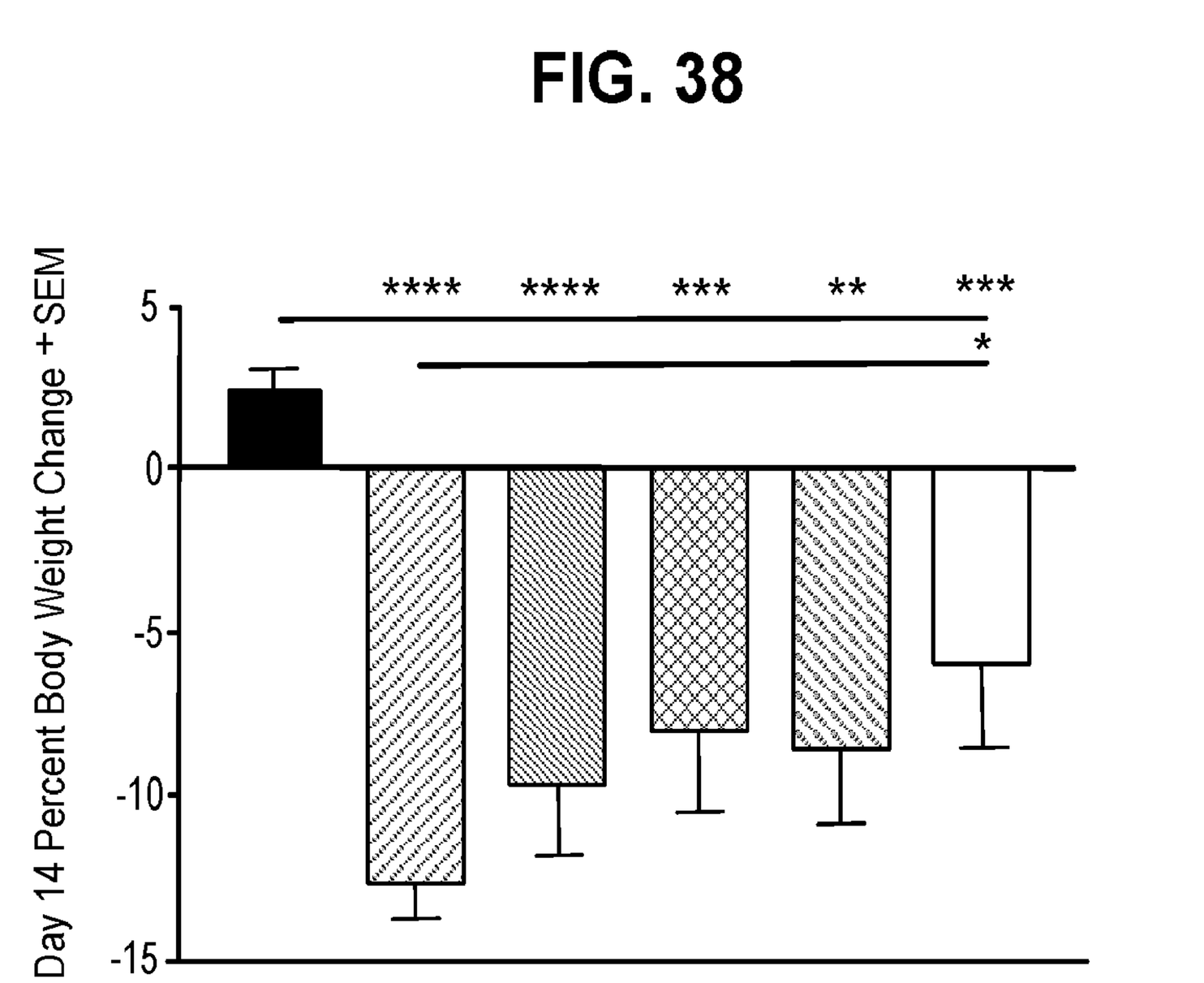

Day 14 Percent Body Weight Change + SEM
5
0
-5
-10
-15
****
****
***
**
***
*
Control
Vehicle
DATK32 (25mg/kg IP Q3D)
DATK32 (25mg/kg IC Q3D)
DATK32 (25mg/kg IC QD)
DATK32 (5mg/kg IC QD)

FIG. 39

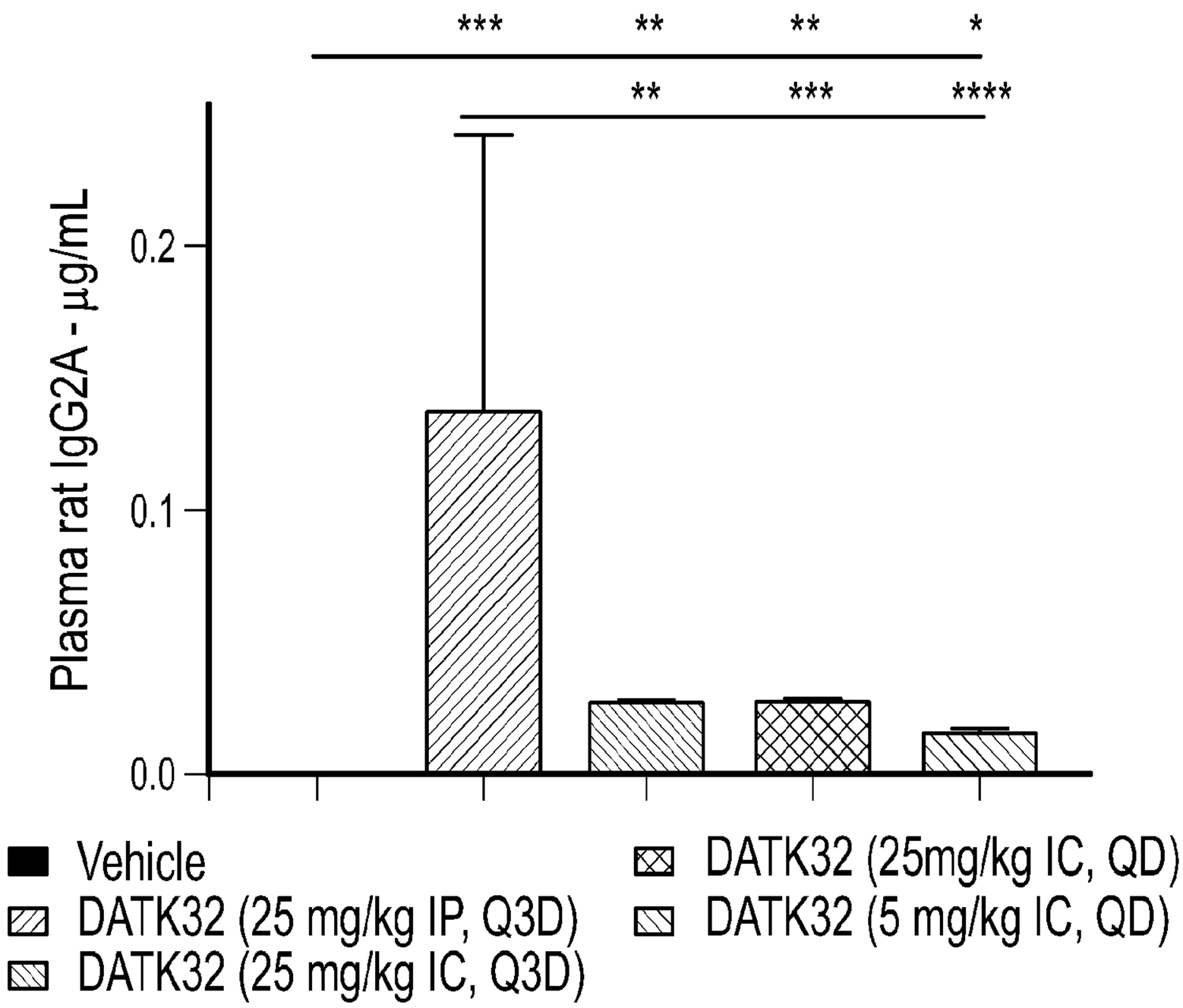

***
**
**
*
**
***
****
Plasma rat IgG2A - µg/mL
0.2
0.1
0.0
Vehicle
DATK32 (25 mg/kg IP, Q3D)
DATK32 (25 mg/kg IC, Q3D)
DATK32 (25mg/kg IC, QD)
DATK32 (5 mg/kg IC, QD)

FIG. 40

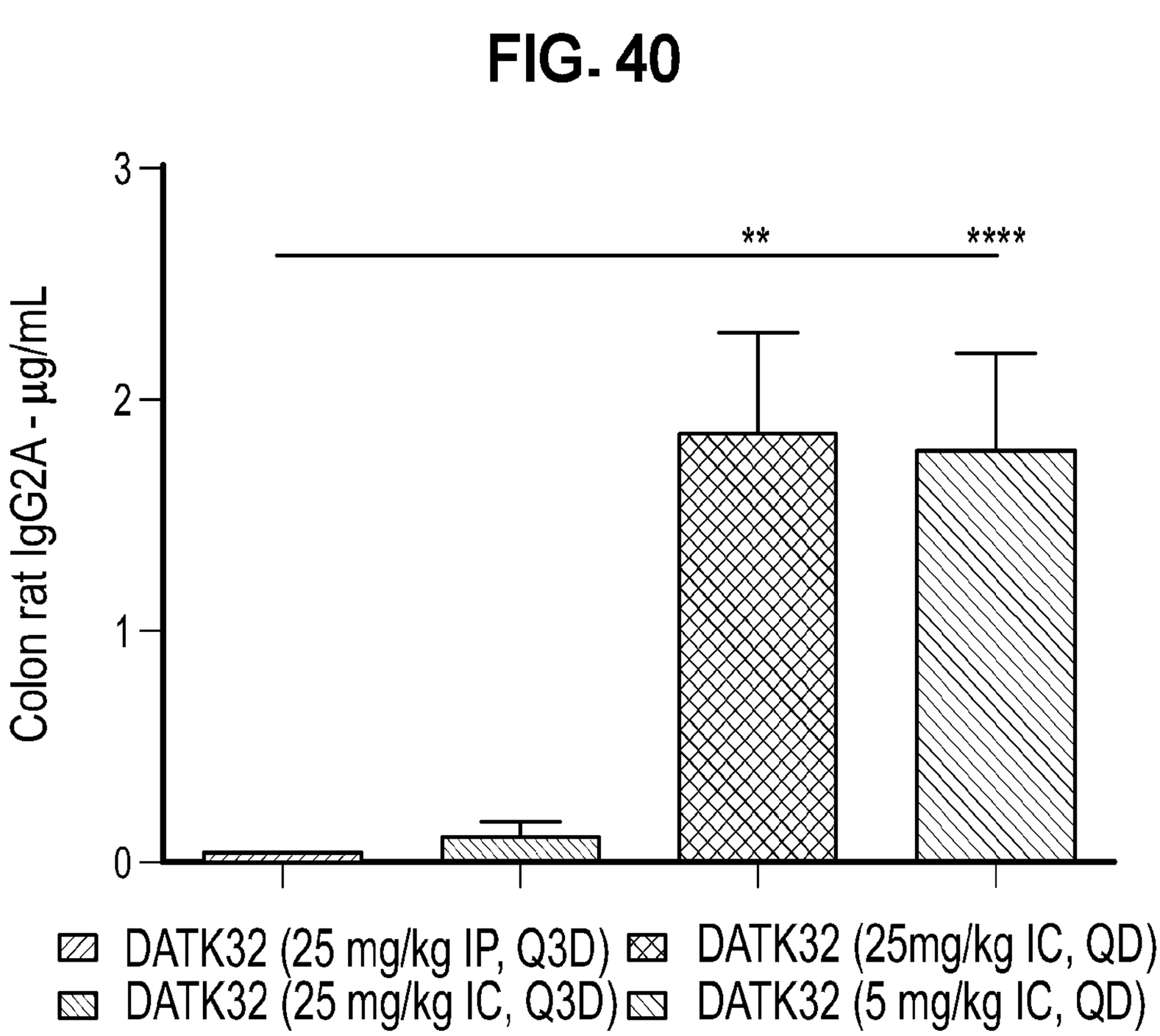

**
****
Colon rat IgG2A - µg/mL
3
2
1
0
DATK32 (25 mg/kg IP, Q3D)
DATK32 (25 mg/kg IC, Q3D)
DATK32 (25mg/kg IC, QD)
DATK32 (5 mg/kg IC, QD)

FIG. 41

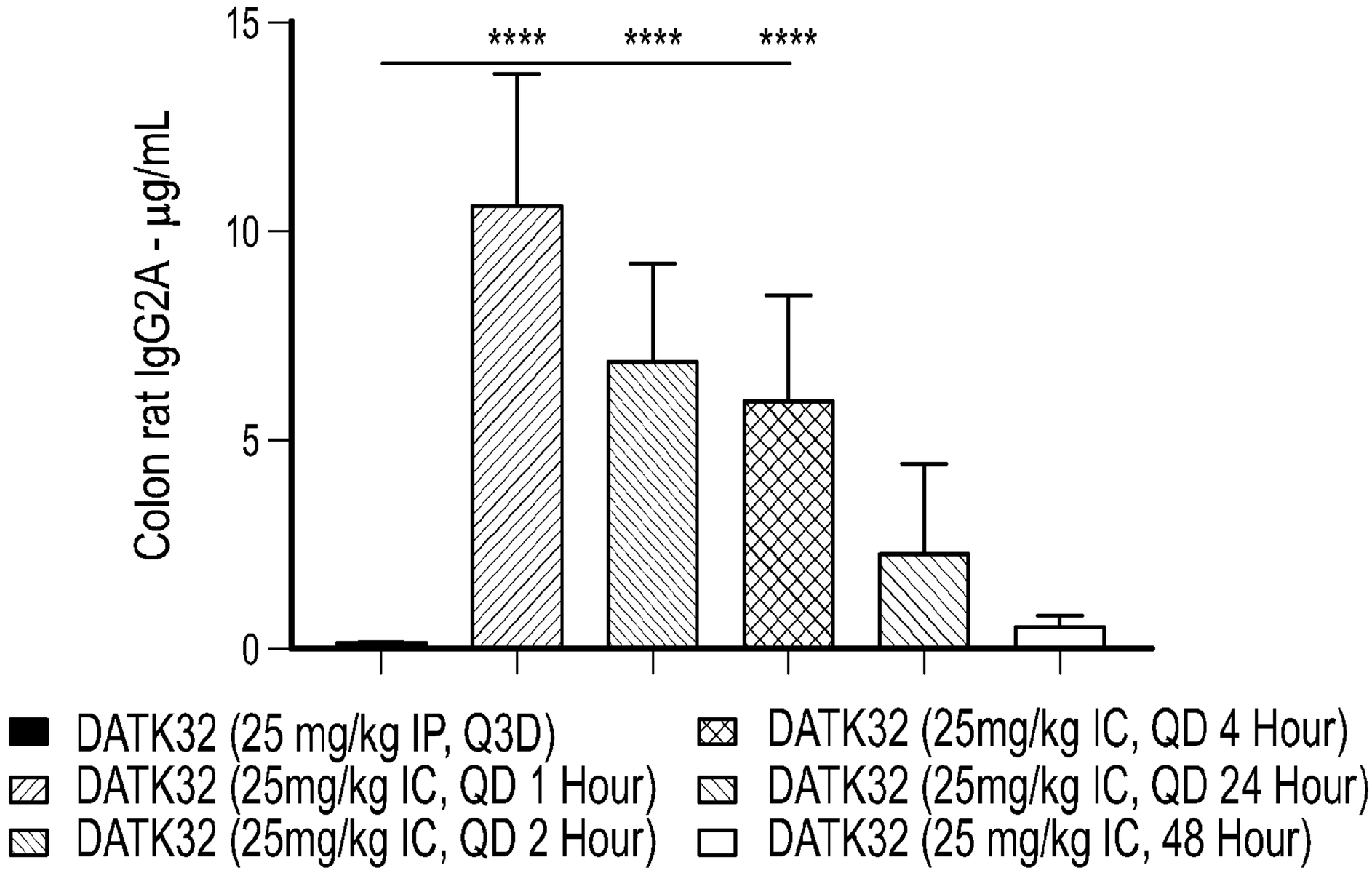

15
10
5
0
Colon rat IgG2A - µg/mL
****
****
****
DATK32 (25 mg/kg IP, Q3D)
DATK32 (25mg/kg IC, QD 1 Hour)
DATK32 (25mg/kg IC, QD 2 Hour)
DATK32 (25mg/kg IC, QD 4 Hour)
DATK32 (25mg/kg IC, QD 24 Hour)
DATK32 (25 mg/kg IC, 48 Hour)

FIG. 42

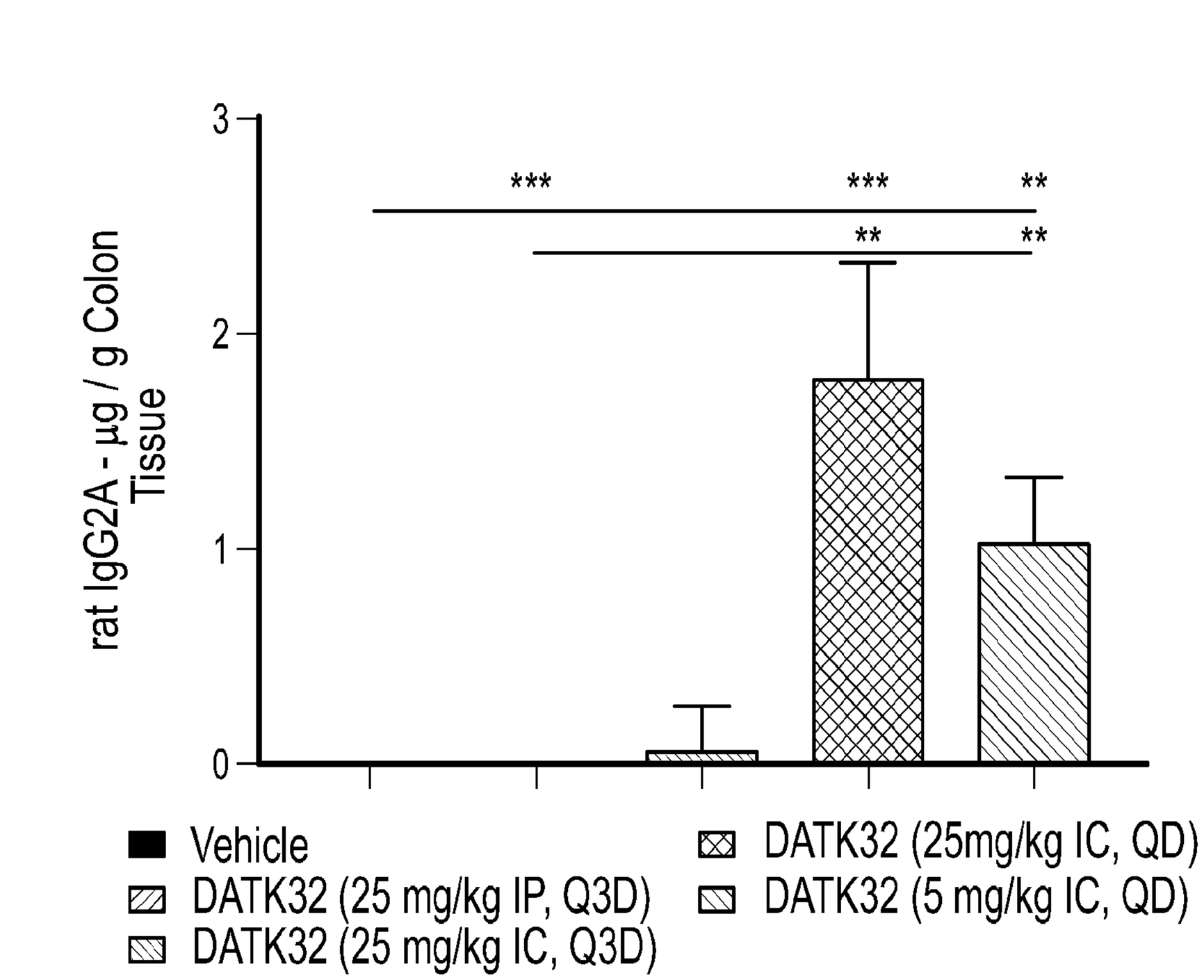

3
2
1
0
rat IgG2A - µg / g Colon Tissue
***
***
**
**
**
Vehicle
DATK32 (25 mg/kg IP, Q3D)
DATK32 (25 mg/kg IC, Q3D)
DATK32 (25mg/kg IC, QD)
DATK32 (5 mg/kg IC, QD)

FIG. 43

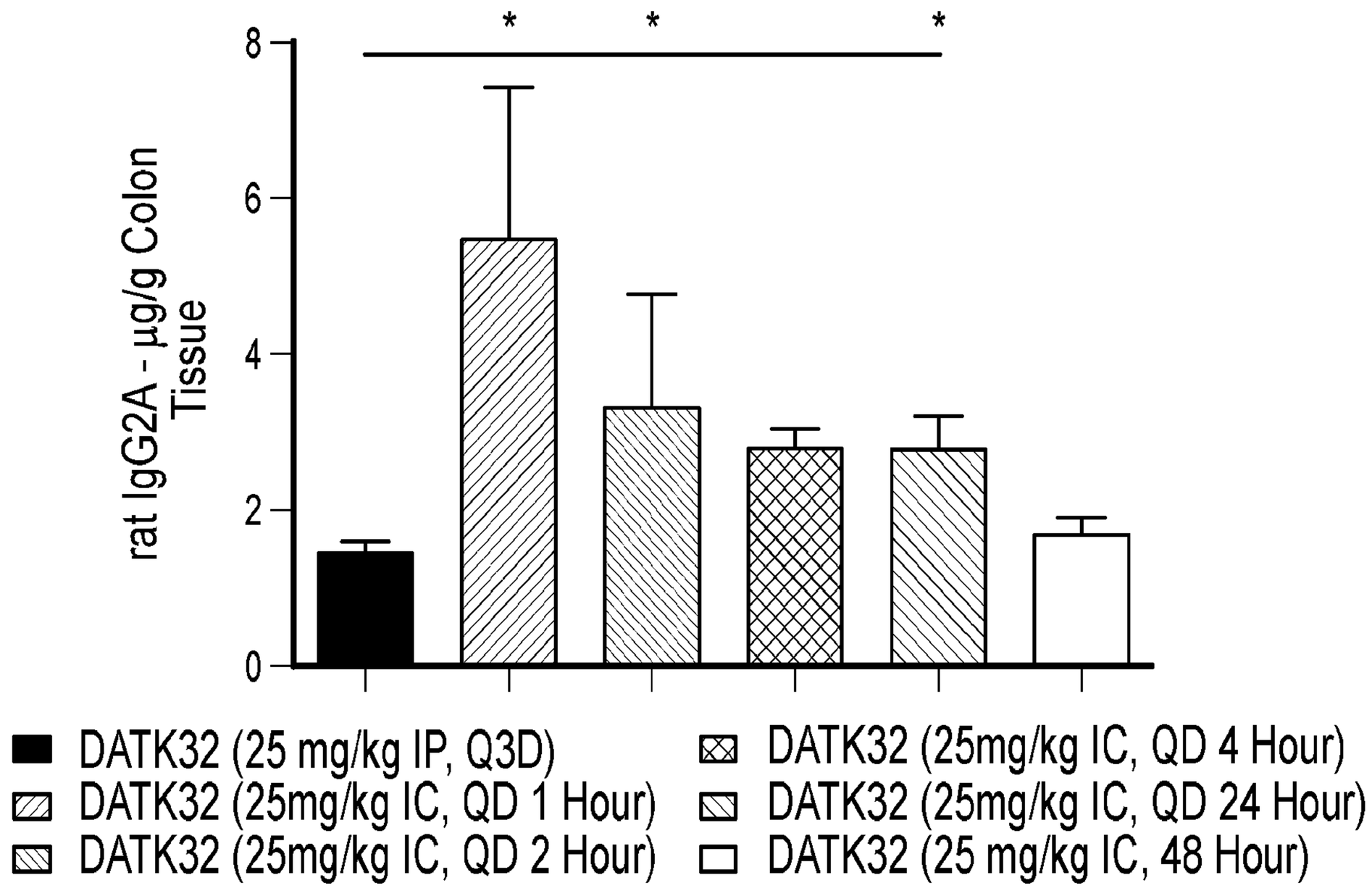

*
*
*
8
6
4
2
0
rat IgG2A - µg/g Colon Tissue
DATK32 (25 mg/kg IP, Q3D)
DATK32 (25mg/kg IC, QD 1 Hour)
DATK32 (25mg/kg IC, QD 2 Hour)
DATK32 (25mg/kg IC, QD 4 Hour)
DATK32 (25mg/kg IC, QD 24 Hour)
DATK32 (25 mg/kg IC, 48 Hour)

FIG. 44

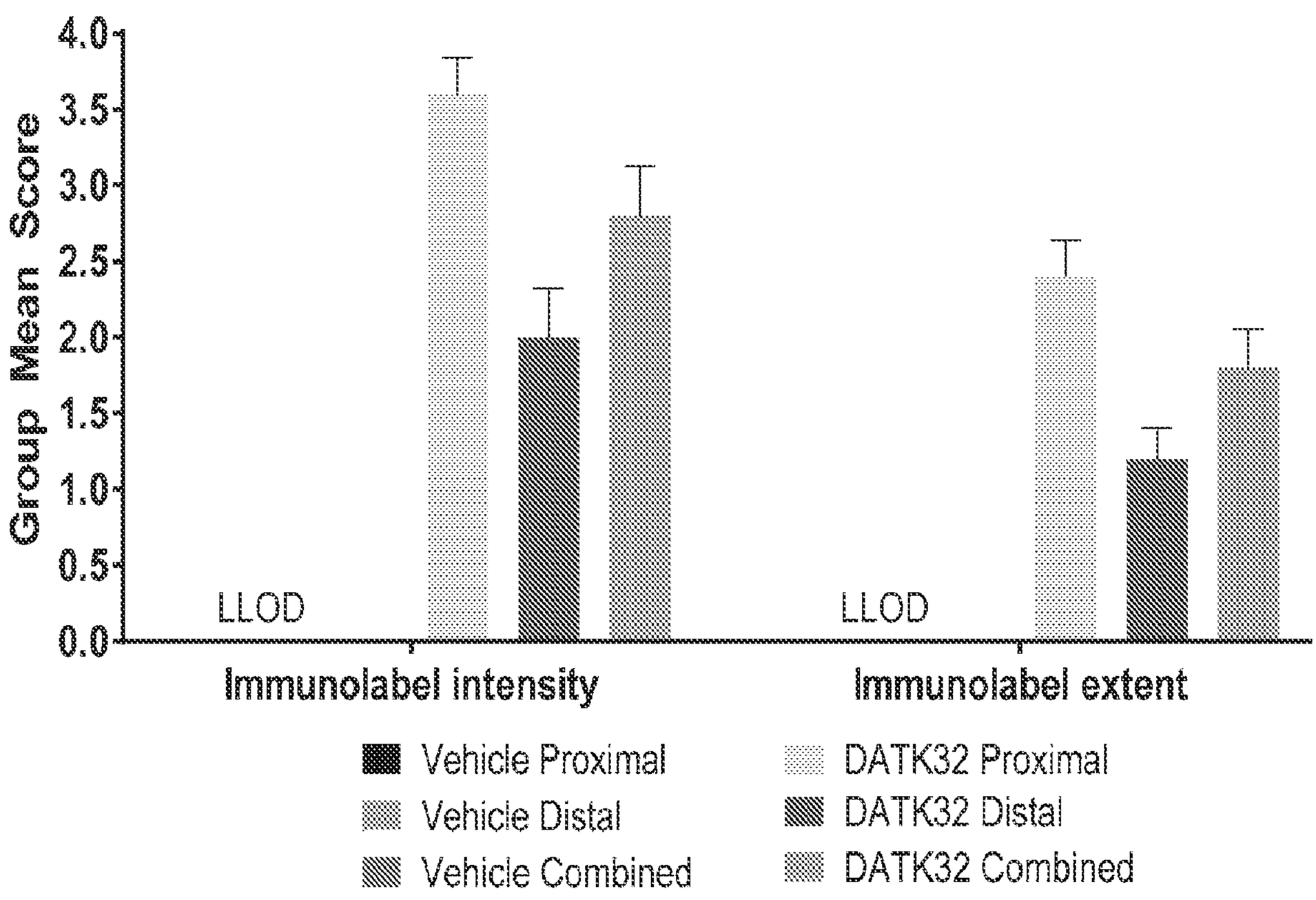

Group Mean Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
LLOD
LLOD
Immunolabel intensity
Immunolabel extent
Vehicle Proximal
Vehicle Distal
Vehicle Combined
DATK32 Proximal
DATK32 Distal
DATK32 Combined

FIG. 45

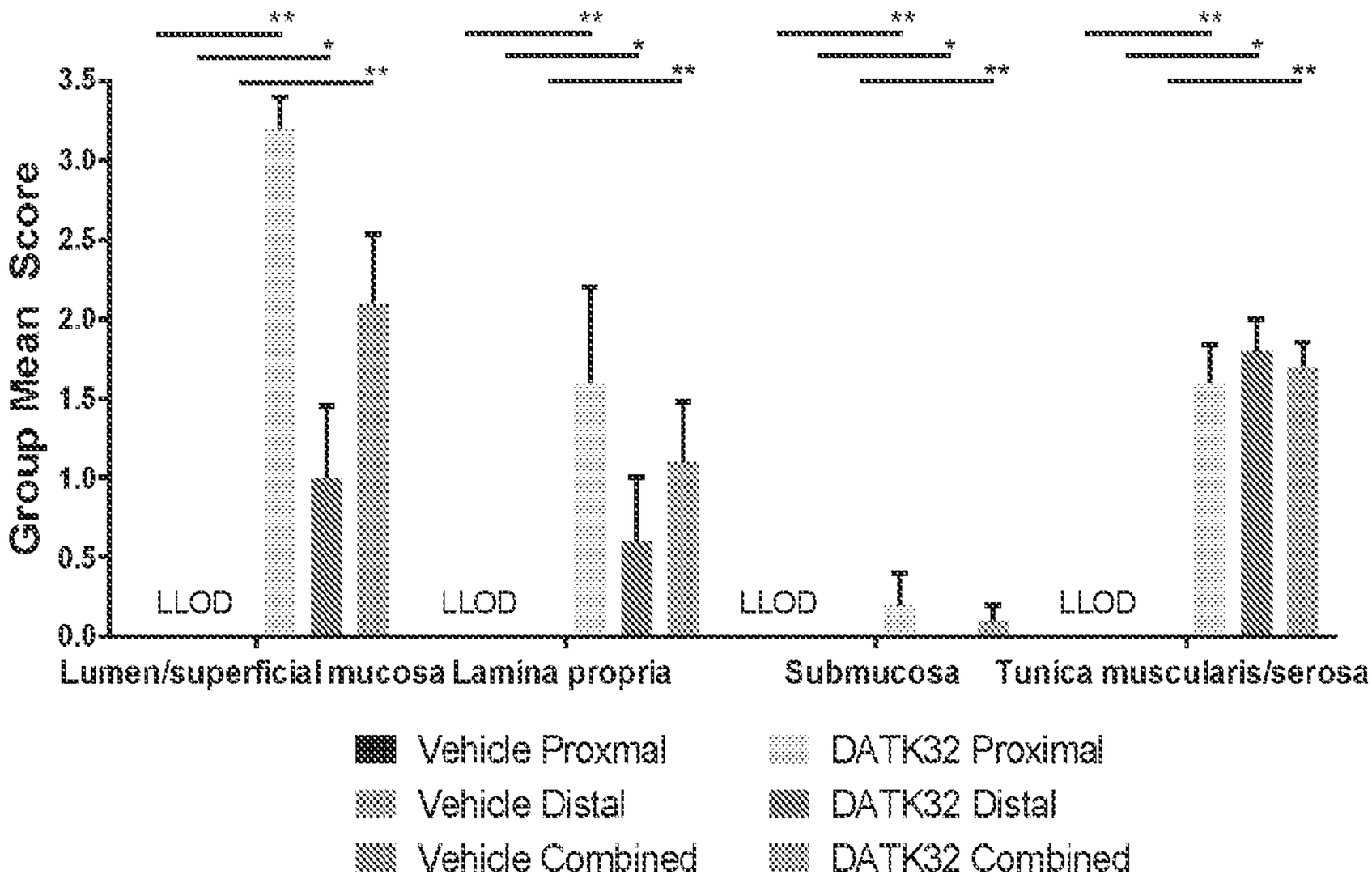

Group Mean Score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
**
LLOD
LLOD
LLOD
LLOD
Lumen/superficial mucosa
Lamina propria
Submucosa
Tunica muscularis/serosa
Vehicle Proxmal
Vehicle Distal
Vehicle Combined
DATK32 Proximal
DATK32 Distal
DATK32 Combined

FIG. 46

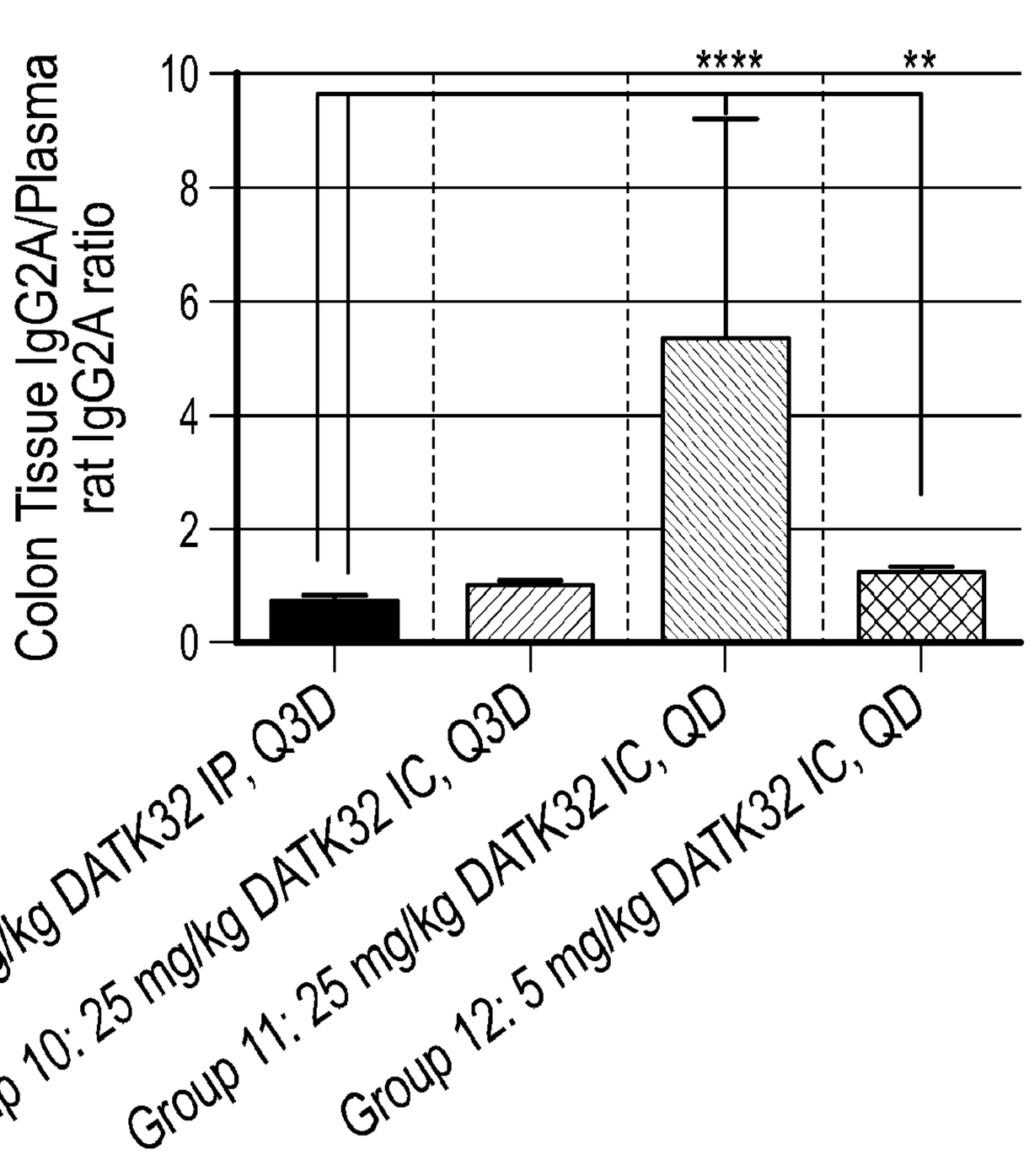

Colon Tissue IgG2A/Plasma rat IgG2A ratio
10
8
6
4
2
0
****
**
Group 9: 25 mg/kg DATK32 IP, Q3D
Group 10: 25 mg/kg DATK32 IC, Q3D
Group 11: 25 mg/kg DATK32 IC, QD
Group 12: 5 mg/kg DATK32 IC, QD

FIG. 47

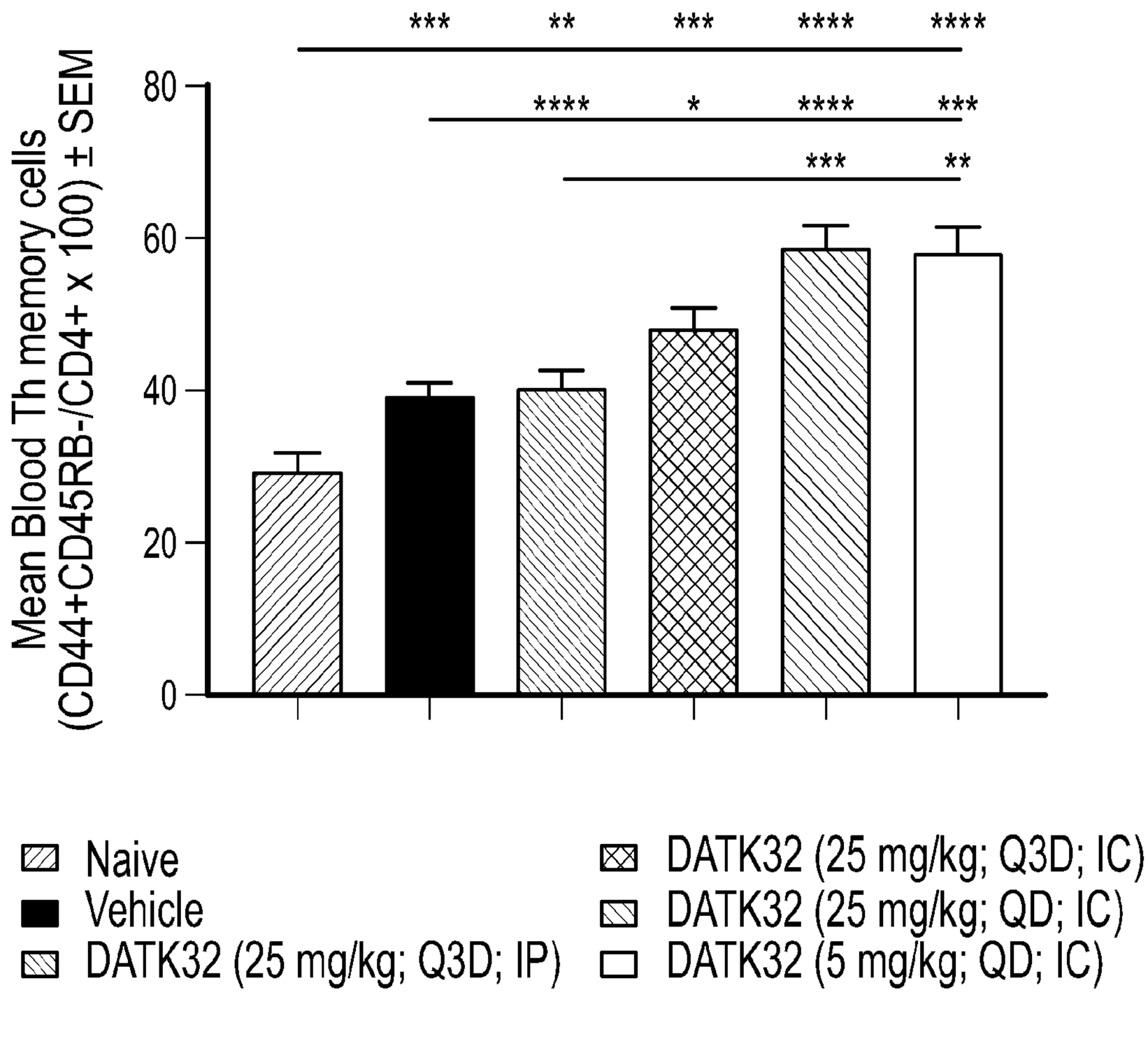

***
**
***
****
****
****
*
****
***
***
**
Mean Blood Th memory cells
(CD44+CD45RB-/CD4+ x 100) ± SEM
80
60
40
20
0
Naive
Vehicle
DATK32 (25 mg/kg; Q3D; IP)
DATK32 (25 mg/kg; Q3D; IC)
DATK32 (25 mg/kg; QD; IC)
DATK32 (5 mg/kg; QD; IC)

FIG. 48

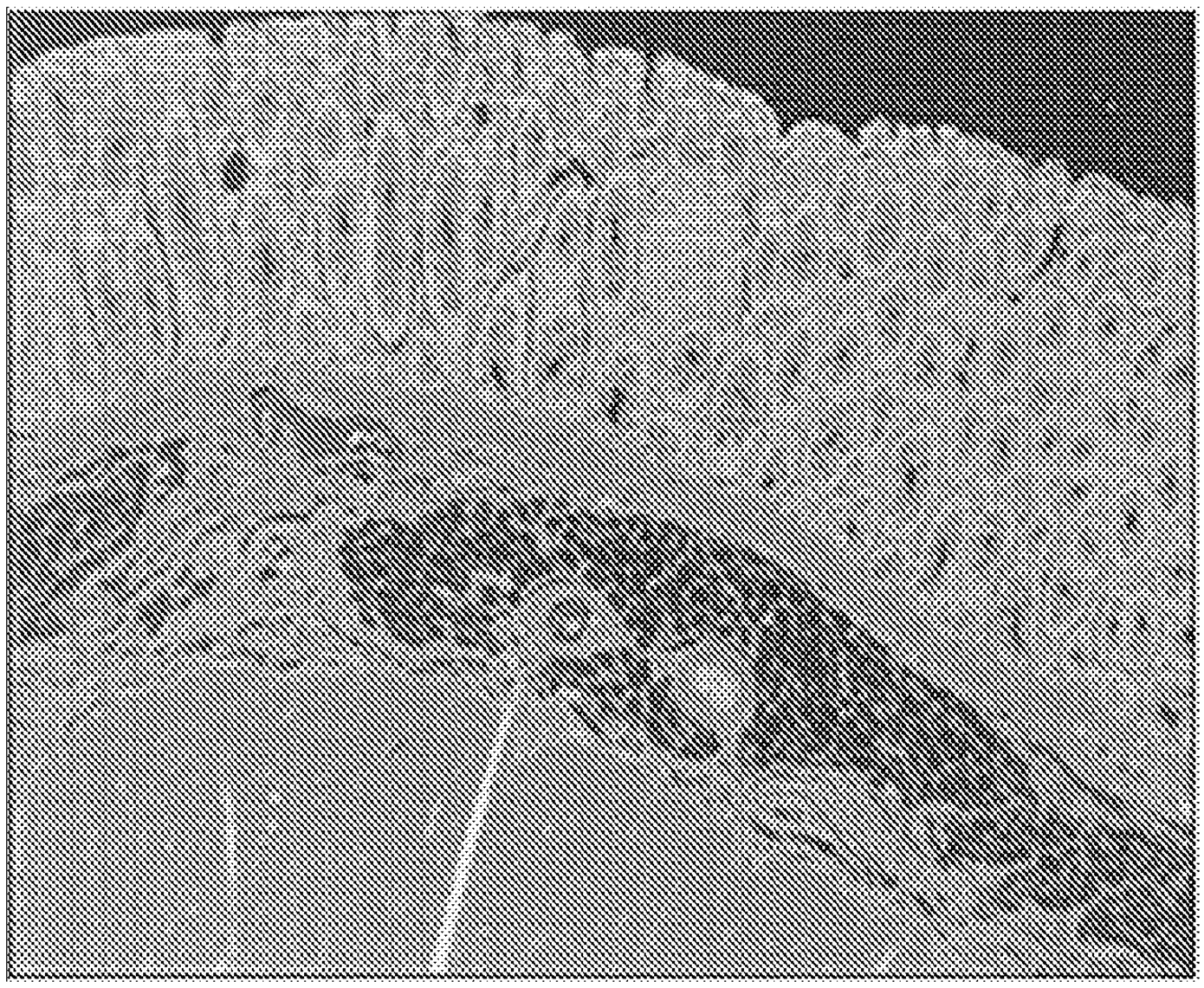


FIG. 49

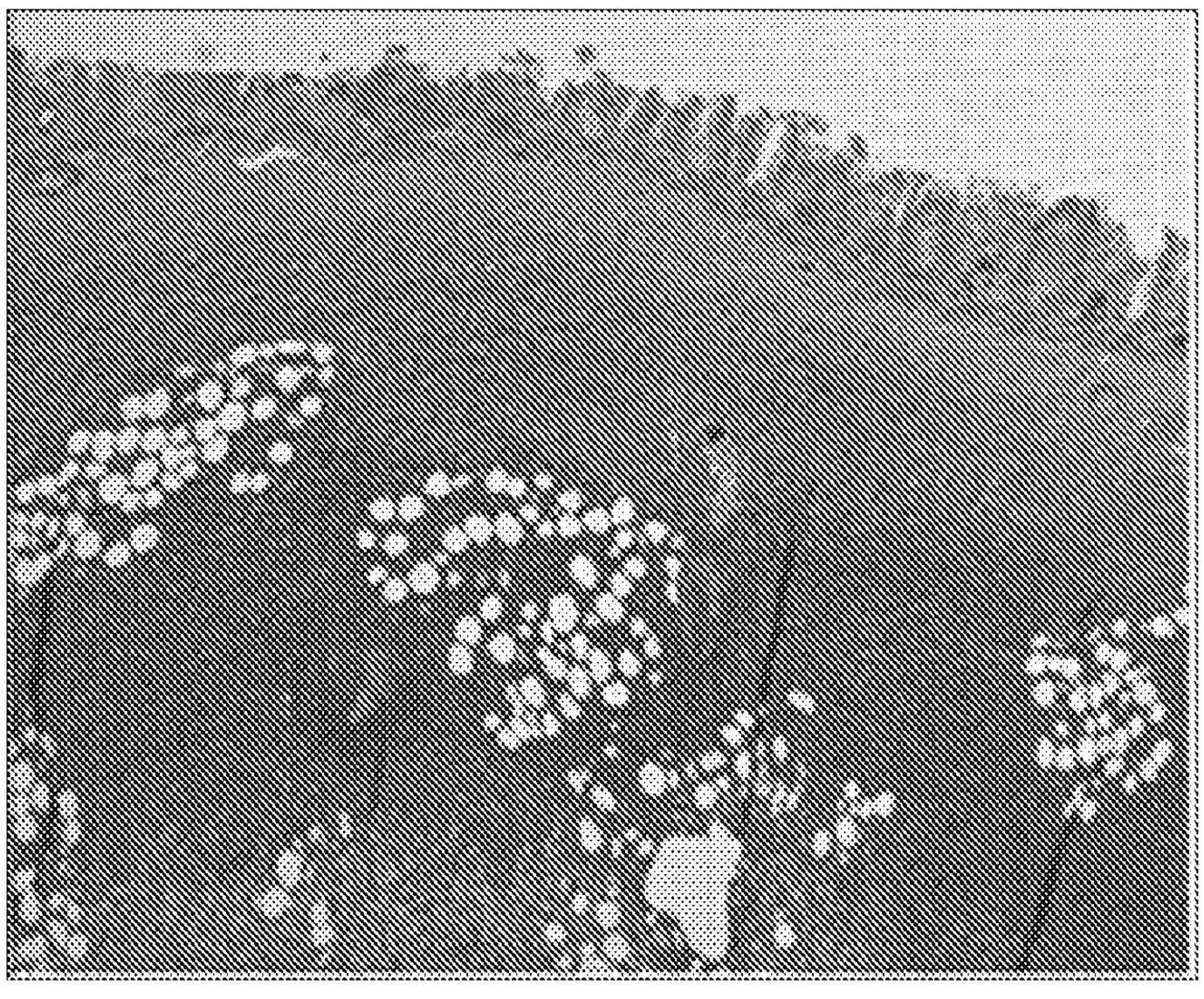


FIG. 50

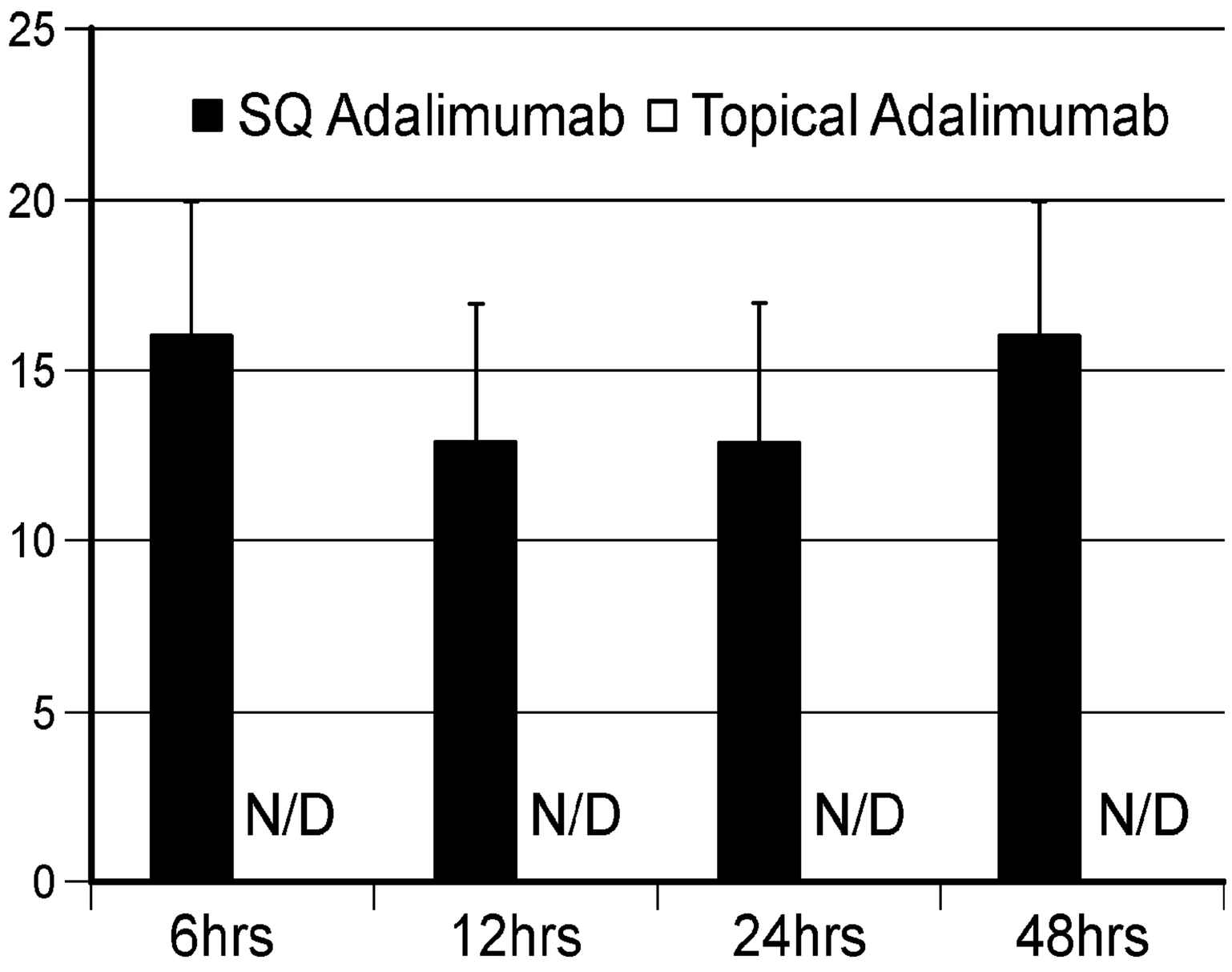

25
20
15
10
5
0
SQ Adalimumab
Topical Adalimumab
N/D
N/D
N/D
N/D
6hrs
12hrs
24hrs
48hrs

FIG. 51

Time	SQ Adalimumab Plasma concentration µgs/ml	Topical Adalimumab Plasma concentration µgs/ml
6hrs	16 +/-8	0.01
12hrs	13 +/-4	0.01
24hrs	13 +/-3	0.01
48hrs	16 +/-5	0.01

FIG. 52

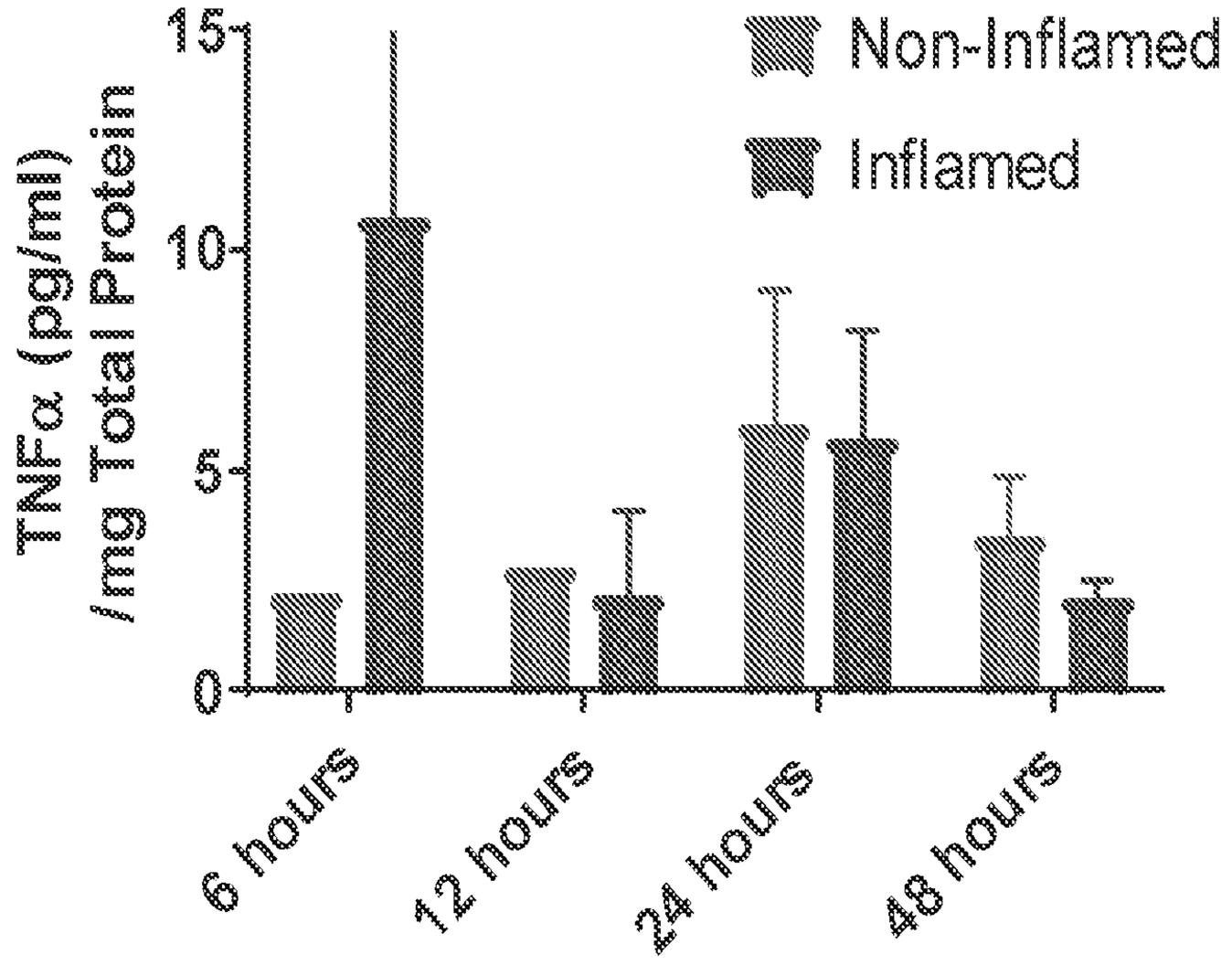

TNFα (pg/ml)
/mg Total Protein
15
10
5
0
6 hours
12 hours
24 hours
48 hours
Non-Inflamed
Inflamed

FIG. 53

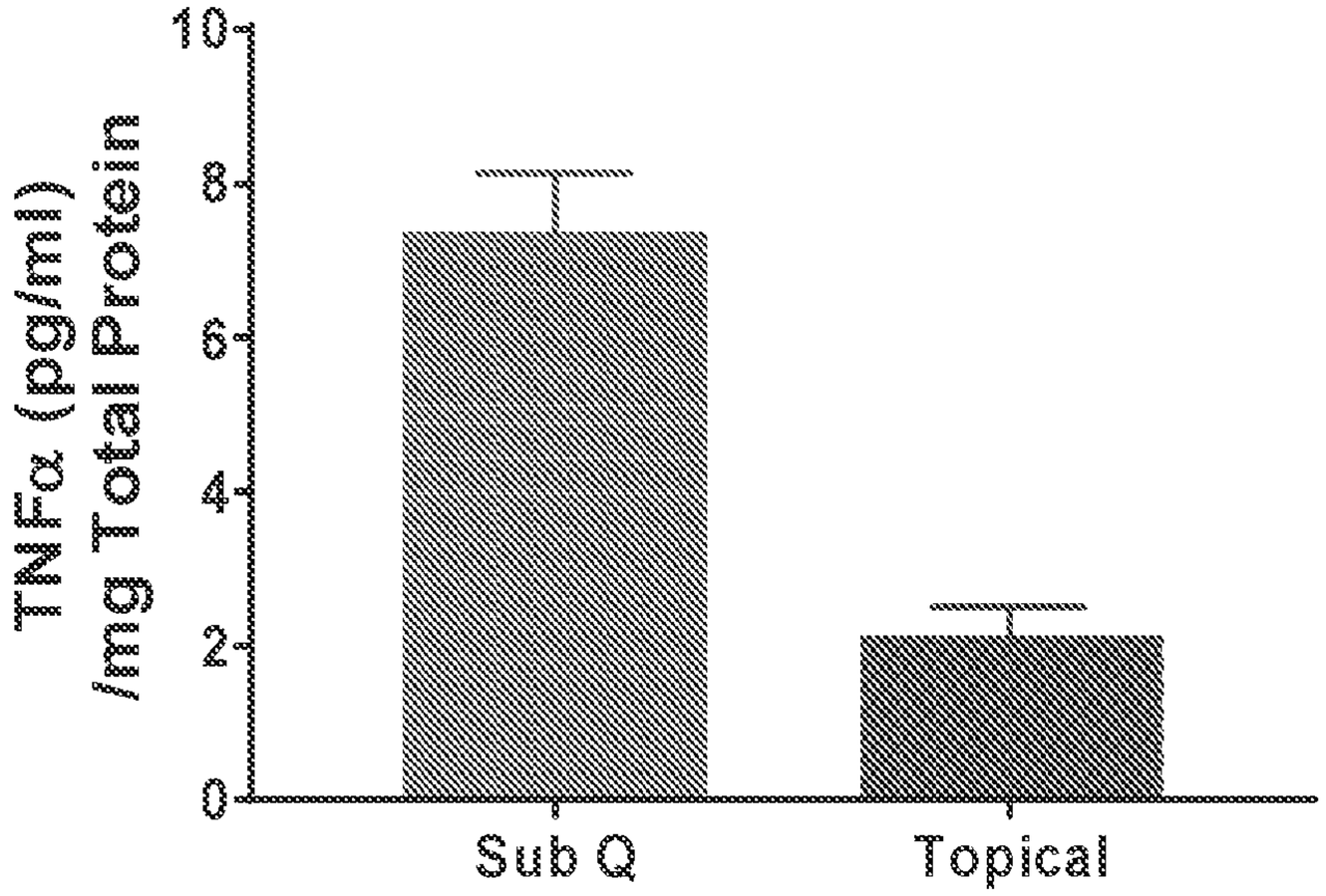


FIG. 54

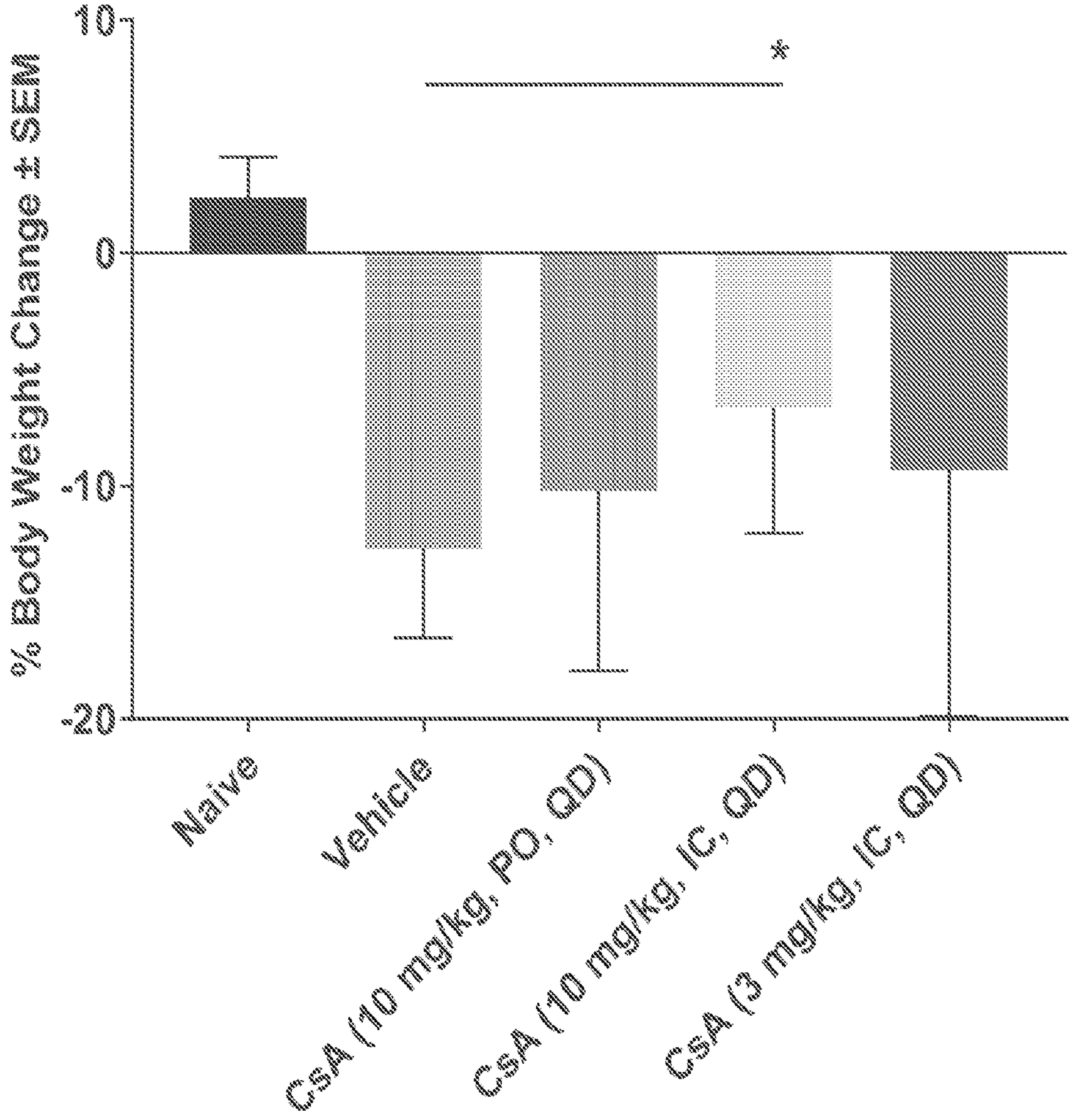

10
0
-10
-20
% Body Weight Change ± SEM
*
Naive
vehicle
CsA (10 mg/kg, PO, QD)
CsA (10 mg/kg, IC, QD)
CsA (3 mg/kg, IC, QD)

FIG. 55

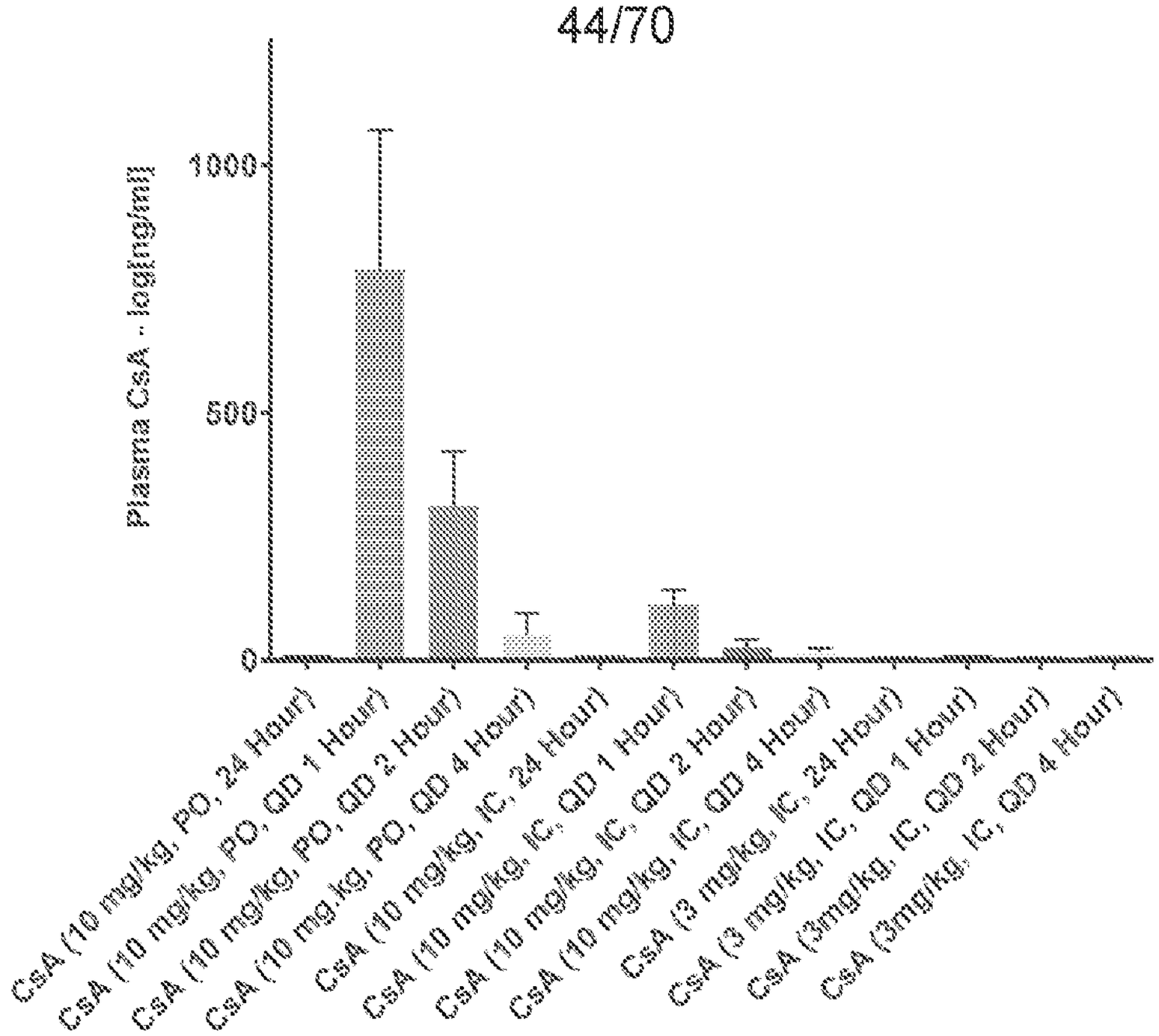

Plasma CsA - ng/ml
1000
500
0
CsA (10 mg/kg, PO, 24 Hour)
CsA (10 mg/kg, PO, QD 1 Hour)
CsA (10 mg/kg, PO, QD 2 Hour)
CsA (10 mg/kg, PO, QD 4 Hour)
CsA (10 mg/kg, IC, 24 Hour)
CsA (10 mg/kg, IC, QD 1 Hour)
CsA (10 mg/kg, IC, QD 2 Hour)
CsA (10 mg/kg, IC, QD 4 Hour)
CsA (3 mg/kg, IC, 24 Hour)
CsA (3 mg/kg, IC, QD 1 Hour)
CsA (3mg/kg, IC, QD 2 Hour)
CsA (3mg/kg, IC, QD 4 Hour)

FIG. 56

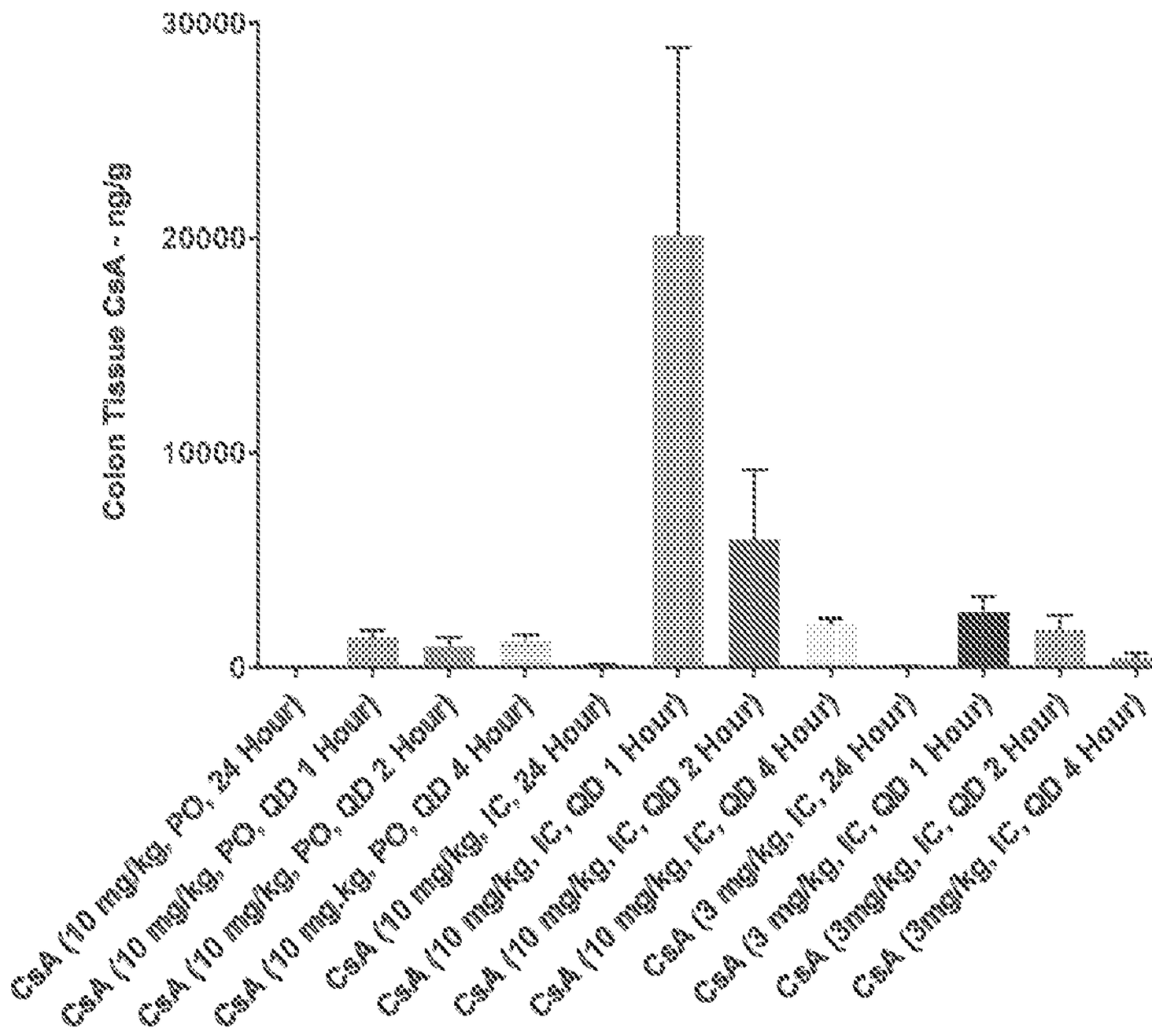

Colon Tissue CsA - ng/g
30000
20000
10000
0
CsA (10 mg/kg, PO, 24 Hour)
CsA (10 mg/kg, PO, QD 1 Hour)
CsA (10 mg/kg, PO, QD 2 Hour)
CsA (10 mg/kg, PO, QD 4 Hour)
CsA (10 mg/kg, IC, 24 Hour)
CsA (10 mg/kg, IC, QD 1 Hour)
CsA (10 mg/kg, IC, QD 2 Hour)
CsA (10 mg/kg, IC, QD 4 Hour)
CsA (3 mg/kg, IC, 24 Hour)
CsA (3 mg/kg, IC, QD 1 Hour)
CsA (3mg/kg, IC, QD 2 Hour)
CsA (3mg/kg, IC, QD 4 Hour)

FIG. 57

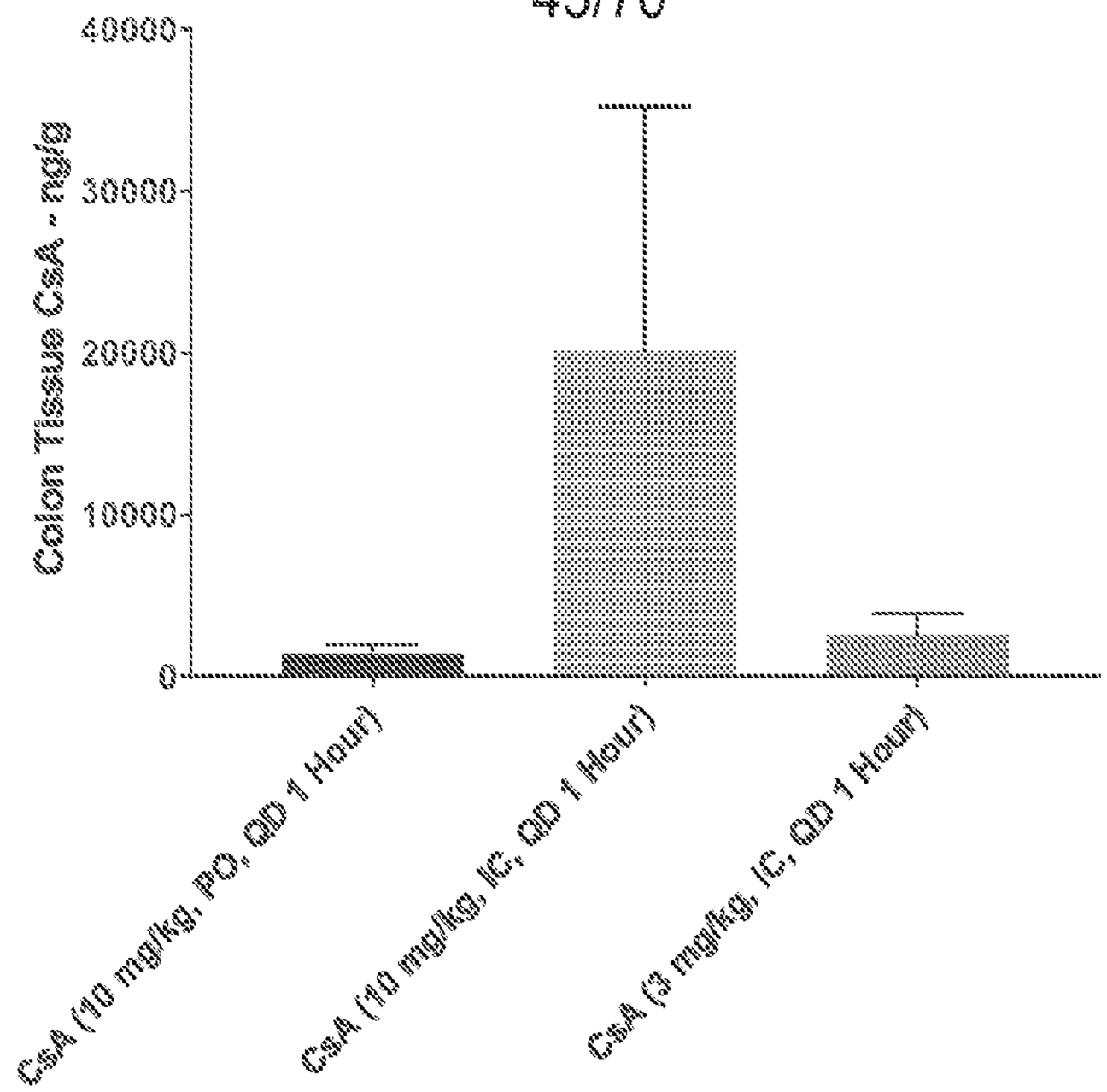

Colon Tissue CsA - ng/g
40000
30000
20000
10000
0
CsA (10 mg/kg, PO, QD 1 Hour)
CsA (10 mg/kg, IC, QD 1 Hour)
CsA (3 mg/kg, IC, QD 1 Hour)

FIG. 58

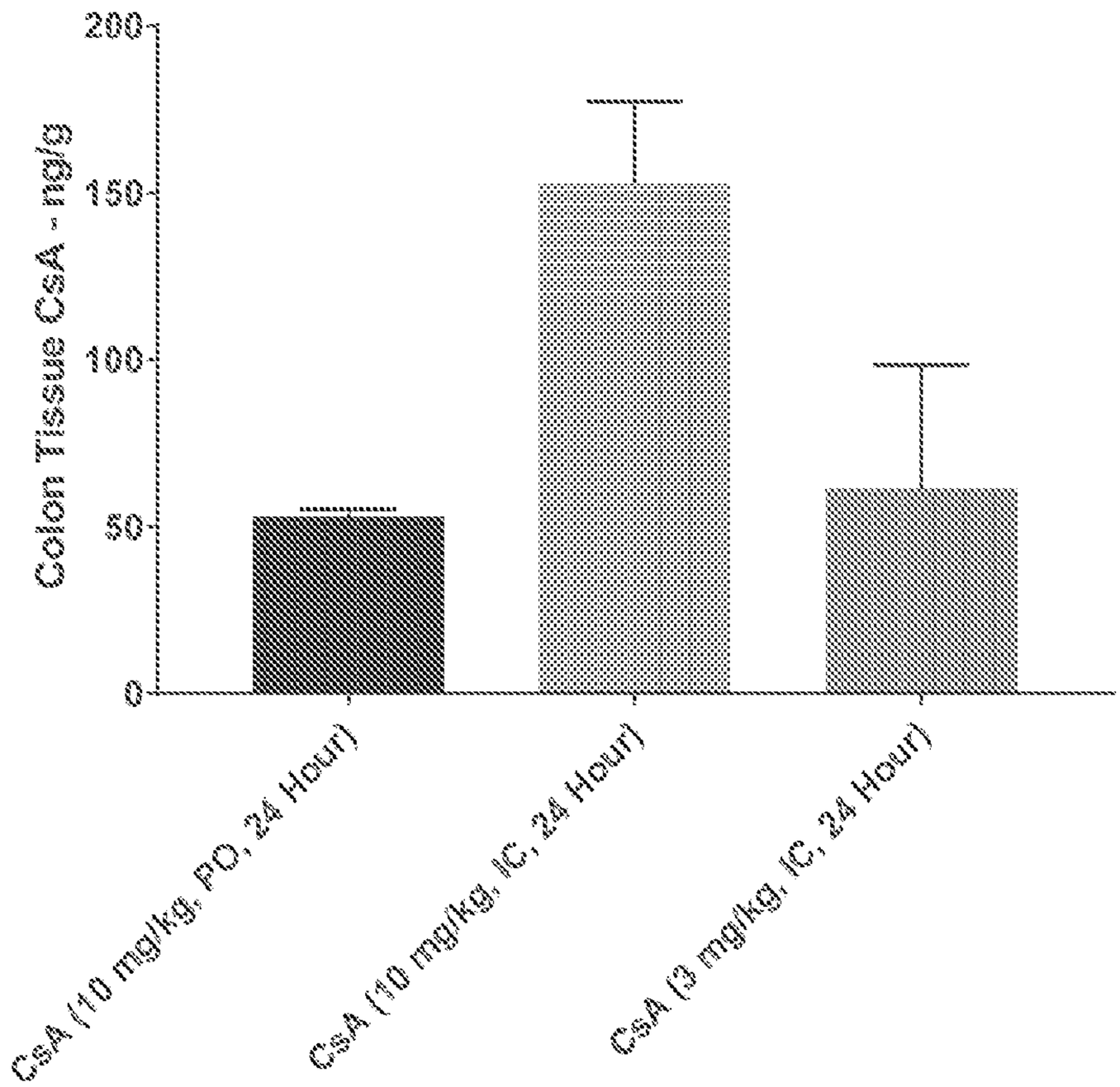

Colon Tissue CsA - ng/g
200
150
100
50
0
CsA (10 mg/kg, PO, 24 Hour)
CsA (10 mg/kg, IC, 24 Hour)
CsA (3 mg/kg, IC, 24 Hour)

FIG. 59

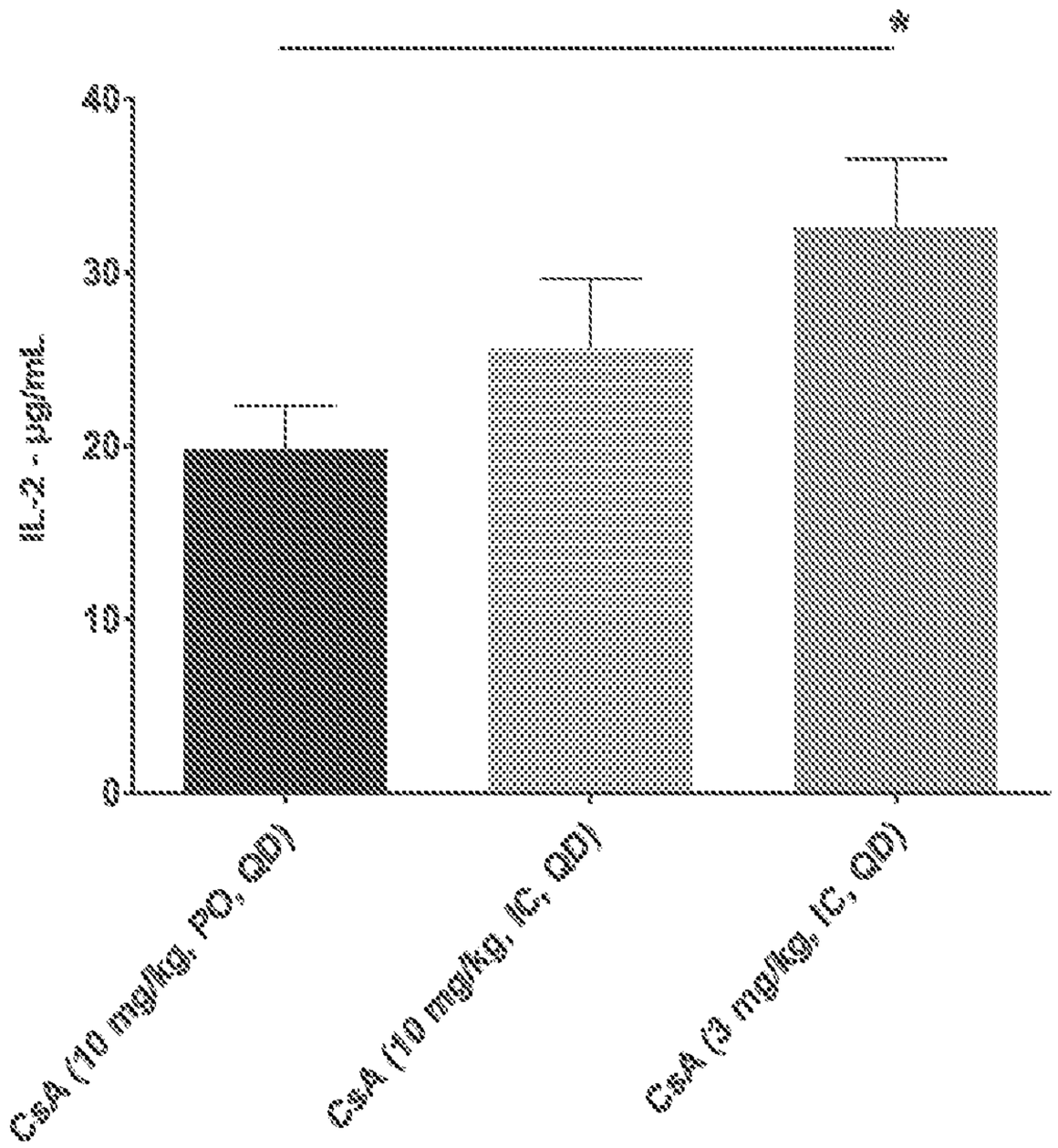

*
IL-2 - µg/mL
40
30
20
10
0
CsA (10 mg/kg, PO, QD)
CsA (10 mg/kg, IC, QD)
CsA (3 mg/kg, IC, QD)

FIG. 60

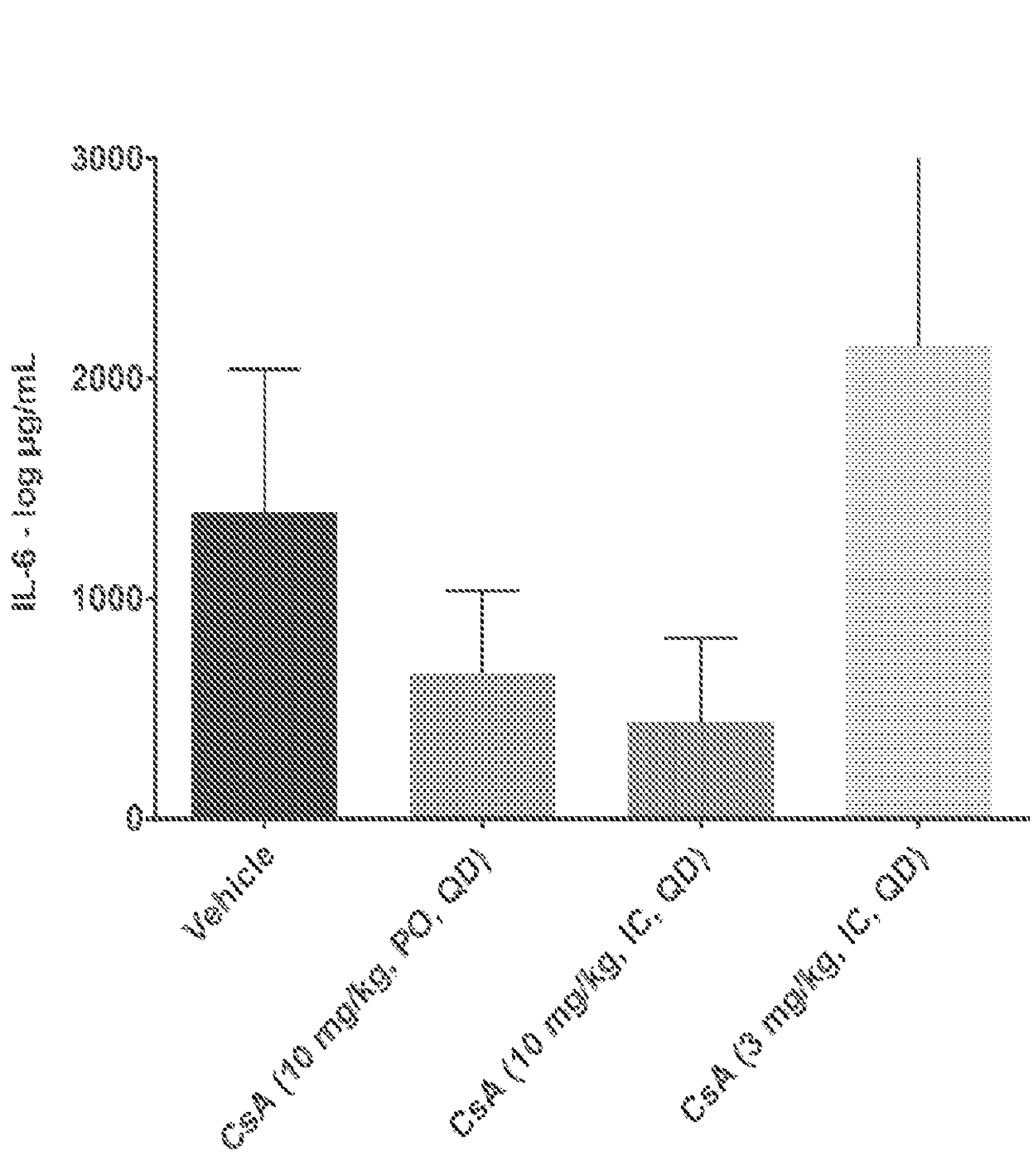

IL-6 - log µg/mL
3000
2000
1000
0
Vehicle
CsA (10 mg/kg, PO, QD)
CsA (10 mg/kg, IC, QD)
CsA (3 mg/kg, IC, QD)

FIG. 61

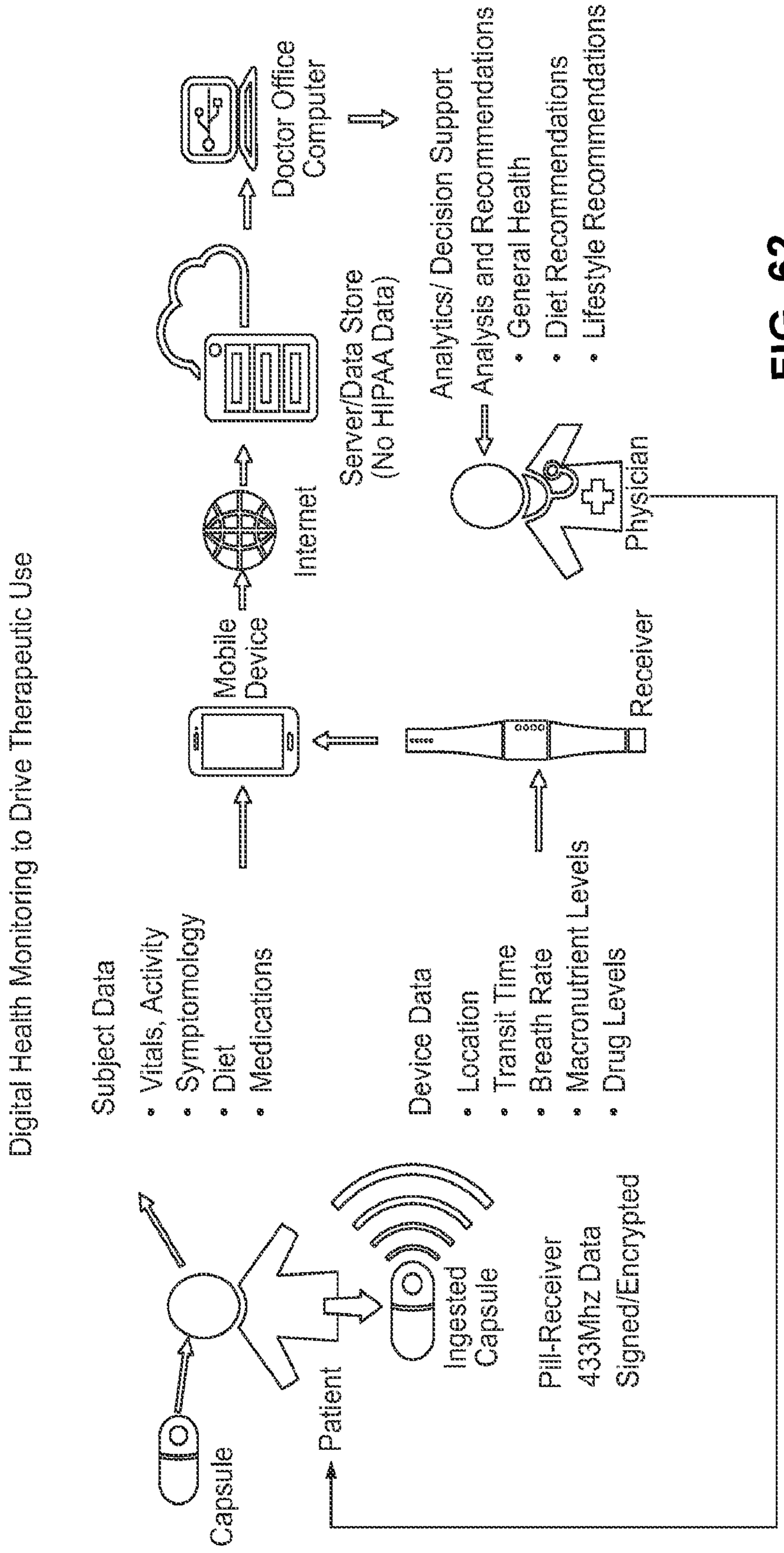

Digital Health Monitoring to Drive Therapeutic Use
Subject Data
• Vitals, Activity
• Symptomology
• Diet
• Medications
Device Data
• Location
• Transit Time
• Breath Rate
• Macronutrient Levels
• Drug Levels
Mobile Device
Internet
Server/Data Store (No HIPAA Data)
Doctor Office Computer
Analytics/ Decision Support
• Analysis and Recommendations
• General Health
• Diet Recommendations
• Lifestyle Recommendations
Physician
Receiver
Capsule
Patient
Ingested Capsule
Pill-Receiver
433Mhz Data
Signed/Encrypted

FIG. 62

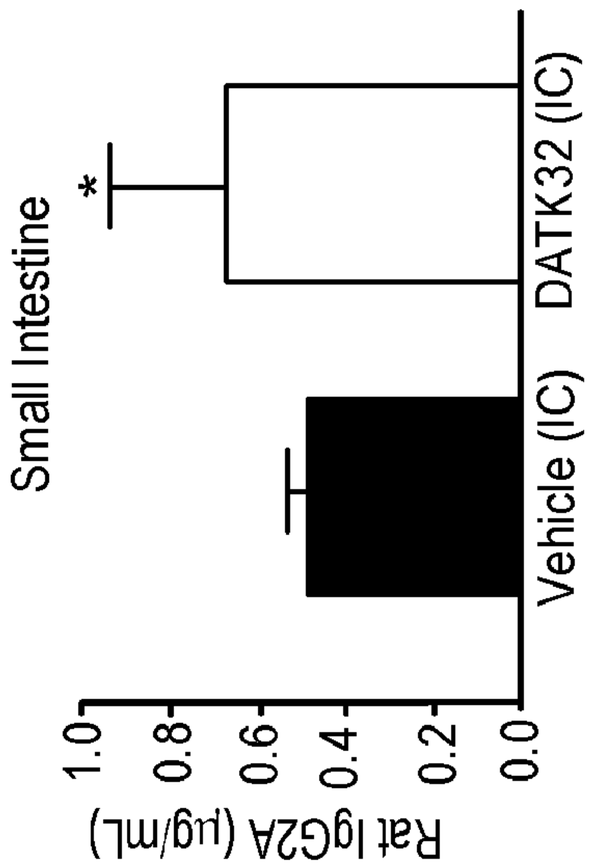


FIG. 63C

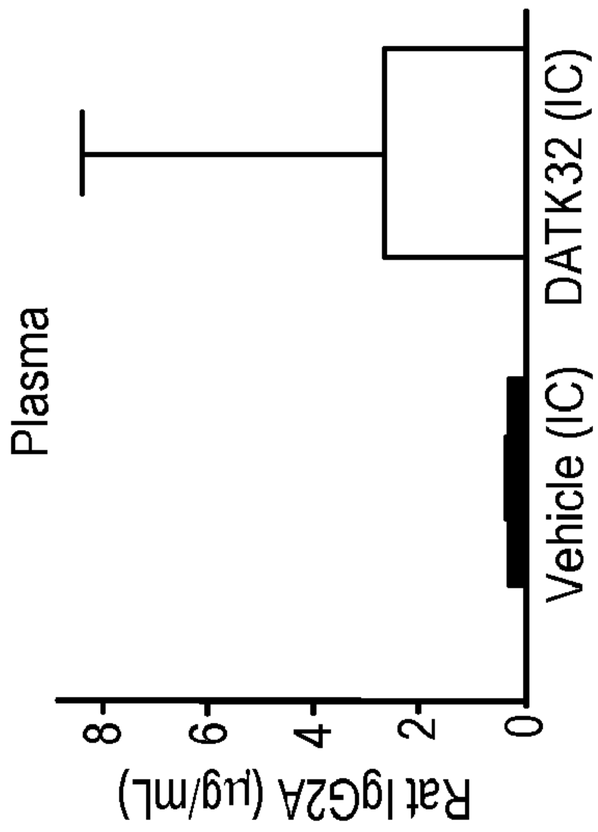


FIG. 63F

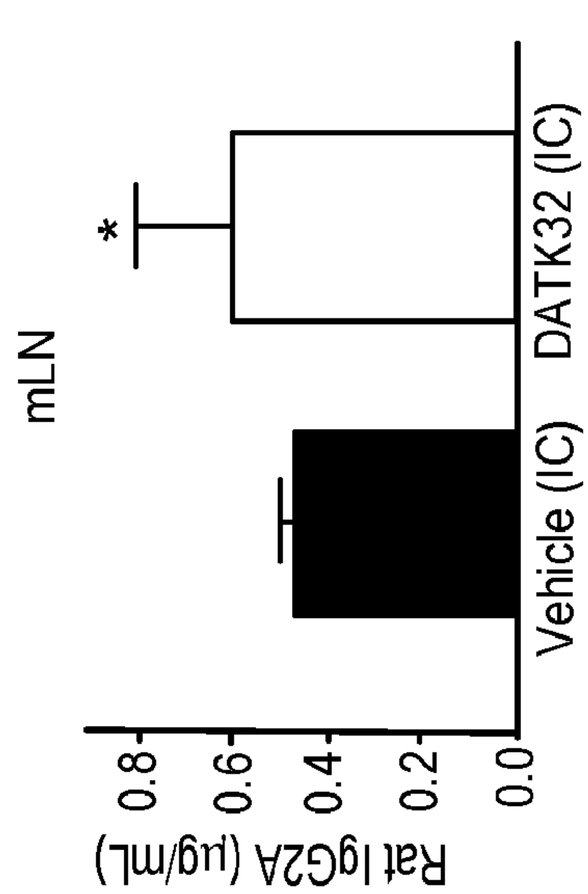


FIG. 63B

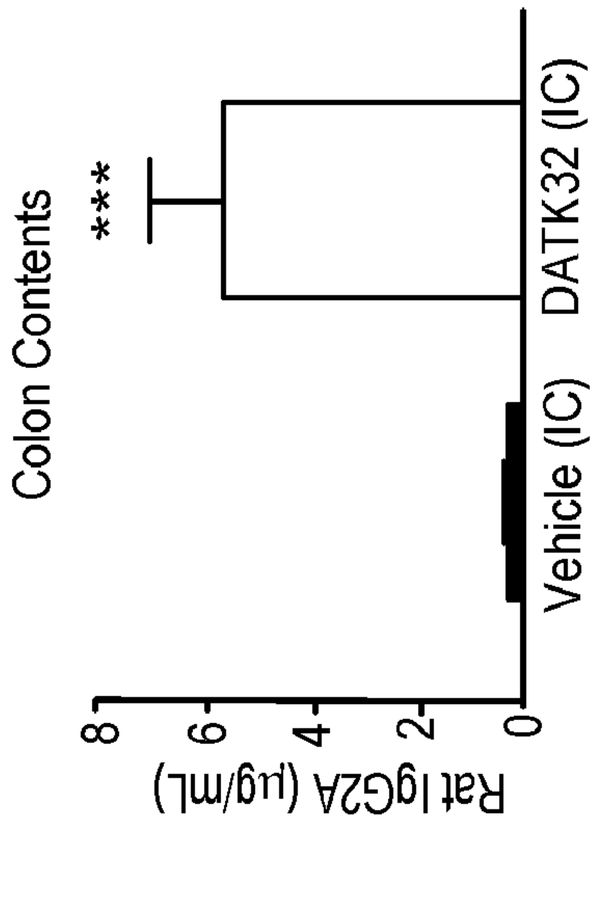


FIG. 63E

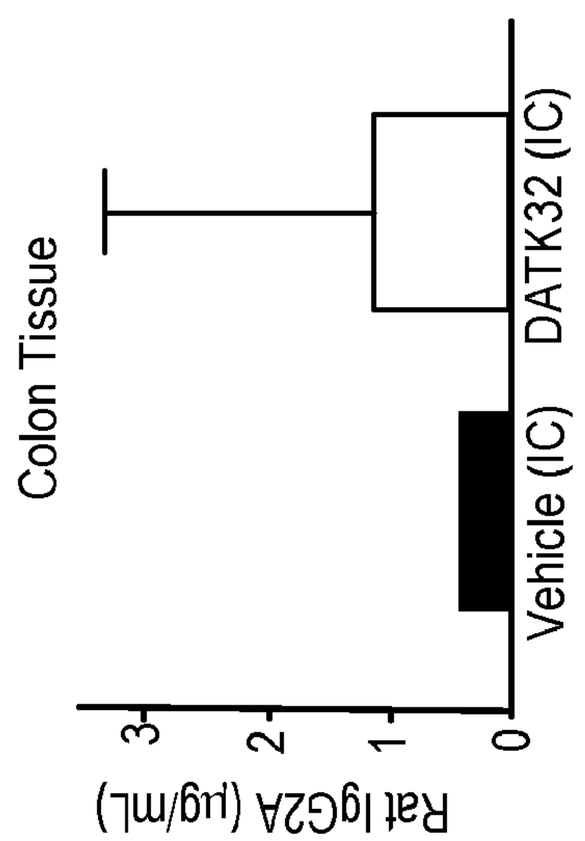


FIG. 63A

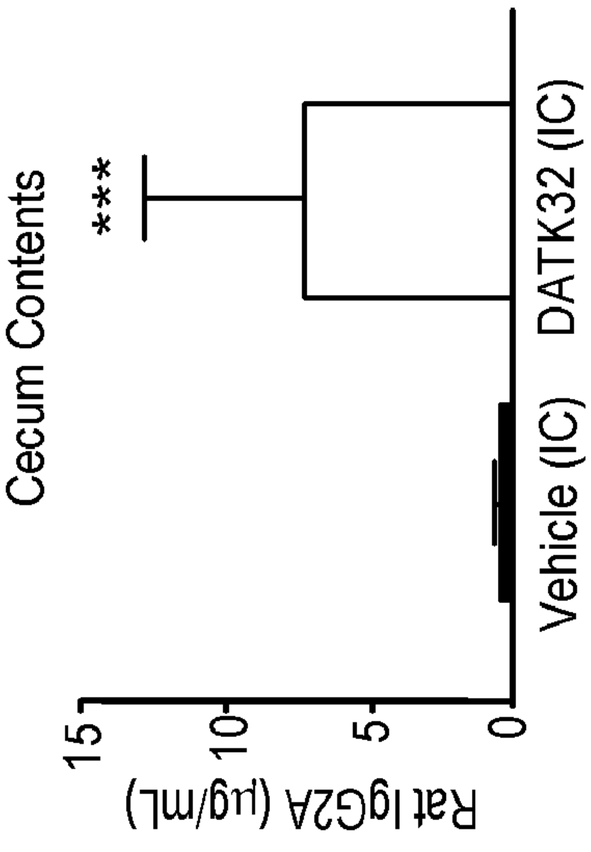


FIG. 63D

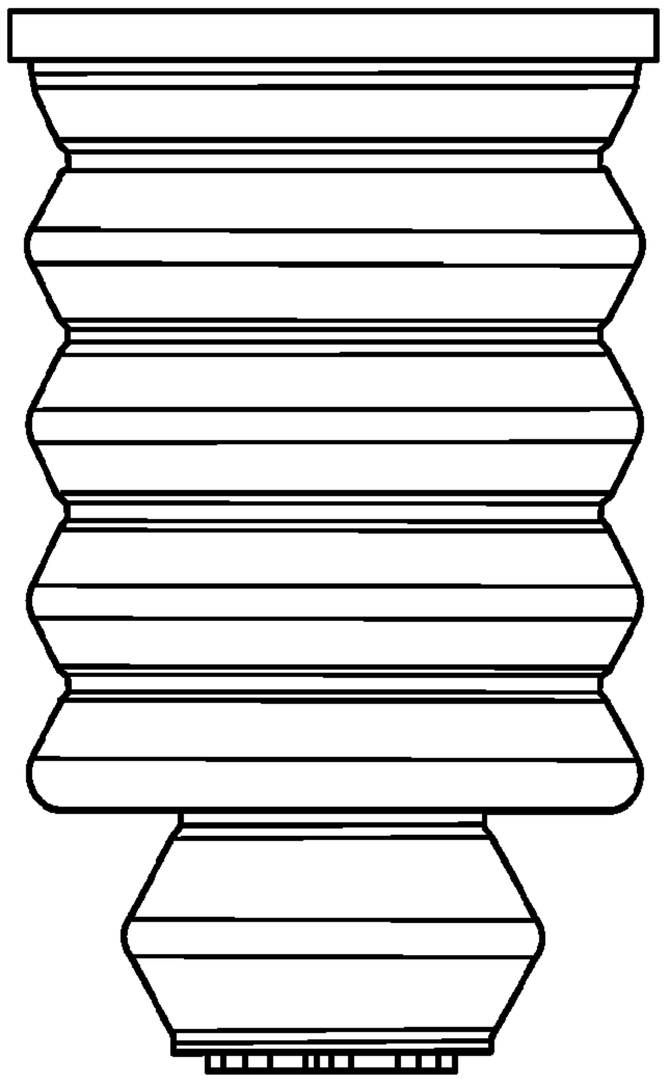


FIG. 64

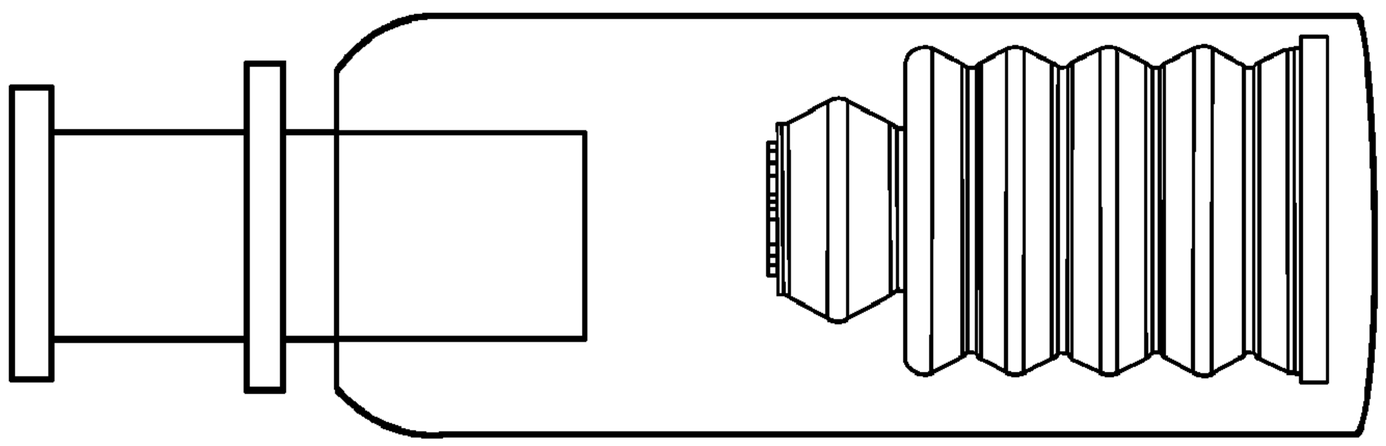


FIG. 65

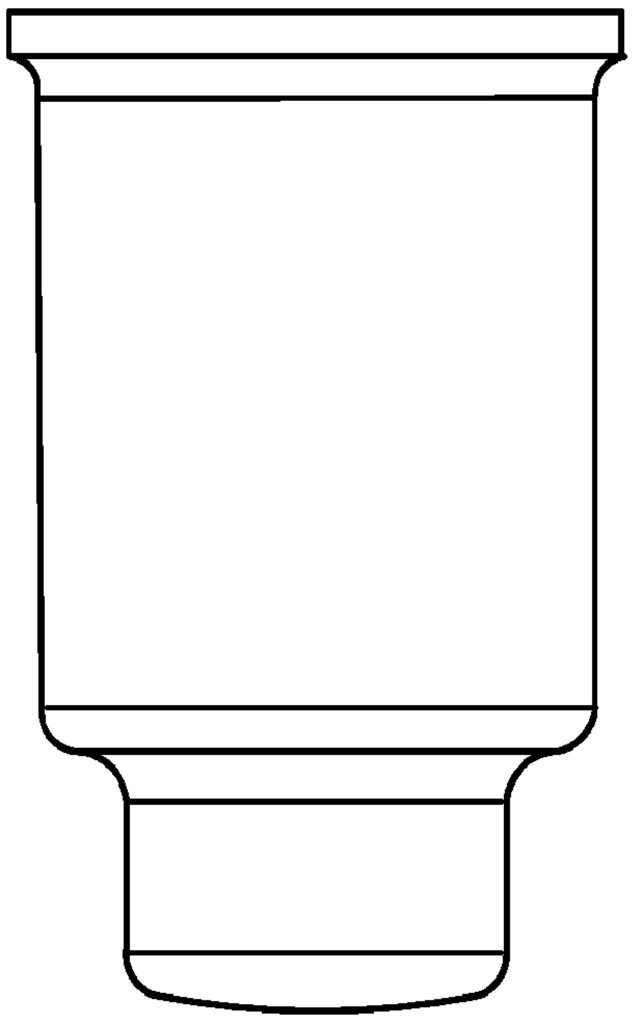


FIG. 66

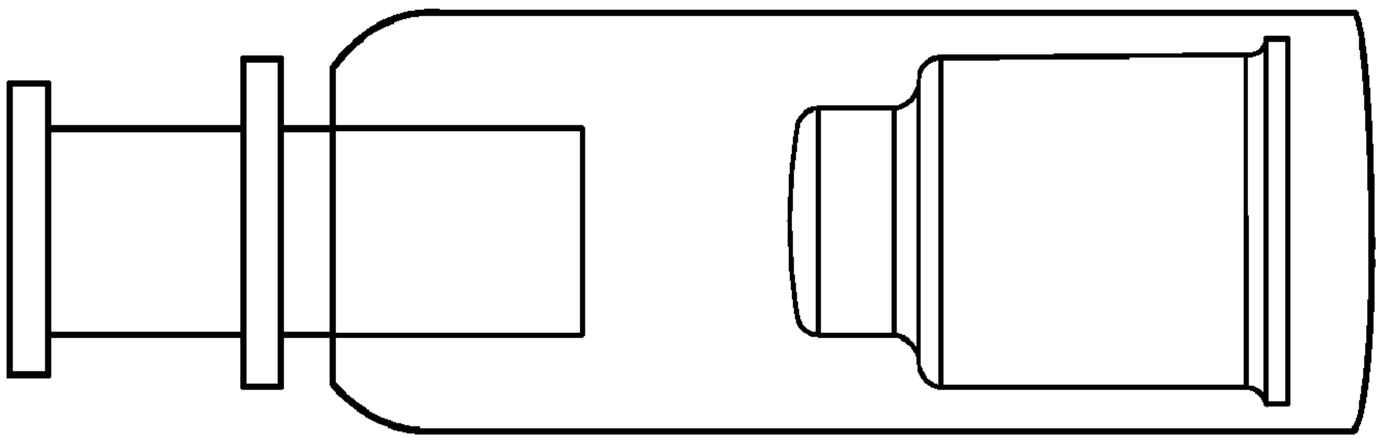


FIG. 67

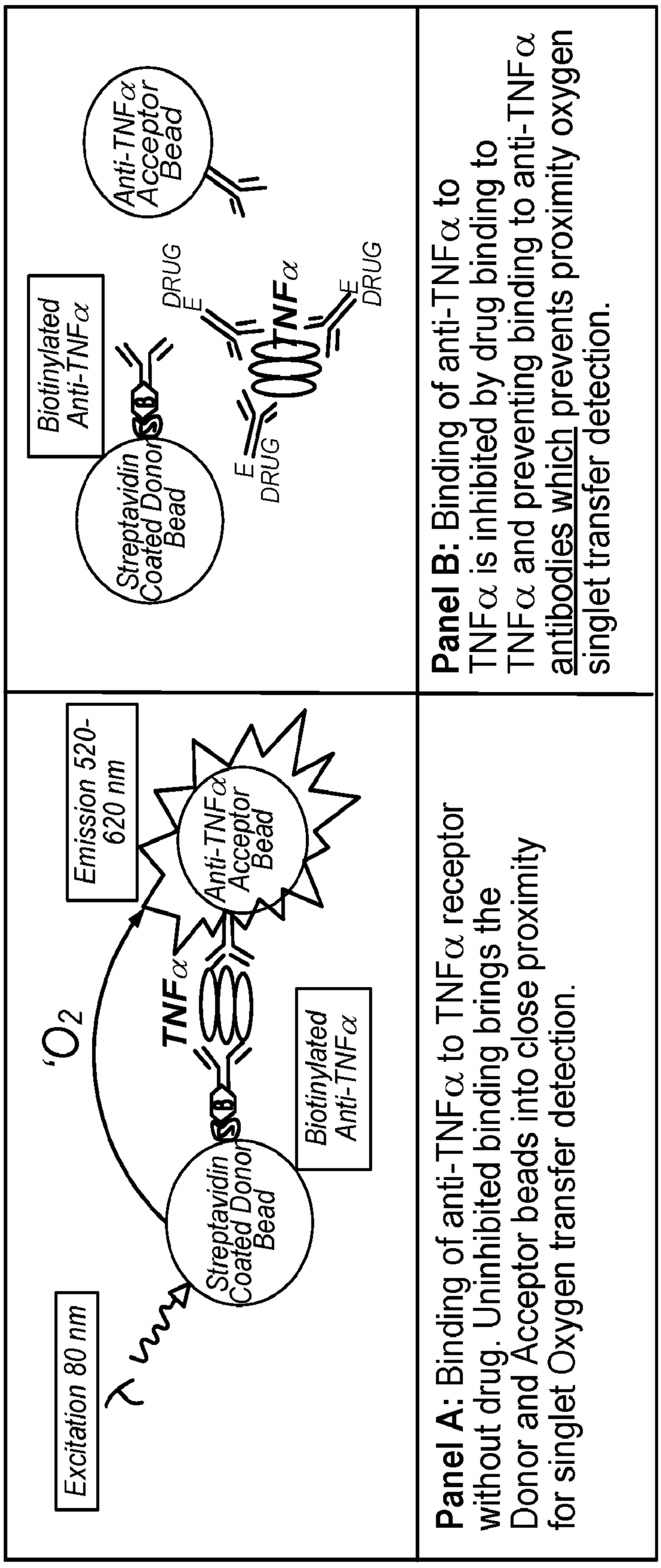

Anti-TNFα Acceptor Bead
Biotinylated Anti-TNFα
Streptavidin Coated Donor Bead
DRUG
E
TNFα
Panel B: Binding of anti-TNFα to TNFα receptor is inhibited by drug binding to TNFα and preventing binding to antibodies which prevents proximity singlet transfer detection.
Excitation 80 nm
λ 680 nm
'O2
Emission 520-620 nm
Panel A: Binding of anti-TNFα to TNFα receptor without drug. Uninhibited binding brings the Donor and Acceptor beads into close proximity for singlet Oxygen transfer detection.

FIG. 68

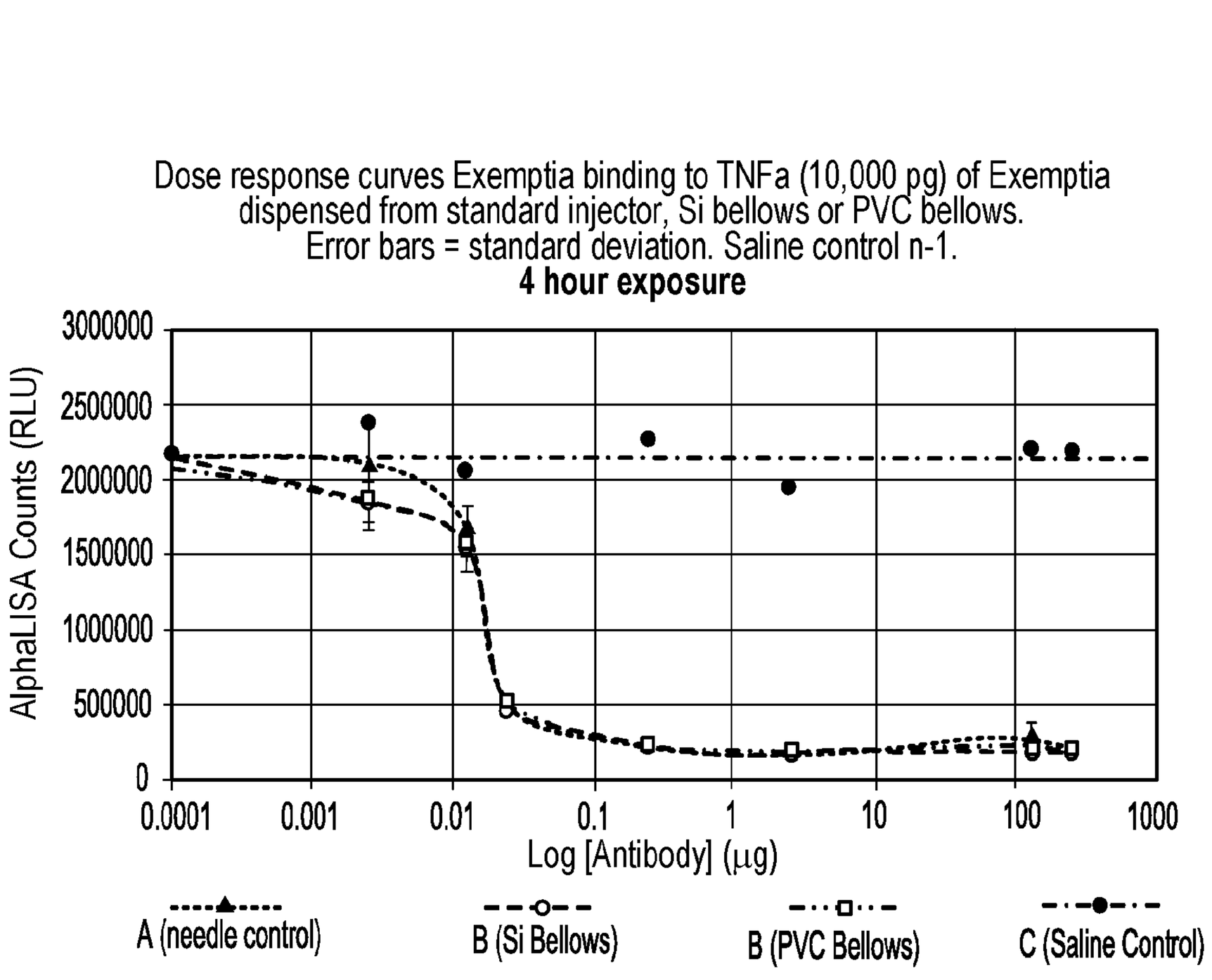

Dose response curves Exemptia binding to TNFa (10,000 pg) of Exemptia dispensed from standard injector, Si bellows or PVC bellows.
Error bars = standard deviation. Saline control n-1.
4 hour exposure
AlphaLISA Counts (RLU)
3000000
2500000
2000000
1500000
1000000
500000
0
0.0001
0.001
0.01
0.1
1
10
100
1000
Log [Antibody] (µg)
A (needle control)
B (Si Bellows)
B (PVC Bellows)
C (Saline Control)

FIG. 69

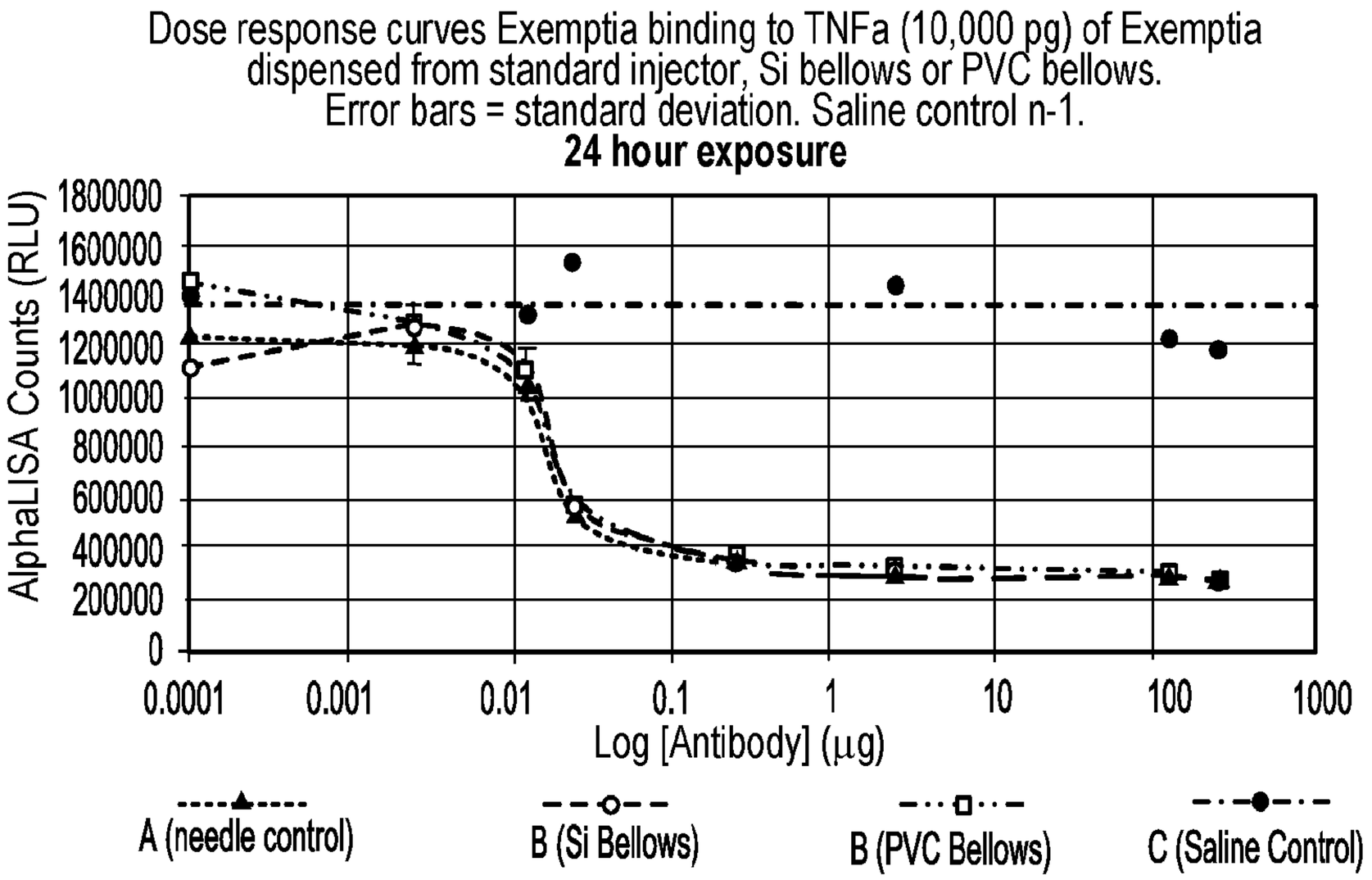

Dose response curves Exemptia binding to TNFa (10,000 pg) of Exemptia dispensed from standard injector, Si bellows or PVC bellows.
Error bars = standard deviation. Saline control n-1.
24 hour exposure
AlphaLISA Counts (RLU)
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
0.0001
0.001
0.01
0.1
1
10
100
1000
Log [Antibody] (µg)
A (needle control)
B (Si Bellows)
B (PVC Bellows)
C (Saline Control)

FIG. 70

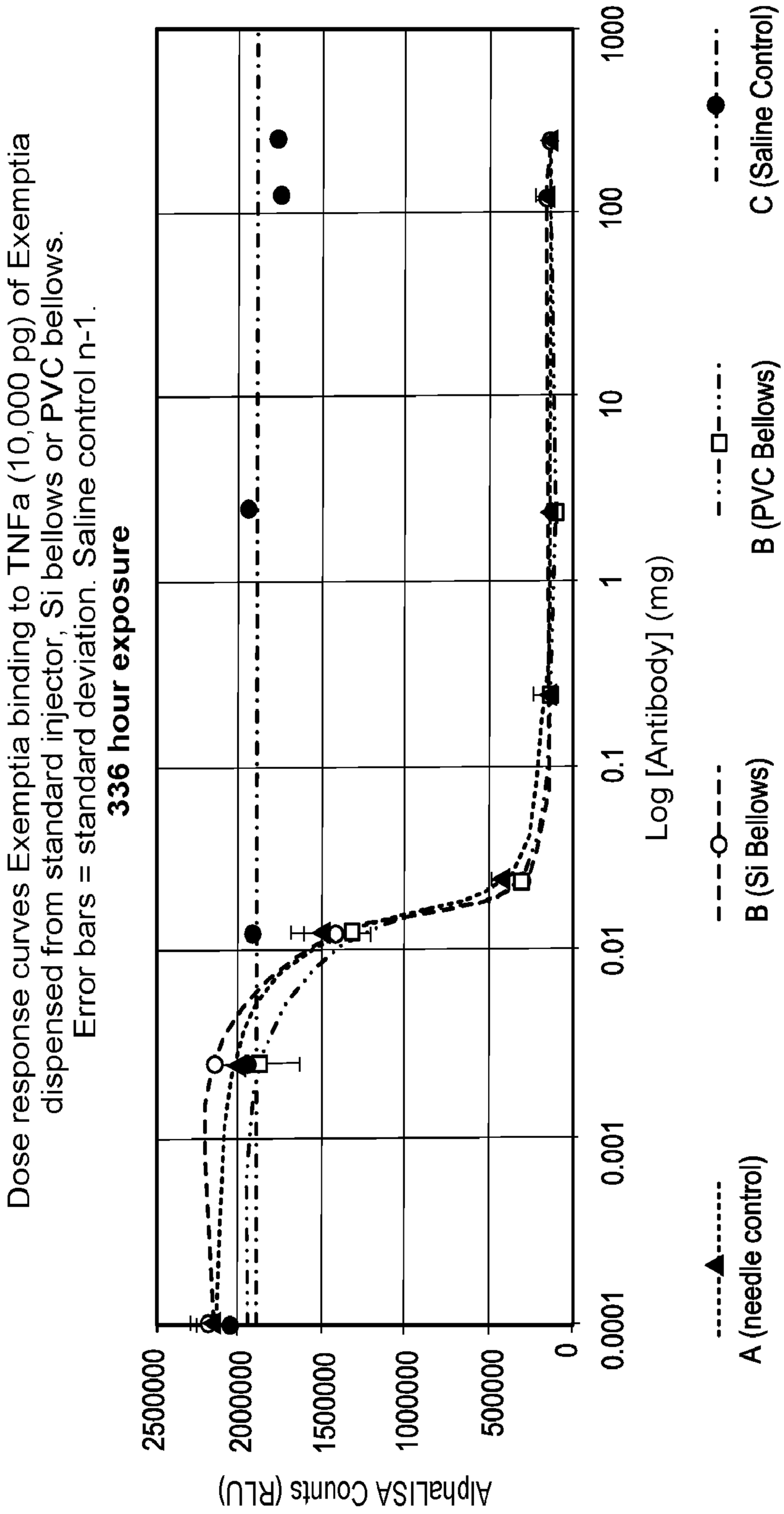

Dose response curves Exemptia binding to TNFa (10,000 pg) of Exemptia dispensed from standard injector, Si bellows or PVC bellows.
Error bars = standard deviation. Saline control n-1.
336 hour exposure
AlphaLISA Counts (RLU)
2500000
2000000
1500000
1000000
500000
0
0.0001
0.001
0.01
0.1
1
10
100
1000
Log [Antibody] (mg)
A (needle control)
B (PVC Bellows)
B (Si Bellows)
C (Saline Control)

FIG. 71

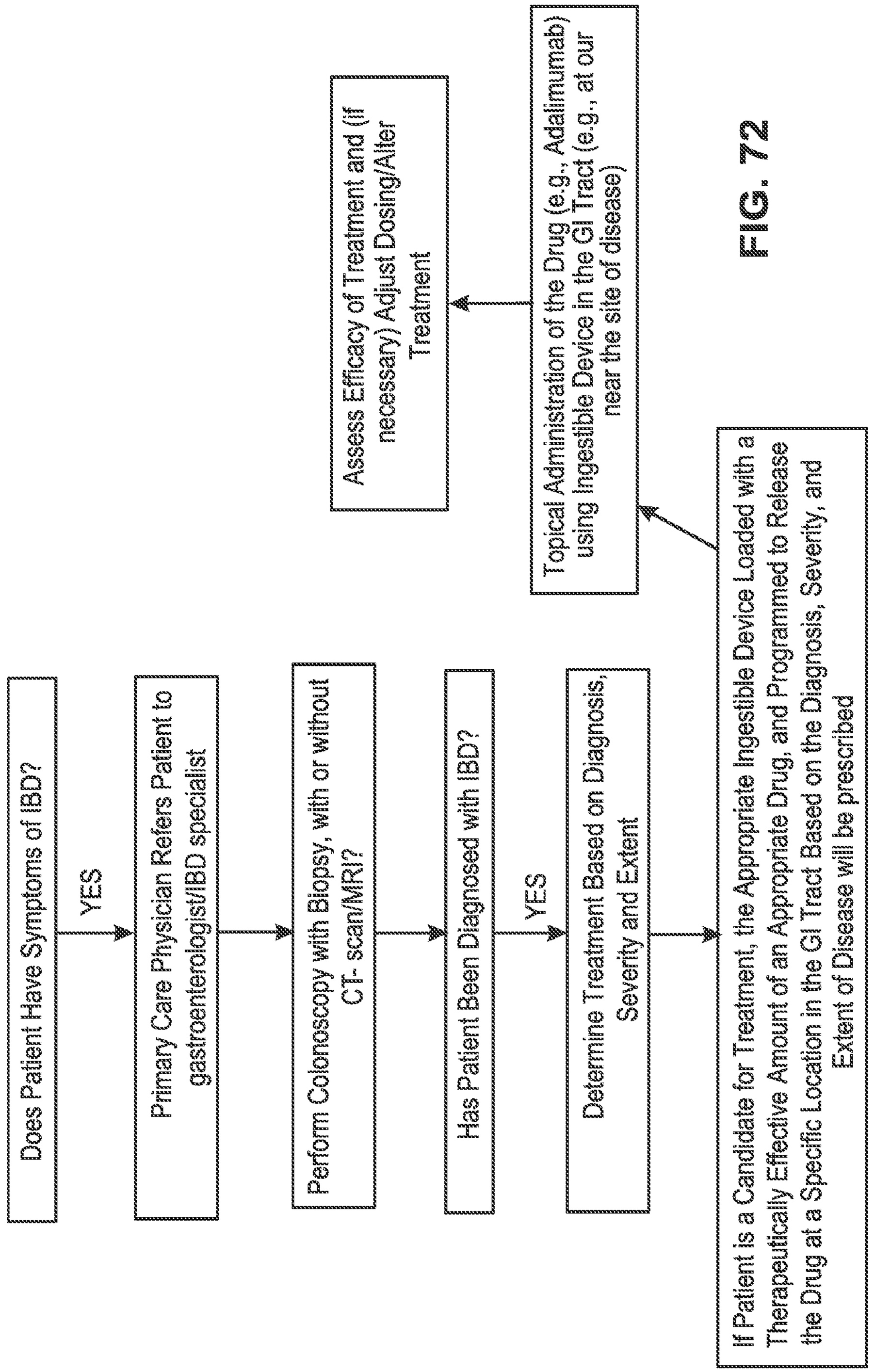

Does Patient Have Symptoms of IBD?
YES
Primary Care Physician Refers Patient to gastroenterologist/IBD specialist
Perform Colonoscopy with Biopsy, with or without CT- scan/MRI?
Has Patient Been Diagnosed with IBD?
YES
Determine Treatment Based on Diagnosis, Severity and Extent
If Patient is a Candidate for Treatment, the Appropriate Ingestible Device Loaded with a Therapeutically Effective Amount of an Appropriate Drug, and Programmed to Release the Drug at a Specific Location in the GI Tract Based on the Diagnosis, Severity, and Extent of Disease will be prescribed
Topical Administration of the Drug (e.g., Adalimumab) using Ingestible Device in the GI Tract (e.g., at our near the site of disease)
Assess Efficacy of Treatment and (if necessary) Adjust Dosing/Alter Treatment

FIG. 72

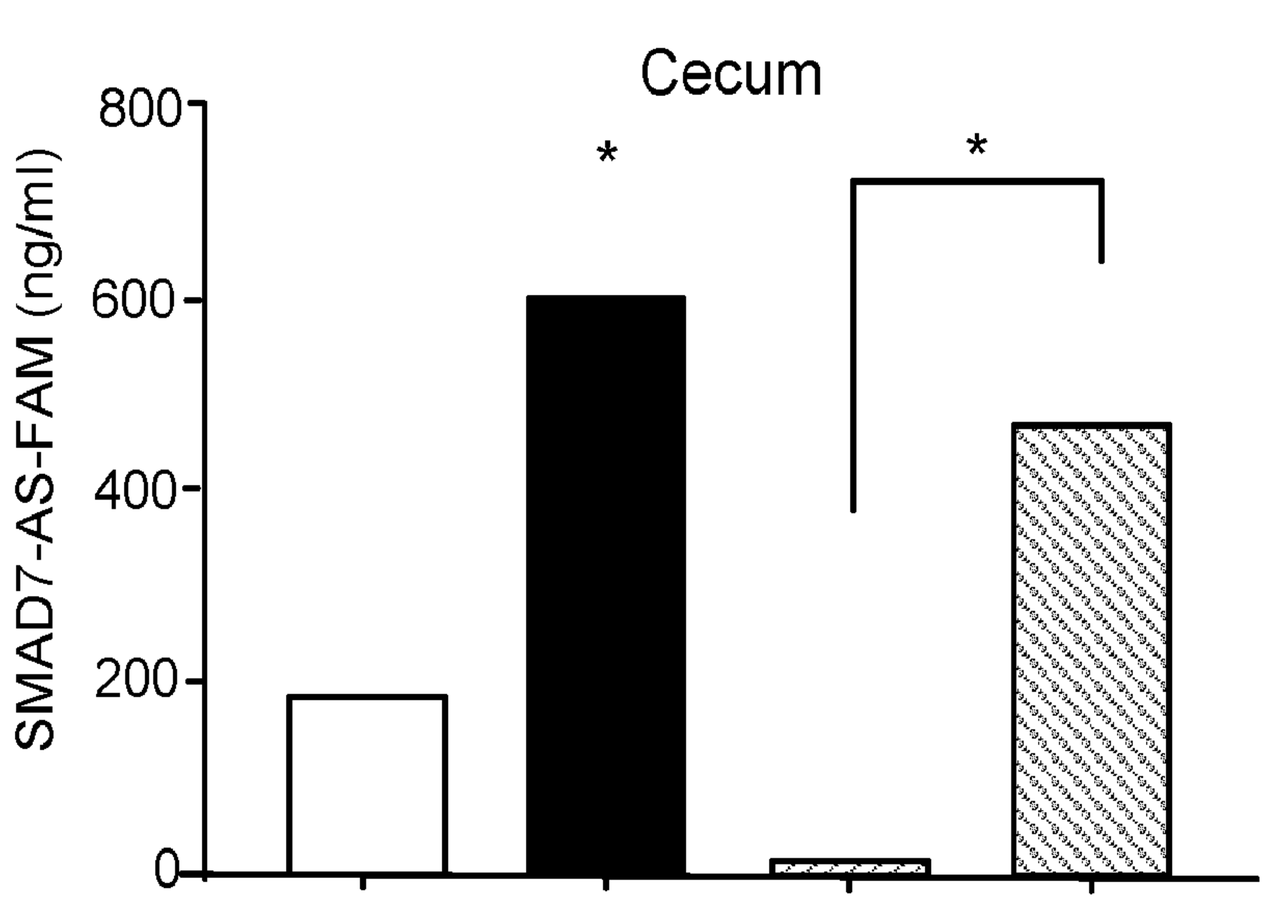

Cecum
SMAD7-AS-FAM (ng/ml)
800
600
400
200
0
*
*

FIG. 73

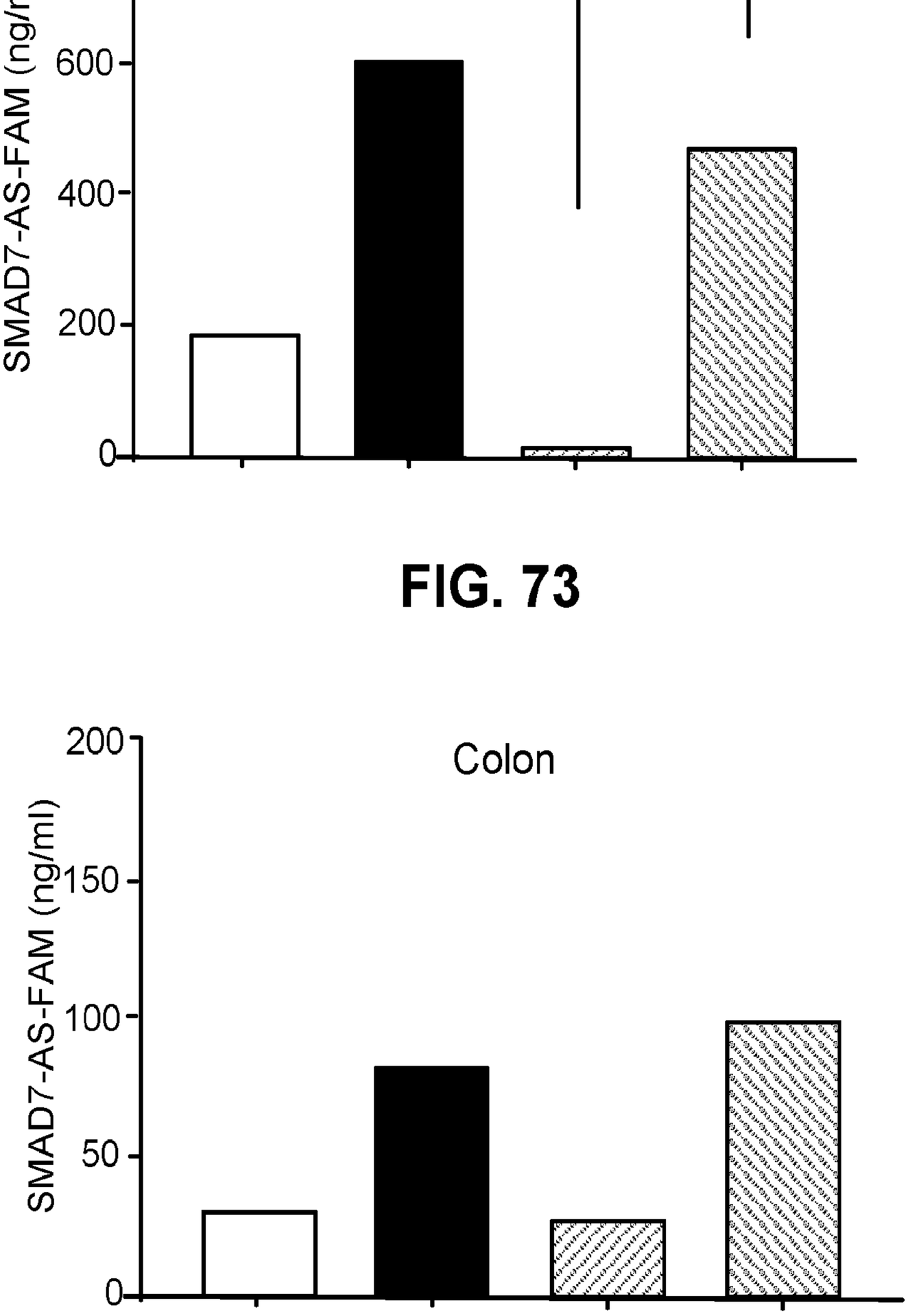

Colon
SMAD7-AS-FAM (ng/ml)
200
150
100
50
0

FIG. 74

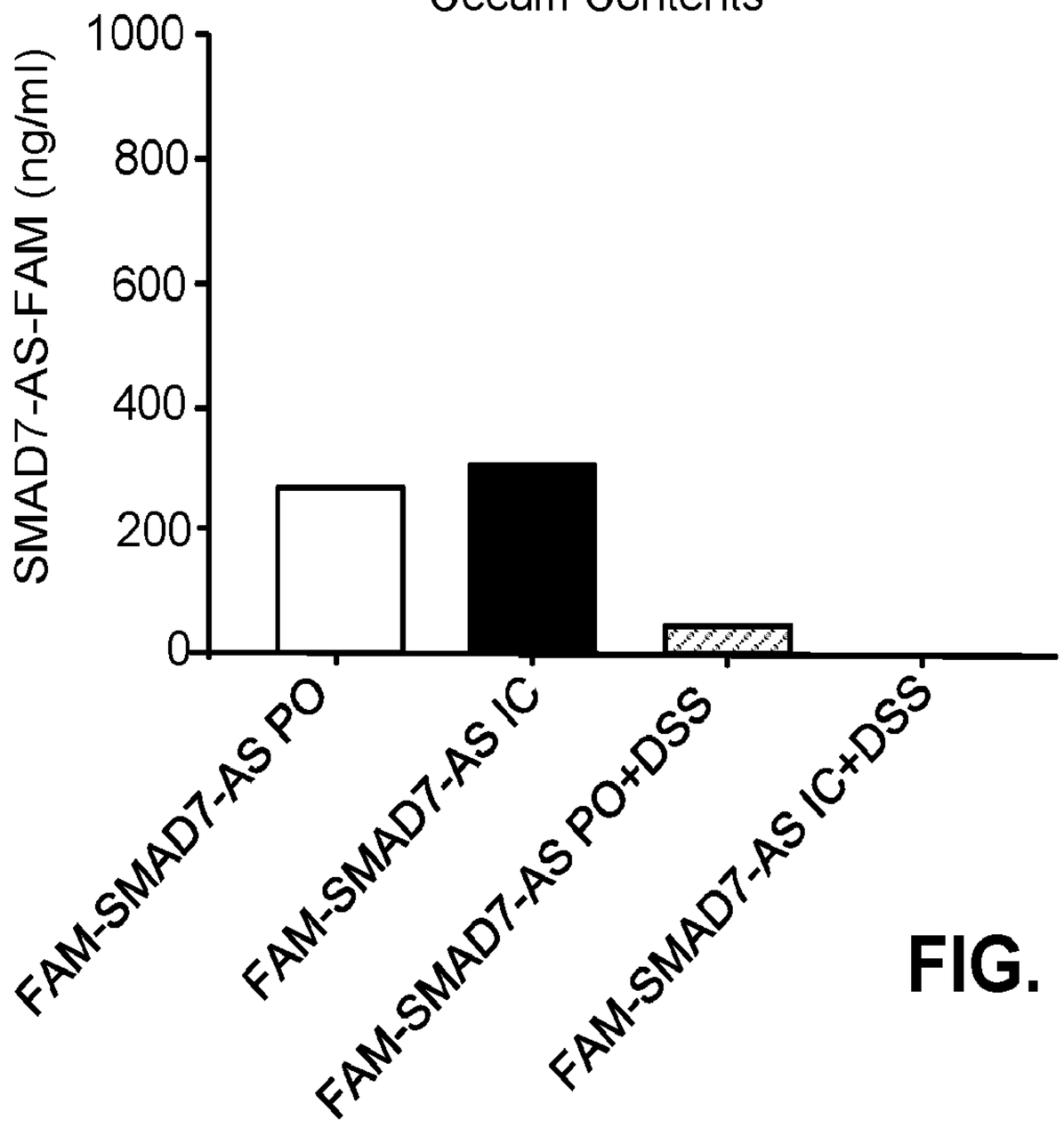

Cecum Contents
SMAD7-AS-FAM (ng/ml)
1000
800
600
400
200
0
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC
FAM-SMAD7-AS PO+DSS
FAM-SMAD7-AS IC+DSS

FIG. 75

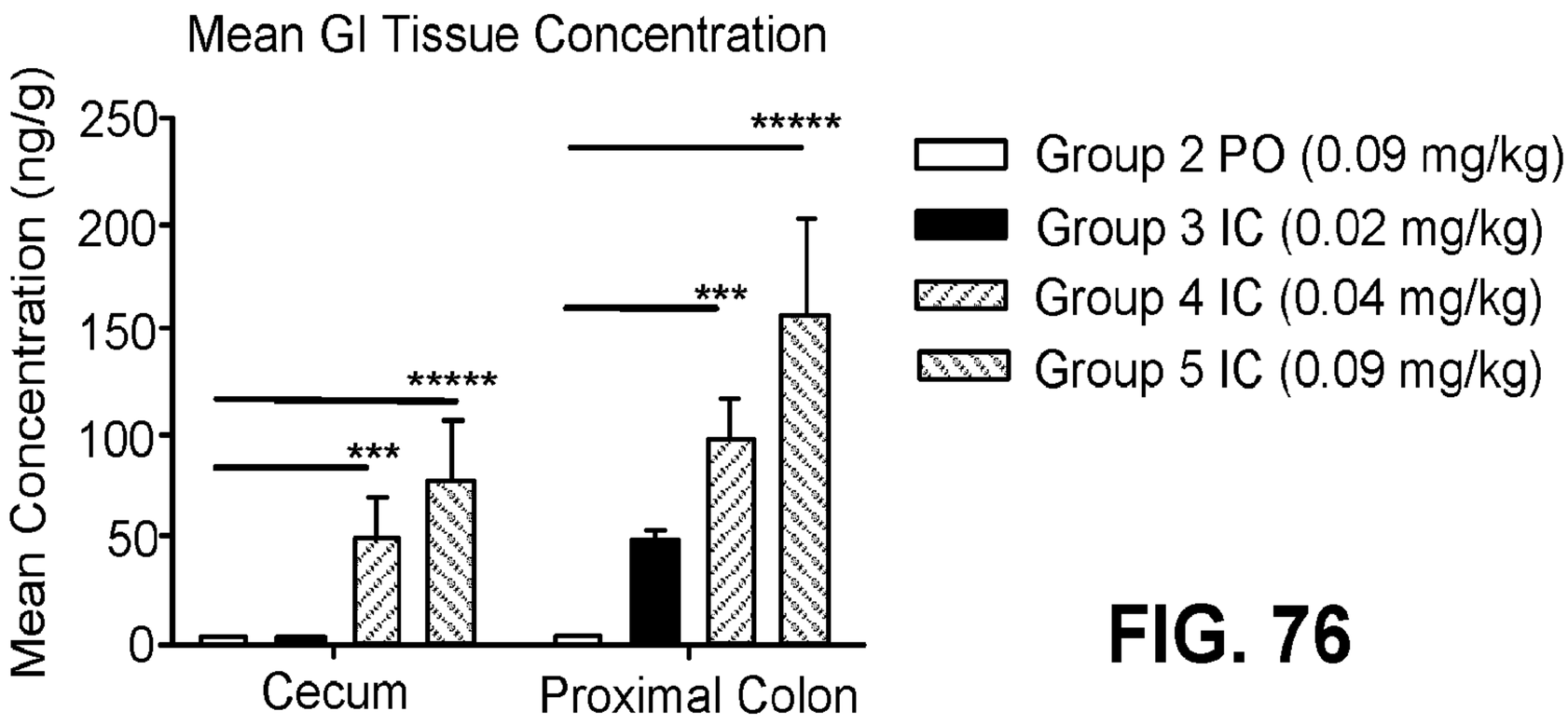

Mean GI Tissue Concentration
Mean Concentration (ng/g)
250
200
150
100
50
0
*****
***
*****
***
Cecum
Proximal Colon
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)

FIG. 76

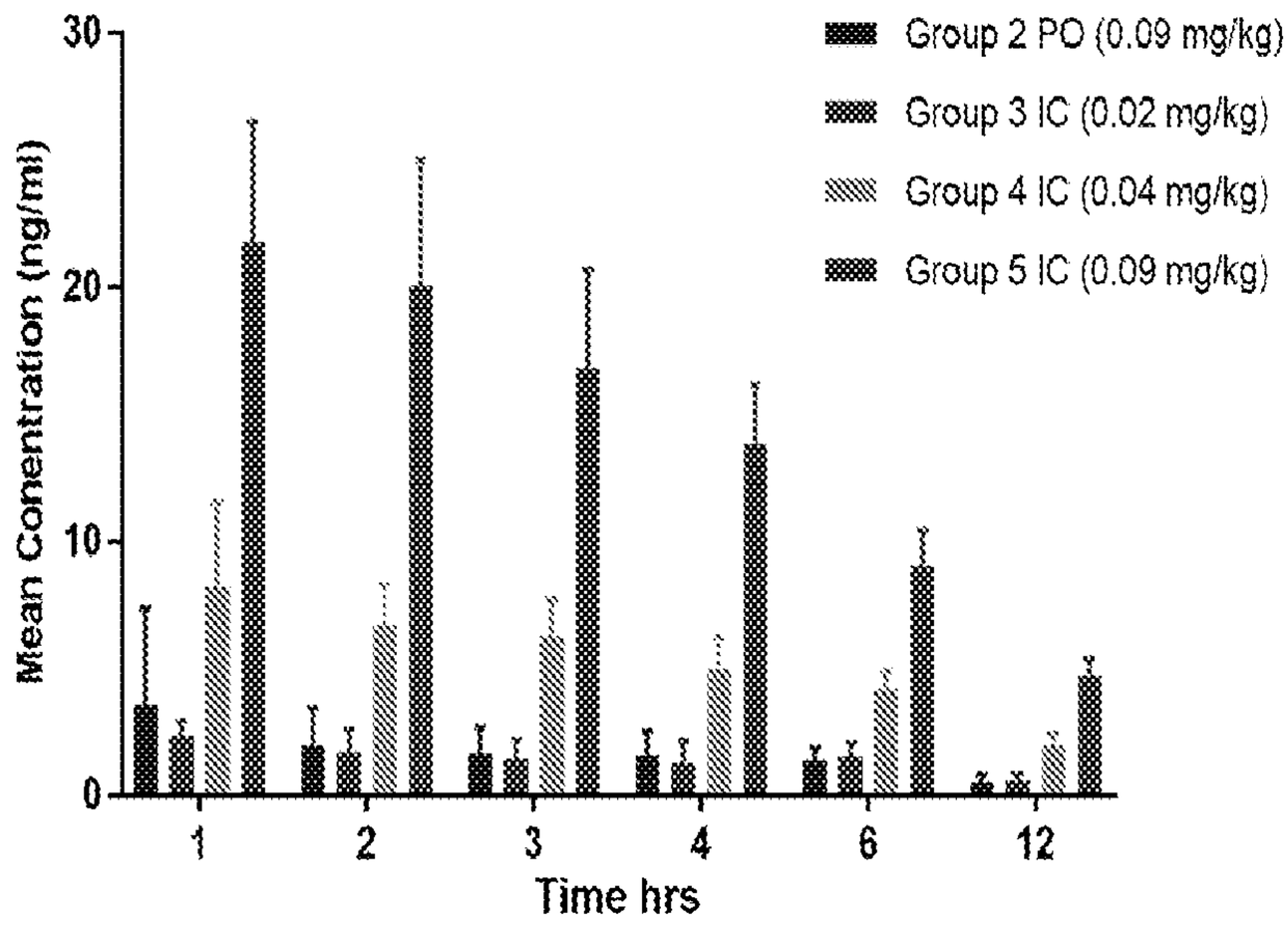

Mean Blood Concentration
of Tacrolimus
Mean Conentration (ng/ml)
30
20
10
0
1
2
3
4
6
12
Time hrs
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)

FIG. 77

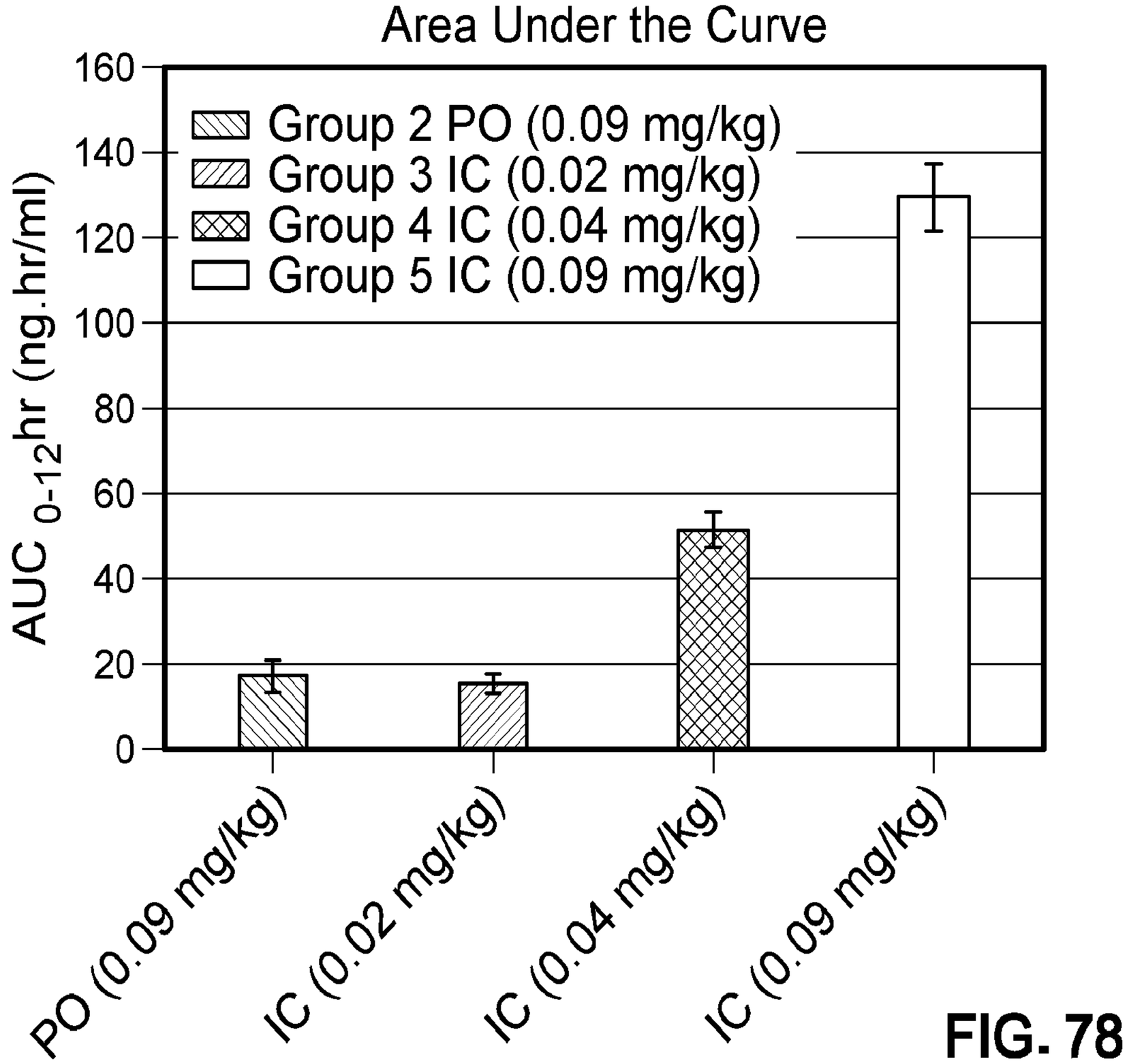

Area Under the Curve
AUC 0-12hr (ng.hr/ml)
160
140
120
100
80
60
40
20
0
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
PO (0.09 mg/kg)
IC (0.02 mg/kg)
IC (0.04 mg/kg)
IC (0.09 mg/kg)

FIG. 78

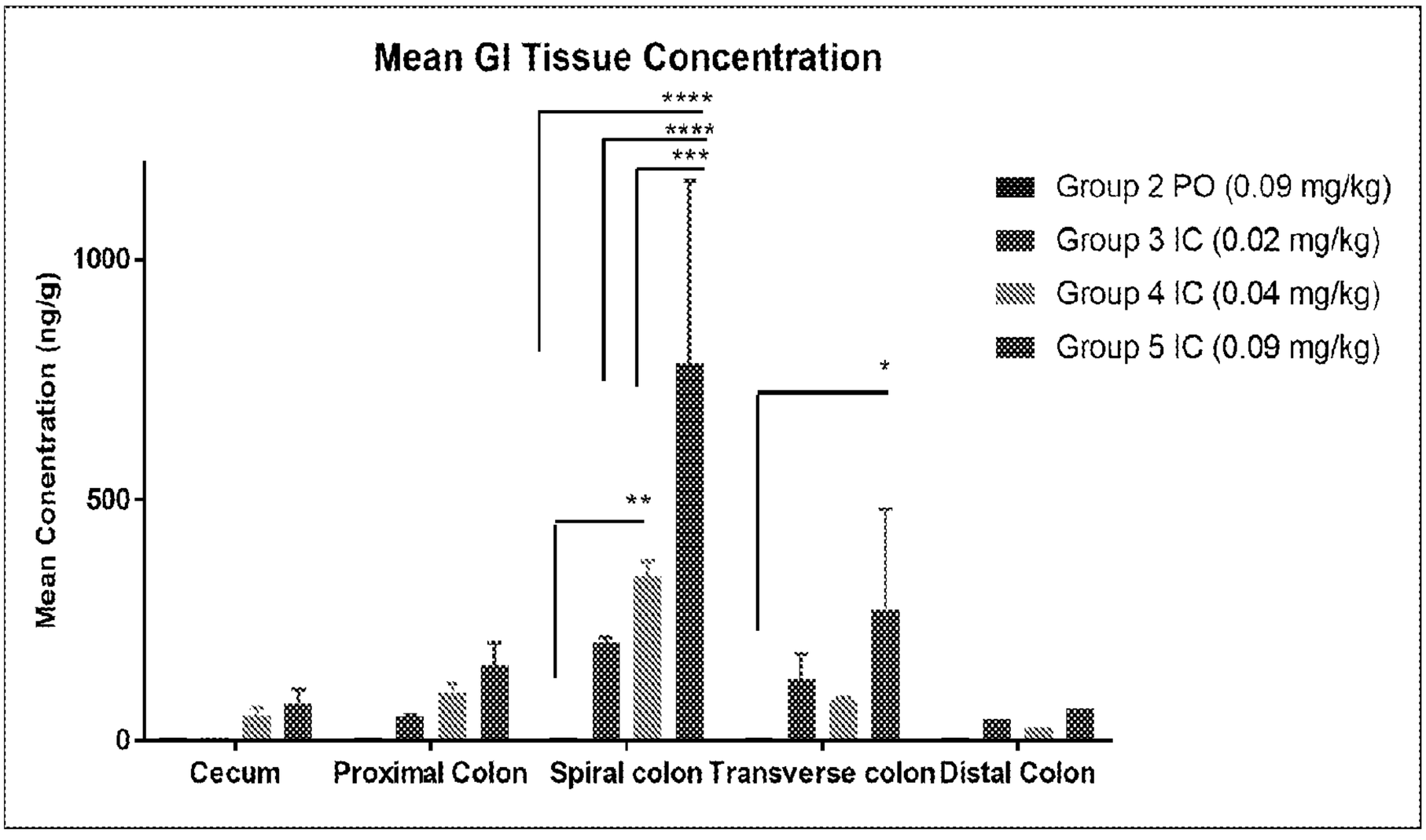

Mean GI Tissue Concentration
Mean Conentration (ng/g)
1000
500
0
****
****
***
**
*
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Cecum
Proximal Colon
Spiral colon
Transverse colon
Distal Colon

FIG. 79

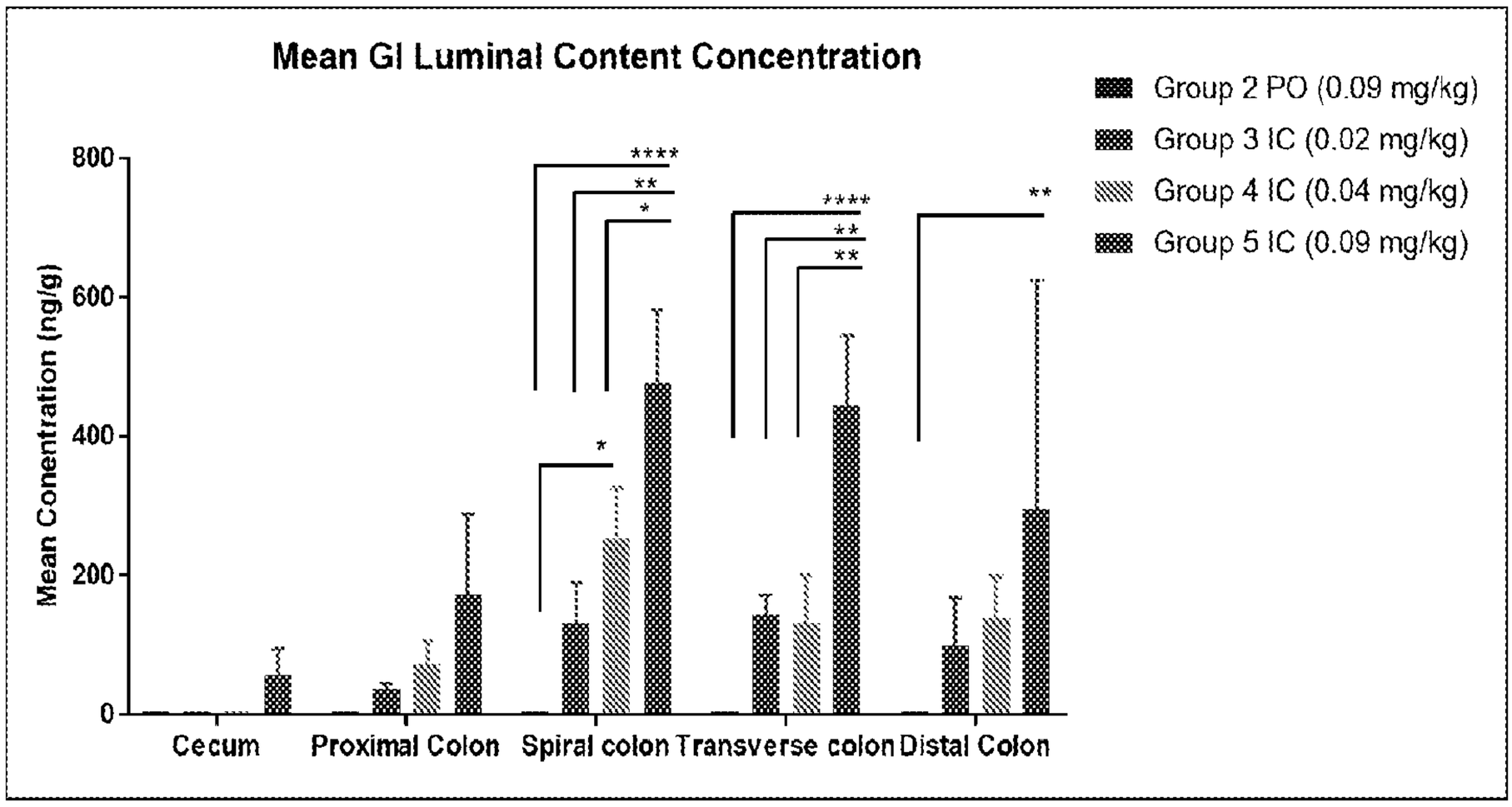

Mean GI Luminal Content Concentration
Mean Conentration (ng/g)
800
600
400
200
0
****
**
*
****
**
**
*
**
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Cecum
Proximal Colon
Spiral colon
Transverse colon
Distal Colon

FIG. 80

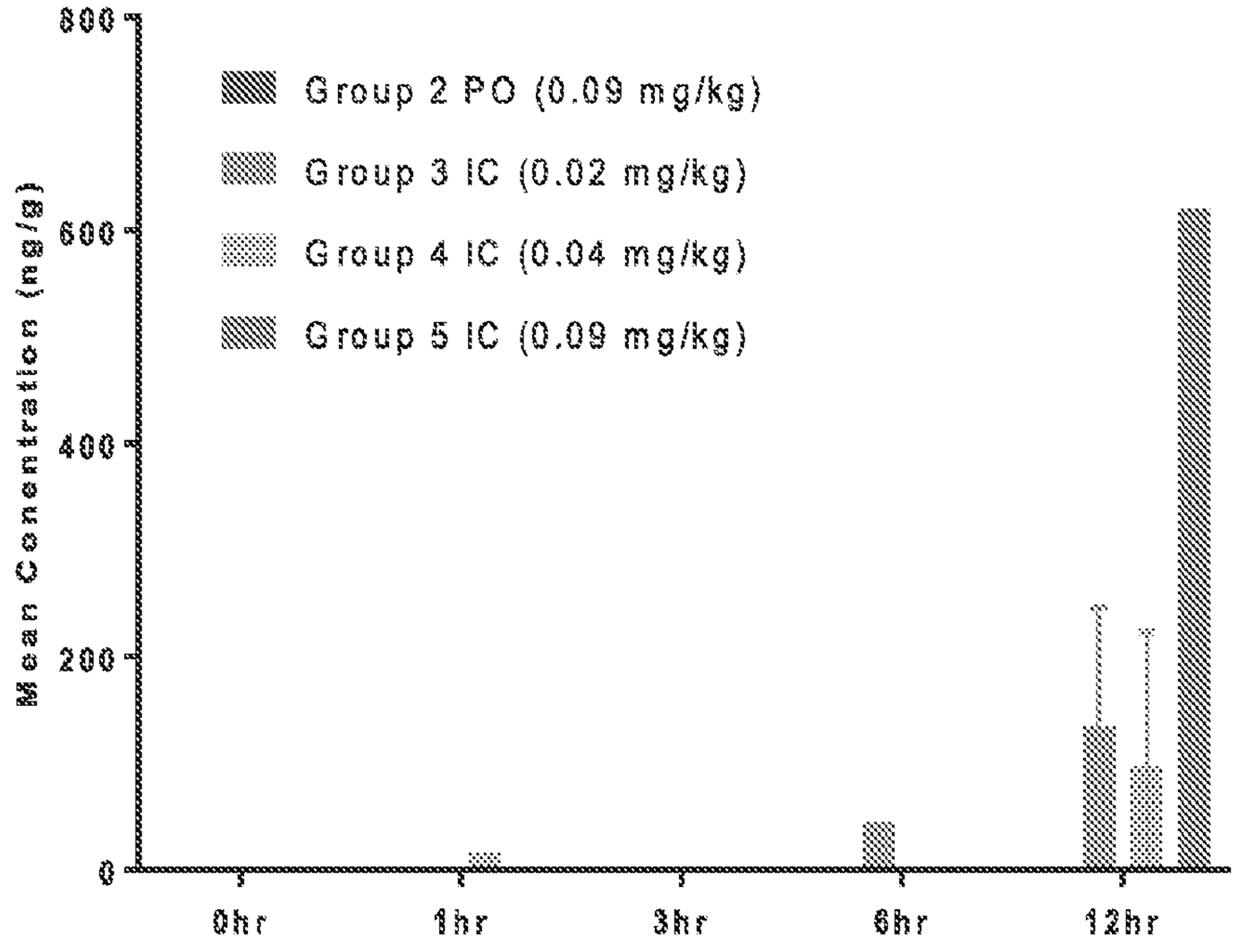

PK of Tacrolimus in Rectal Content
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Mean Conentration (ng/g)
800
600
400
200
0
0hr
1hr
3hr
6hr
12hr

FIG. 81

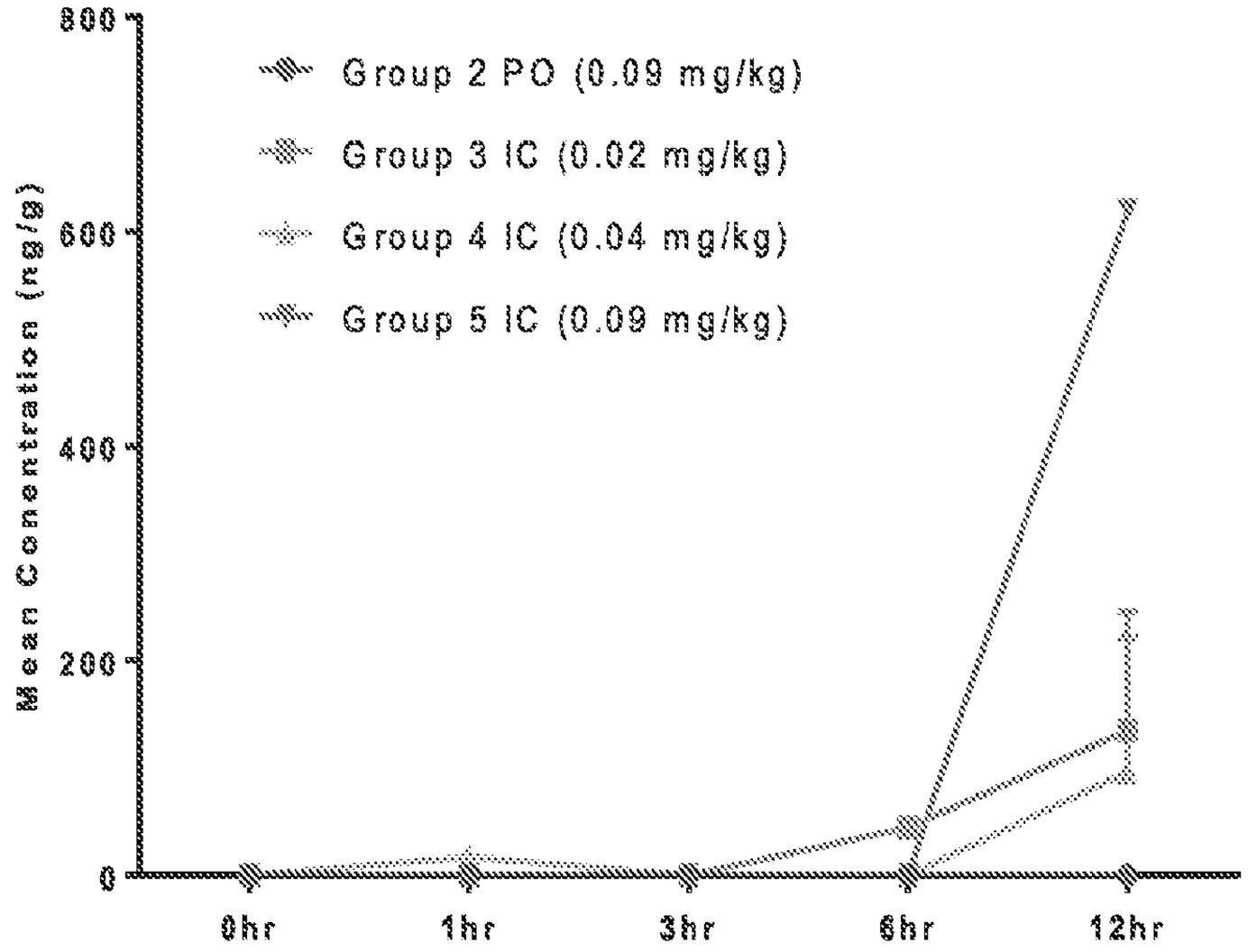

PK of Tacrolimus in Rectal Content
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Mean Conentration (ng/g)
800
600
400
200
0
0hr
1hr
3hr
6hr
12hr

FIG. 82

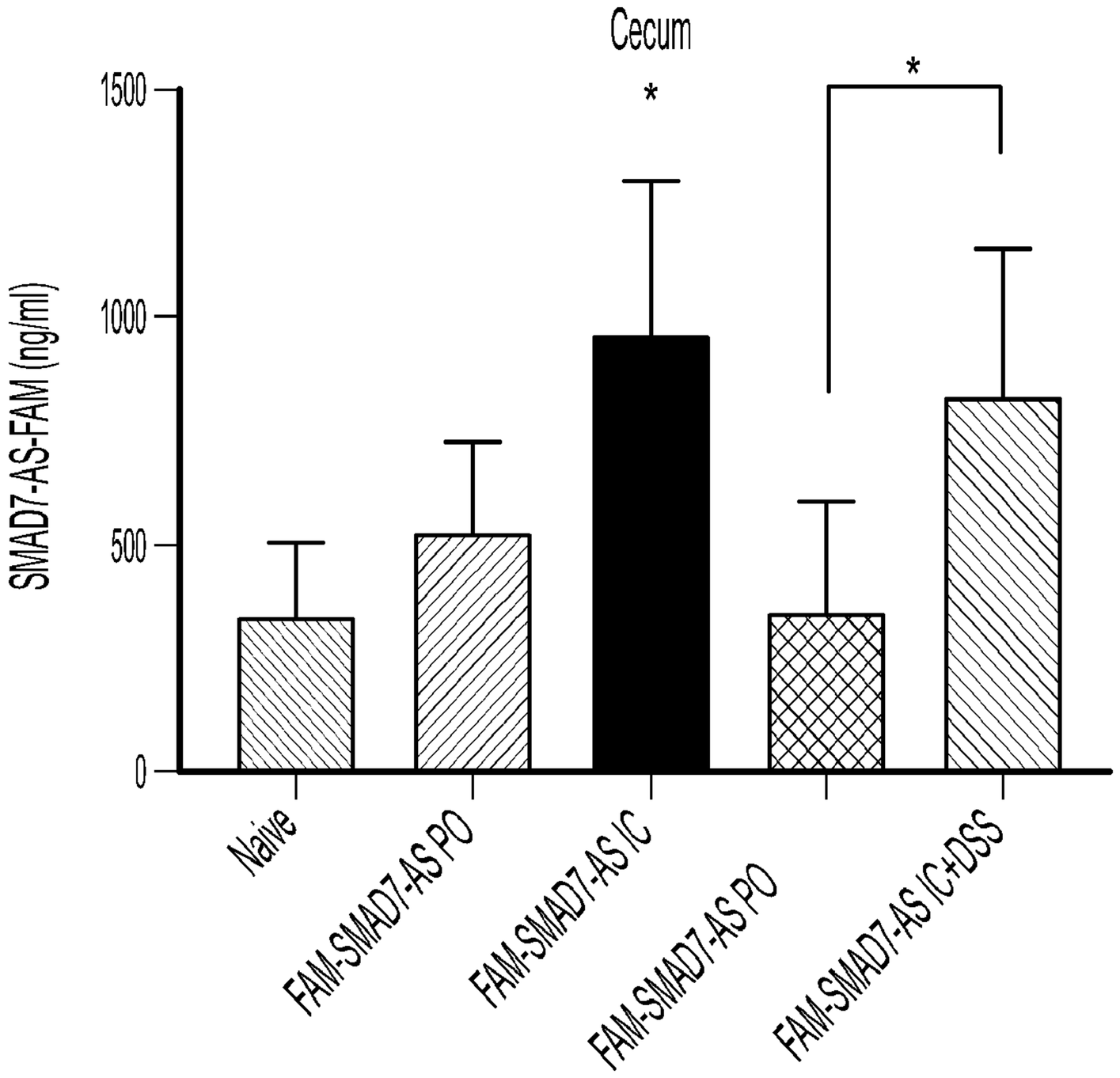

Cecum
SMAD7-AS-FAM (ng/ml)
1500
1000
500
0
*
*
Naive
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC+DSS

FIG. 83

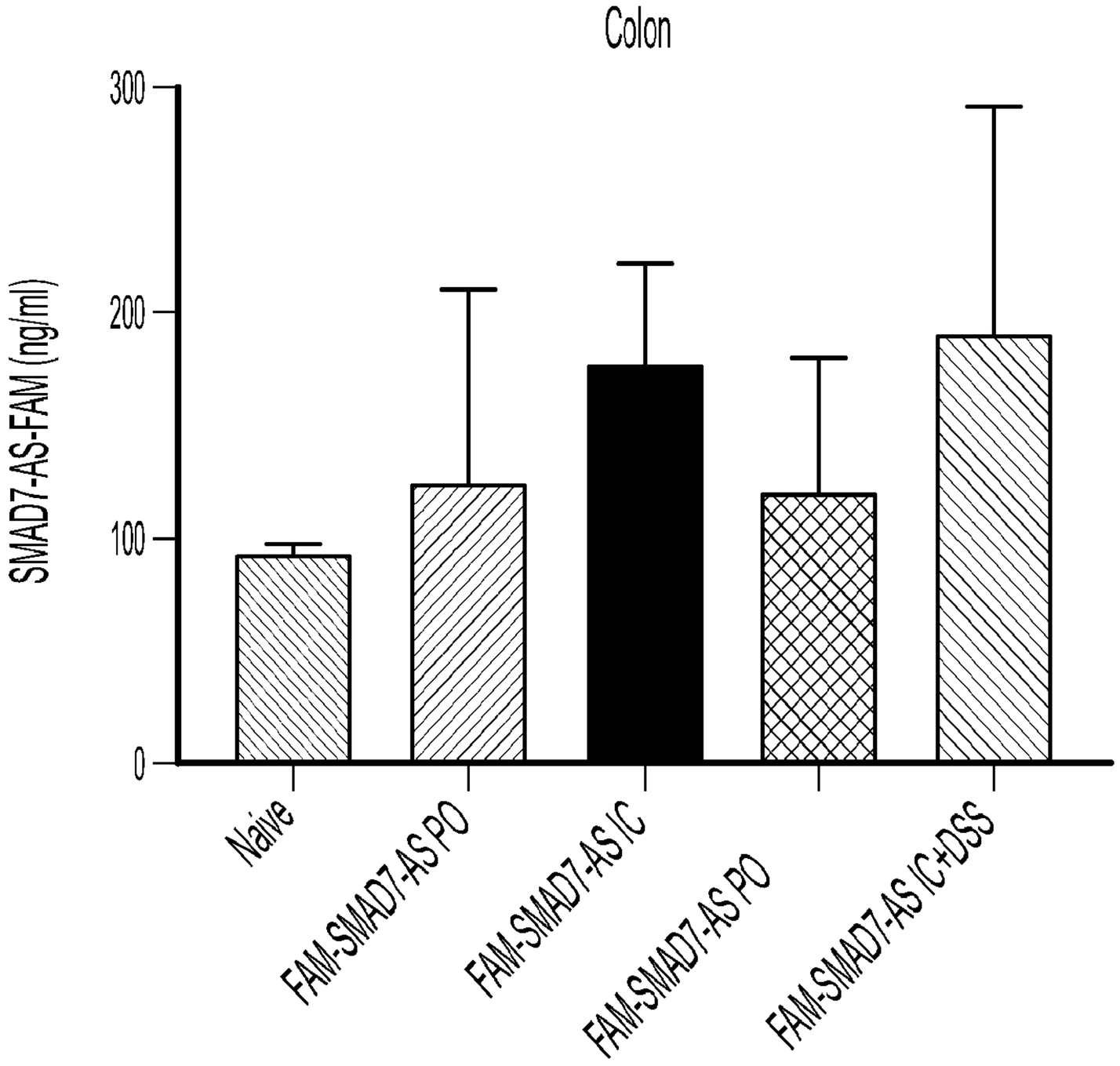

Colon
SMAD7-AS-FAM (ng/ml)
300
200
100
0
Naive
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC+DSS

FIG. 84

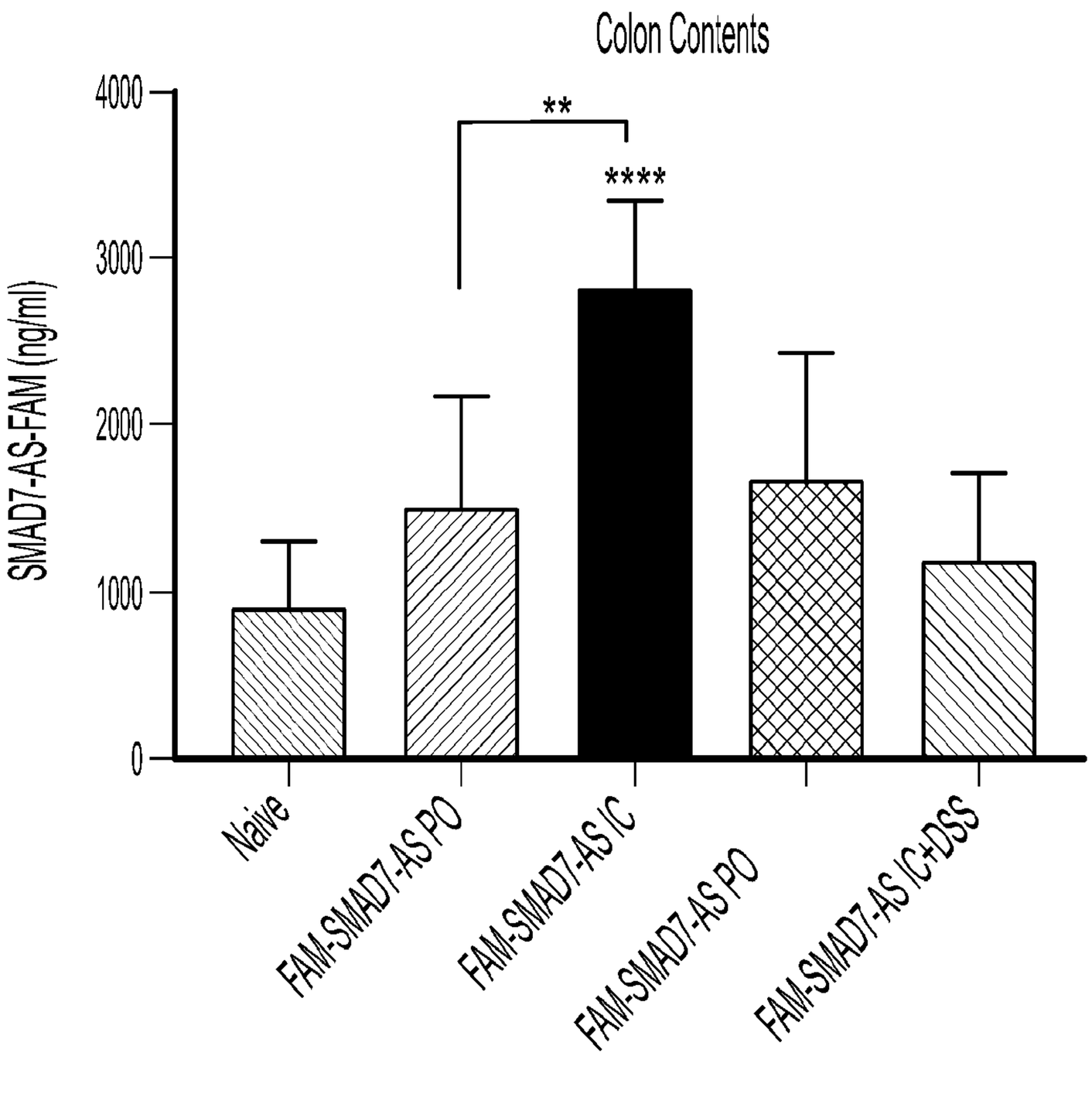

Colon Contents
SMAD7-AS-FAM (ng/ml)
4000
3000
2000
1000
0
**
****
Naive
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC+DSS

FIG. 85

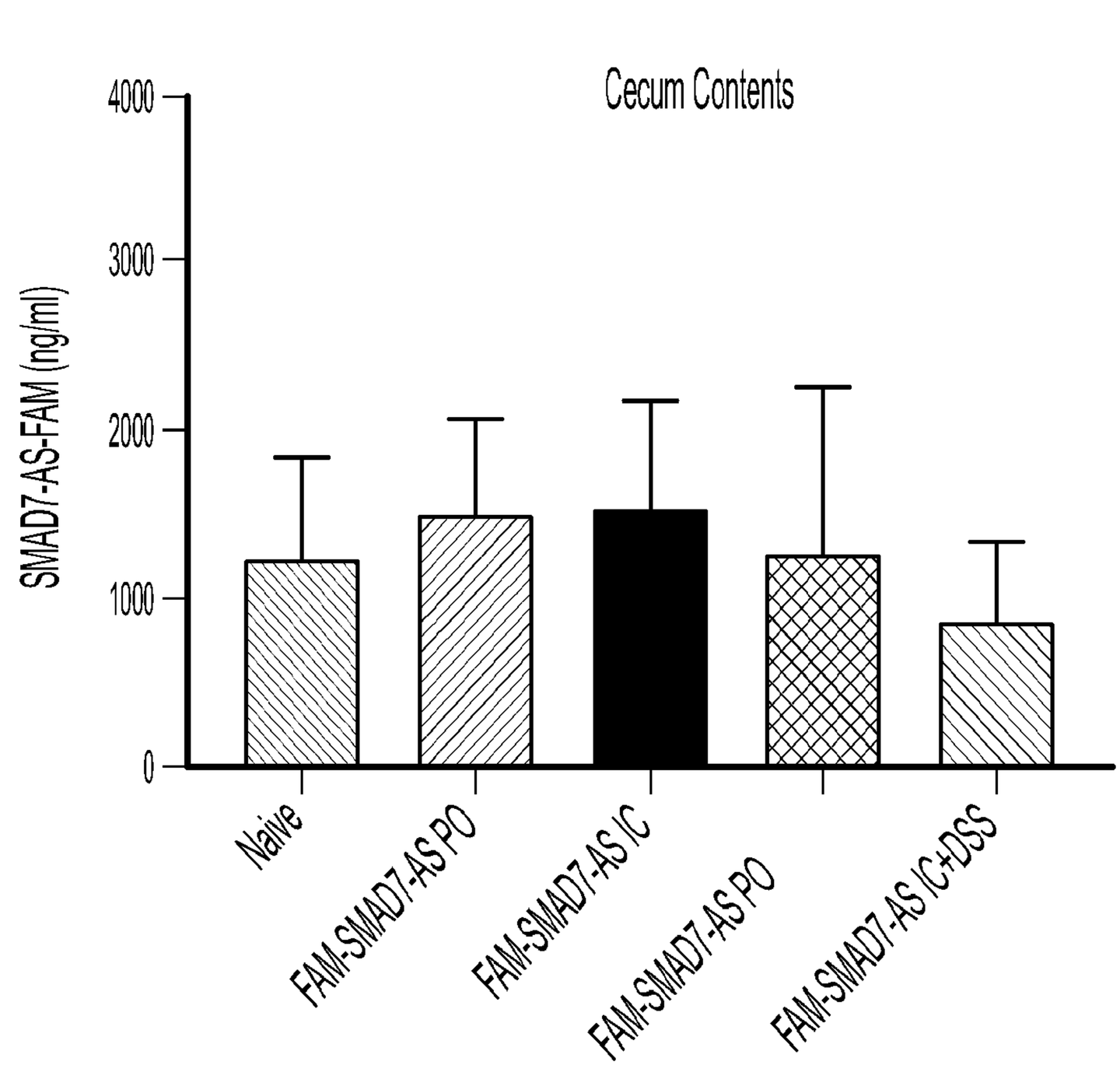

Cecum Contents
SMAD7-AS-FAM (ng/ml)
4000
3000
2000
1000
0
Naive
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC
FAM-SMAD7-AS PO
FAM-SMAD7-AS IC+DSS

FIG. 86

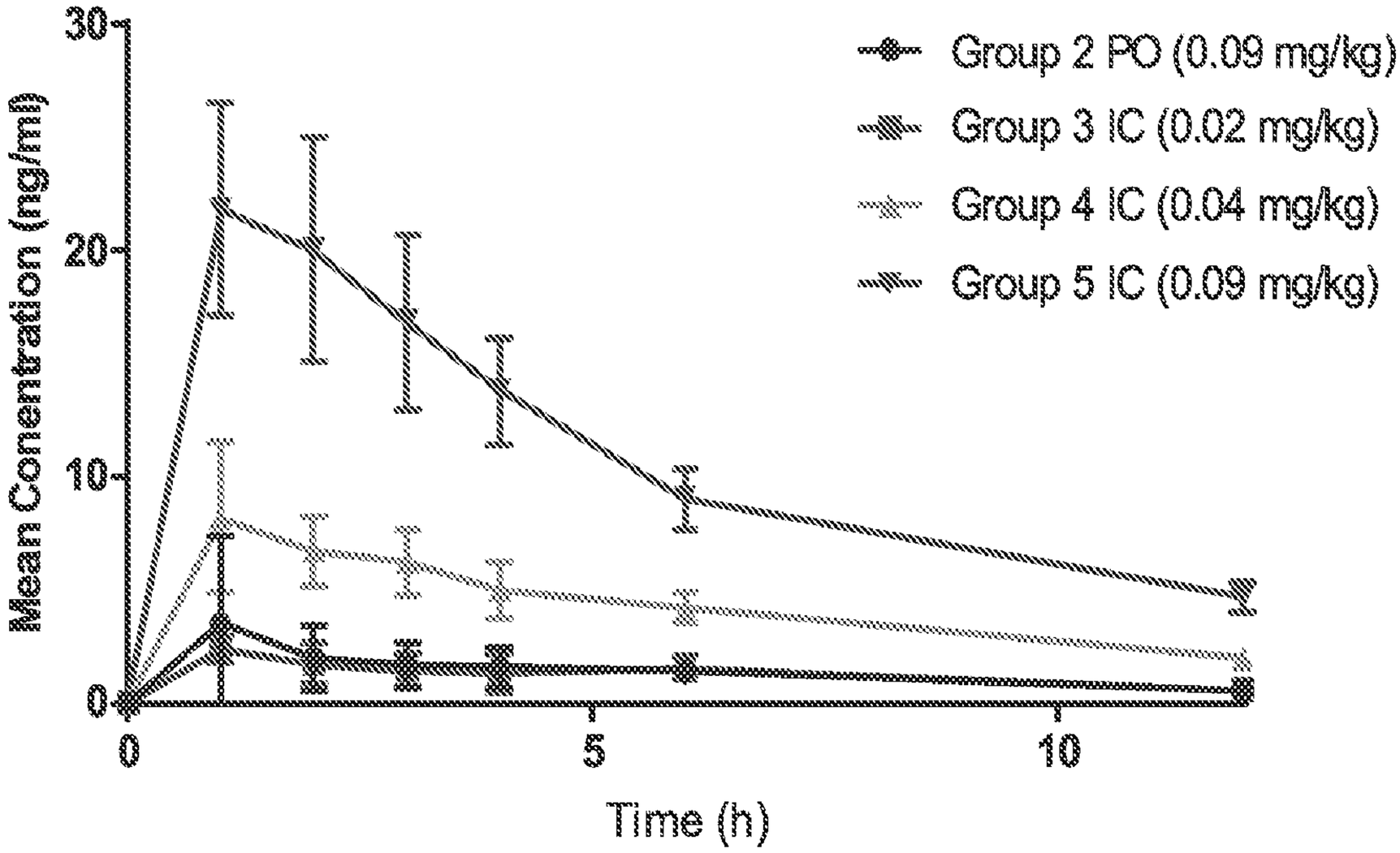

Mean Blood Concentration of Tacrolimus
Mean Conentration (ng/ml)
30
20
10
0
0
5
10
Time (h)
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)

FIG. 87

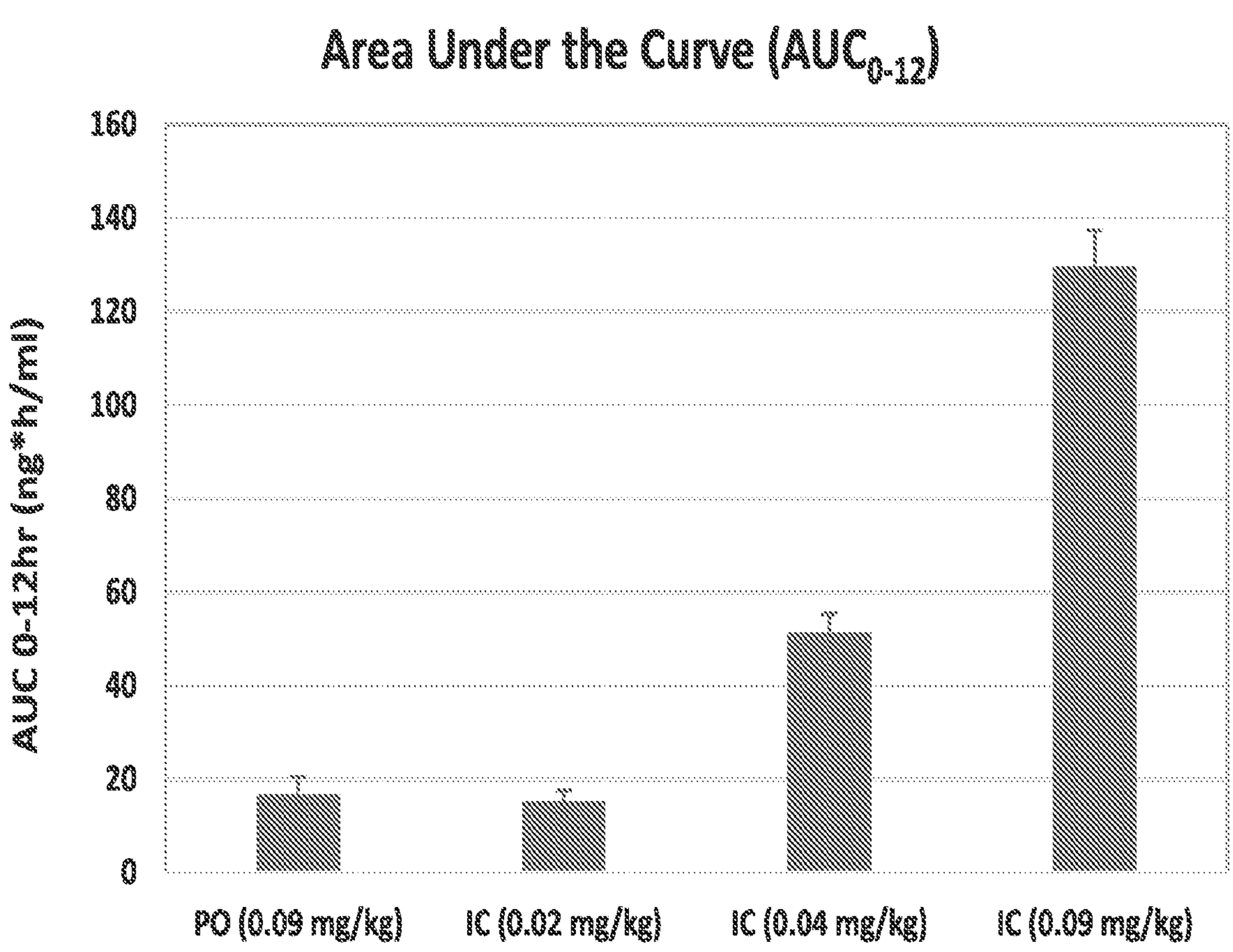

Area Under the Curve (AUC_{0-12})
AUC 0-12hr (ng*h/ml)
160
140
120
100
80
60
40
20
0
PO (0.09 mg/kg)
IC (0.02 mg/kg)
IC (0.04 mg/kg)
IC (0.09 mg/kg)

FIG. 88

Route	PO	IC	IC	IC
Dose (mg.kg)	0.09	0.02	0.04	0.09
Tmax	1	1	1	1
Cmax	3.531 ± 3.84	2.39 ± 0.565	9.197 ± 3.30	21.8 ± 4.73
Trough (12hr)	0.568 ± 0.291	0.746 ± 0.038	1.96 ± 0.491	4.35 ± 0.516
AUC 0-12hr (ng*h/ml)	16.83 ± 3.641	15.29 ± 2.356	51.35 ± 4.04	129.6 ± 7.827

FIG. 89

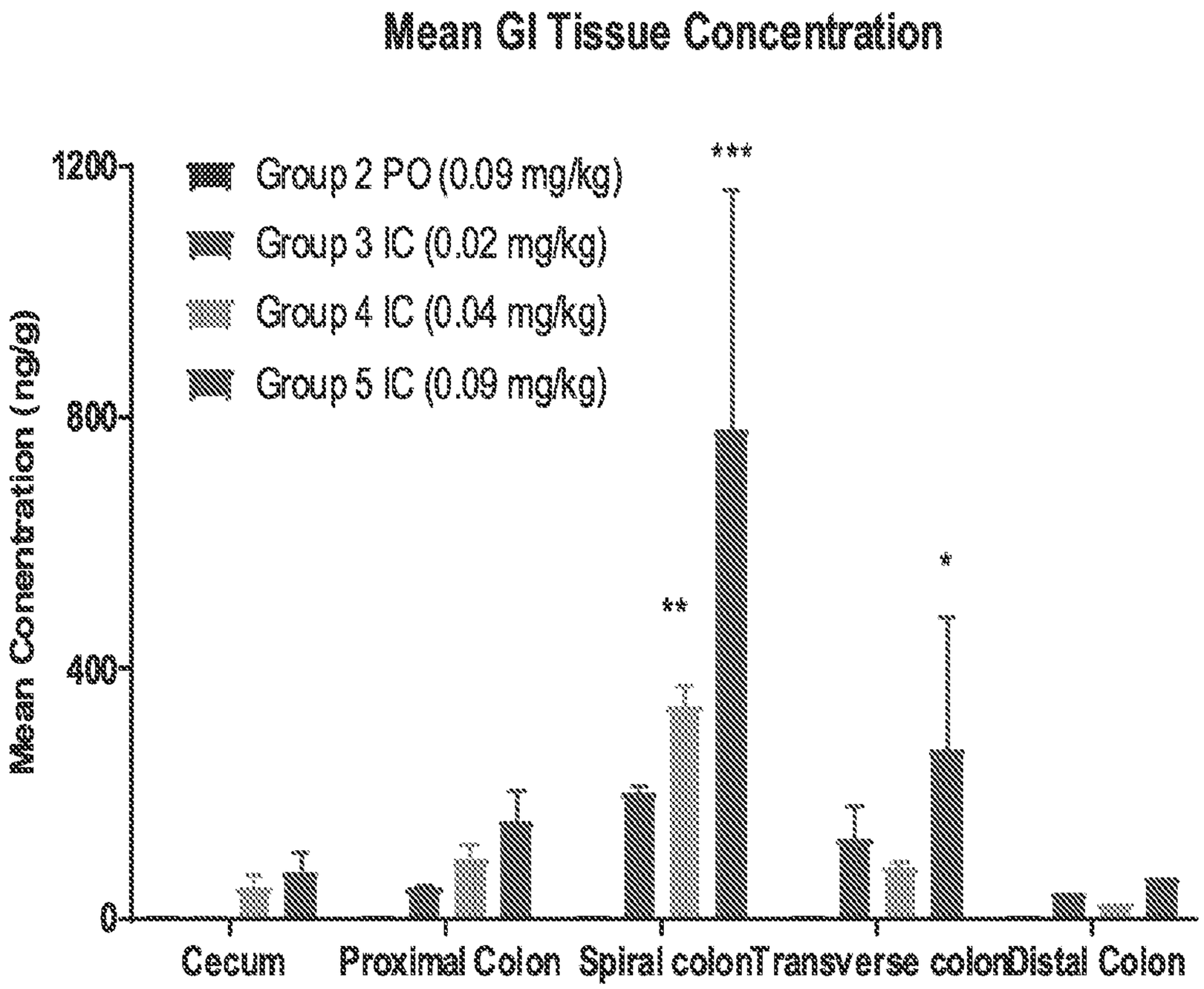


FIG. 90

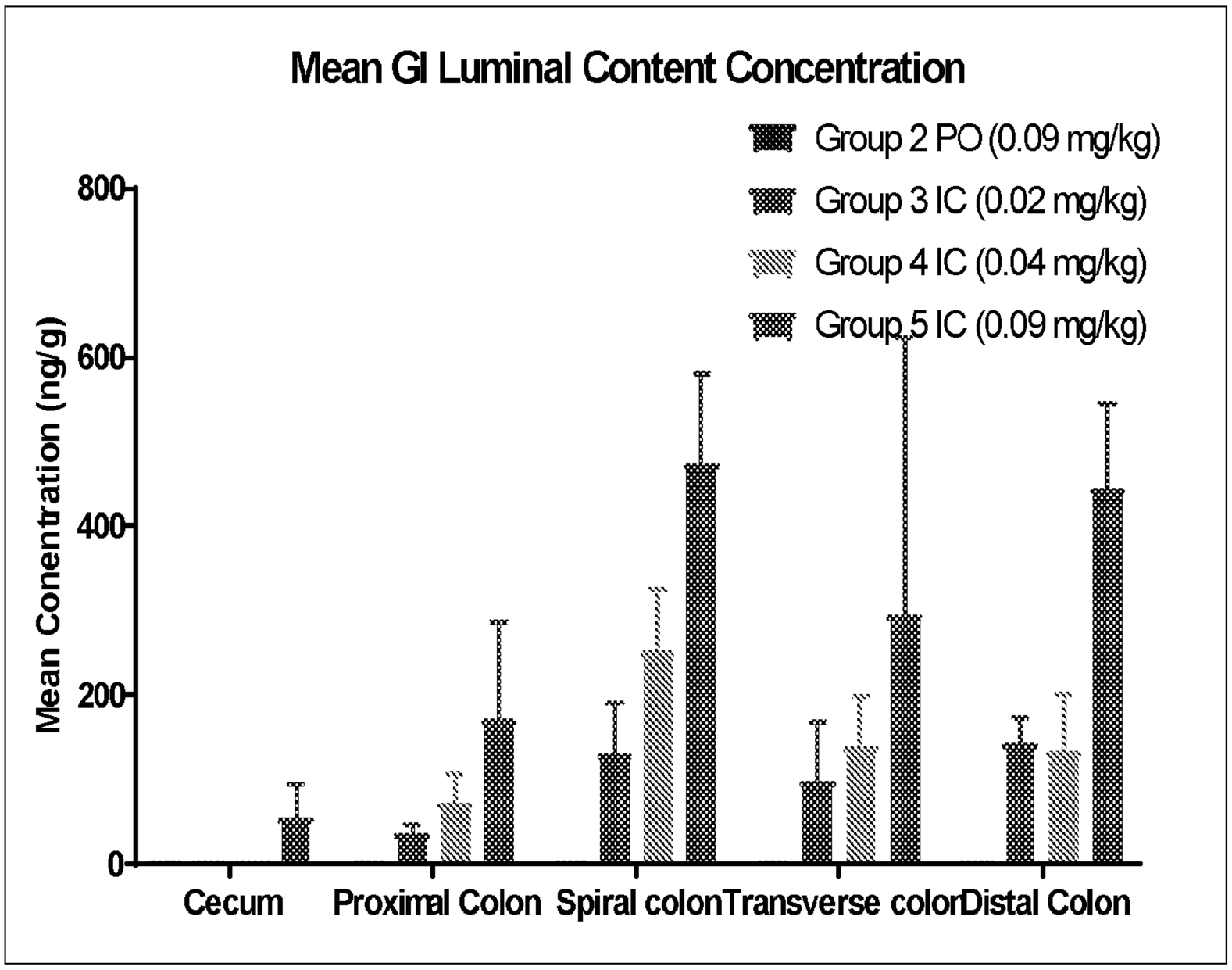

Mean GI Luminal Content Concentration
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Mean Conentration (ng/g)
800
600
400
200
0
Cecum
Proximal Colon
Spiral colon
Transverse colon
Distal Colon

FIG. 91

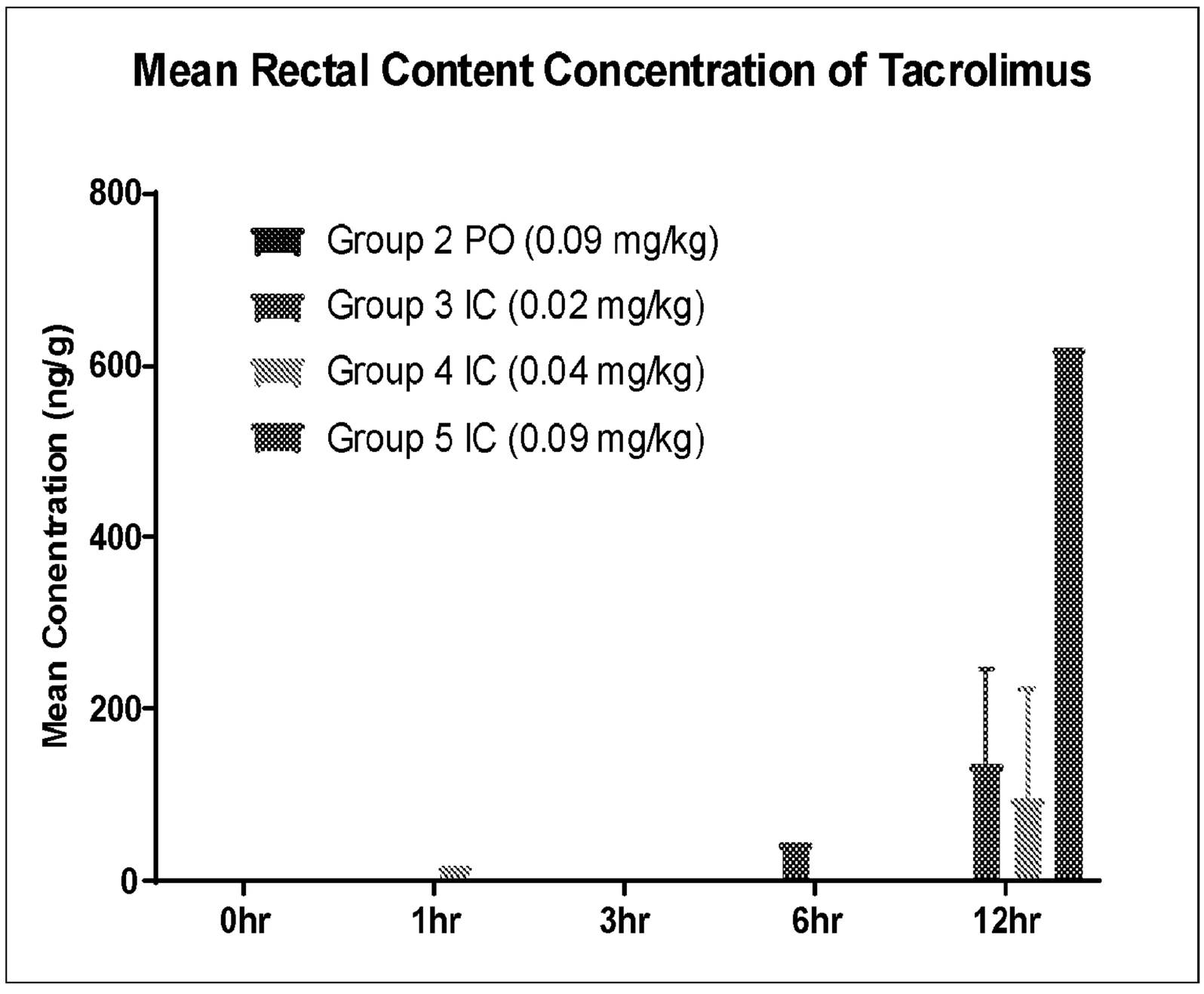

Mean Rectal Content Concentration of Tacrolimus
Group 2 PO (0.09 mg/kg)
Group 3 IC (0.02 mg/kg)
Group 4 IC (0.04 mg/kg)
Group 5 IC (0.09 mg/kg)
Mean Conentration (ng/g)
800
600
400
200
0
0hr
1hr
3hr
6hr
12hr

FIG. 92

Quantitative histological grading of colitis.

Feature graded	Grade	Description
Inflammation	0	None
	1	Slight
	2	Moderate
	3	Severe
Extent	0	None
	1	Mucosa
	2	Mucosa and submucosa
	3	Transmural
Regeneration	0	Complete regeneration or normal tissue
	1	Almost complete regeneration
	2	Regeneration with crypt depletion
	3	Surface epithelium not intact
	4	No tissue repair
Crypt damage	0	None
	1	Basal 1/3 damaged
	2	Basal 2/3 damaged
	3	Only surface epithelium intact
	4	Entire crypt and epithelium lost
Percent involvement	1	1-25%
	2	26-50%
	3	51-75%
	4	76-100%

FIG. 93

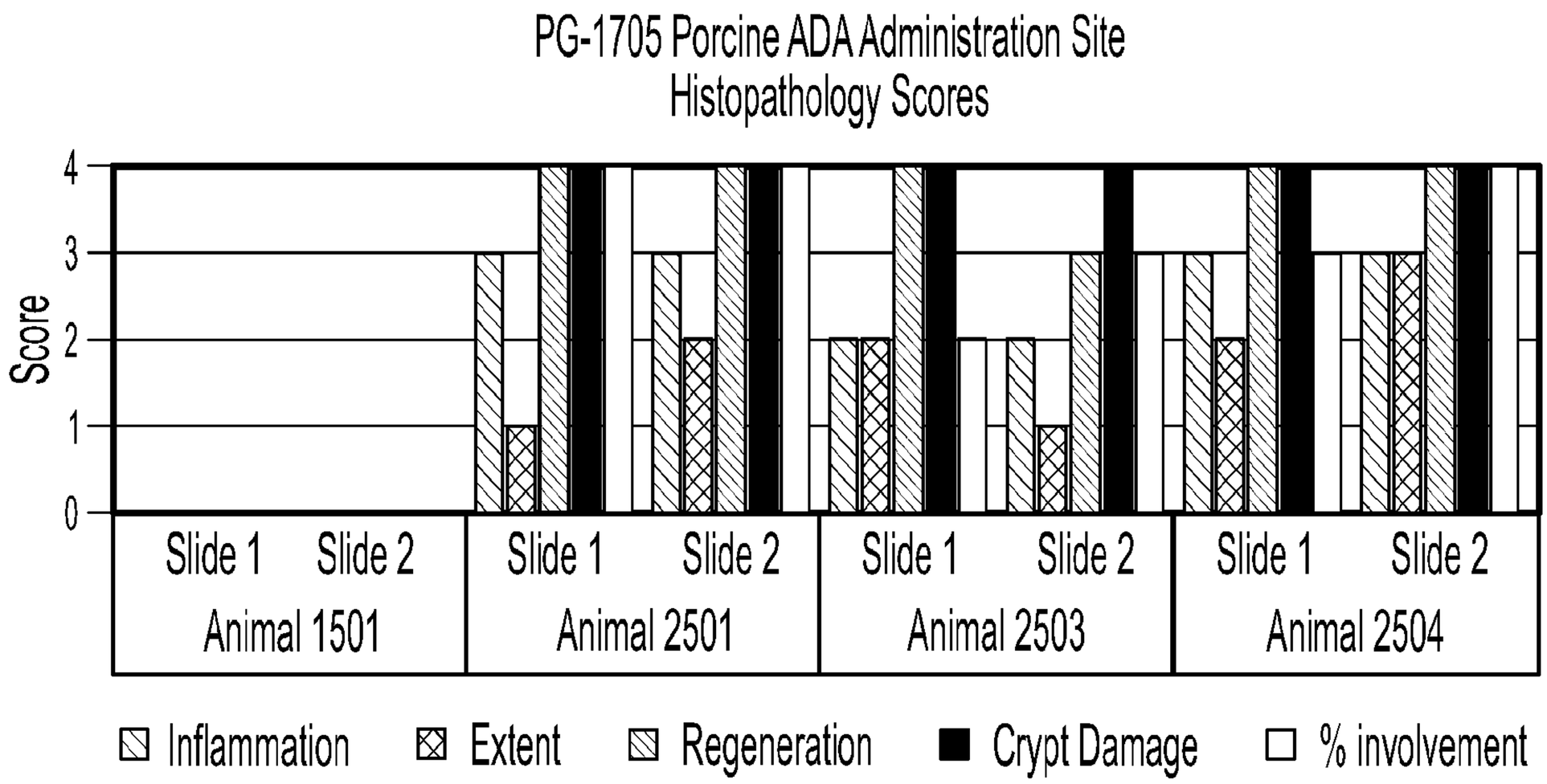

PG-1705 Porcine ADA Administration Site
Histopathology Scores
Score
4
3
2
1
0
Slide 1
Slide 2
Animal 1501
Animal 2501
Animal 2503
Animal 2504
Inflammation
Extent
Regeneration
Crypt Damage
% involvement

FIG. 94

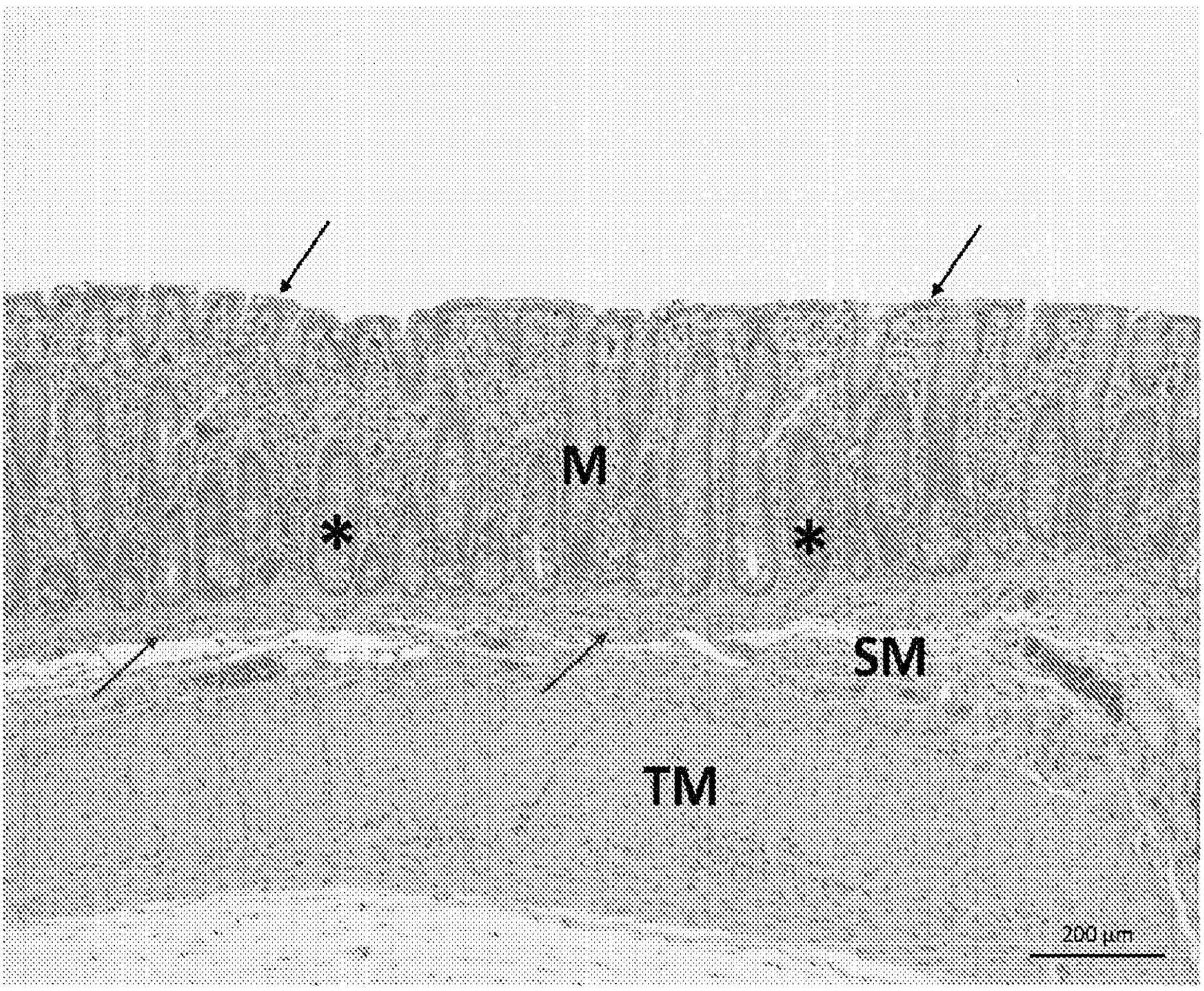

M
SM
TM
200 µm

FIG. 95

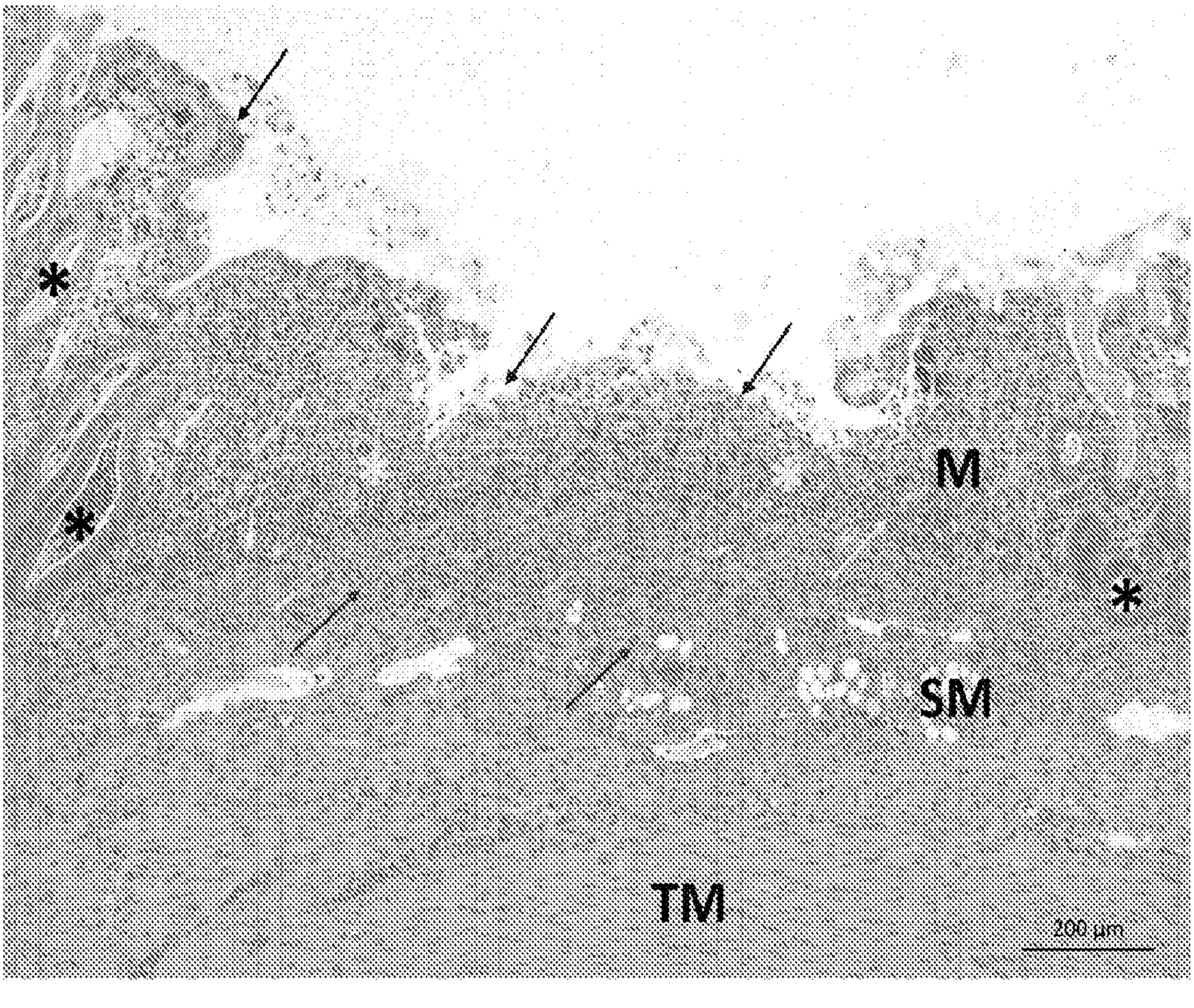


FIG. 96

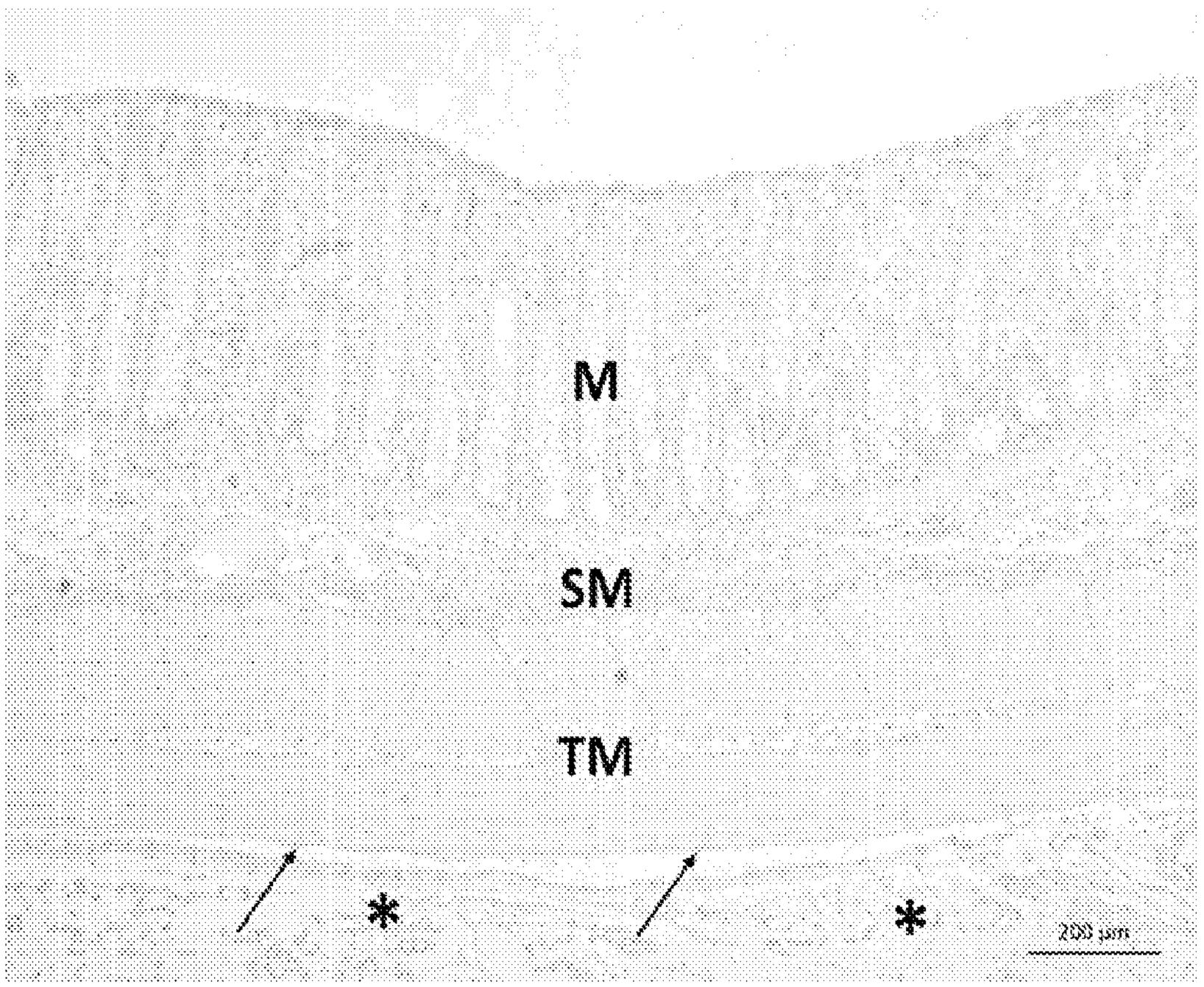


FIG. 97

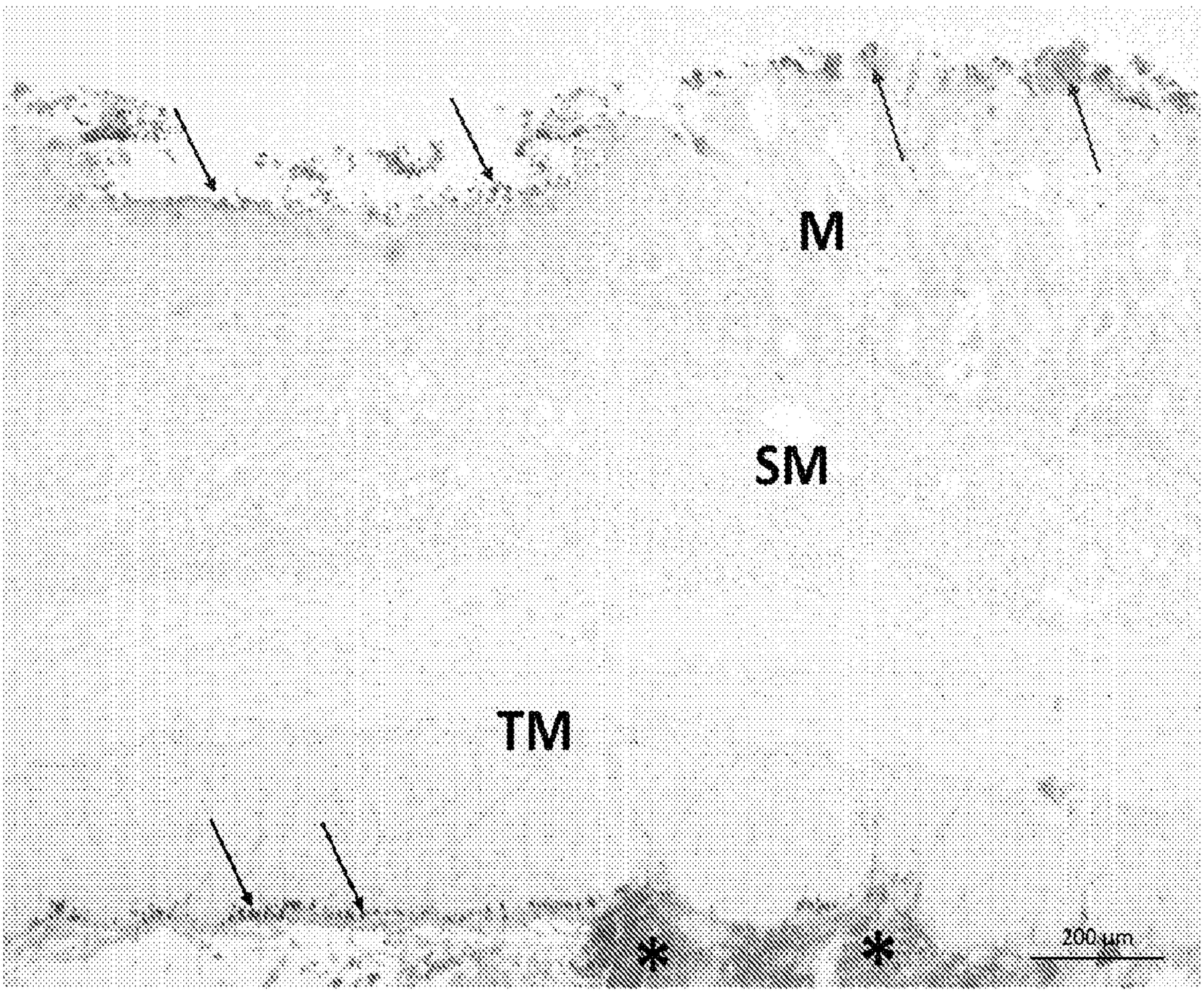


FIG. 98

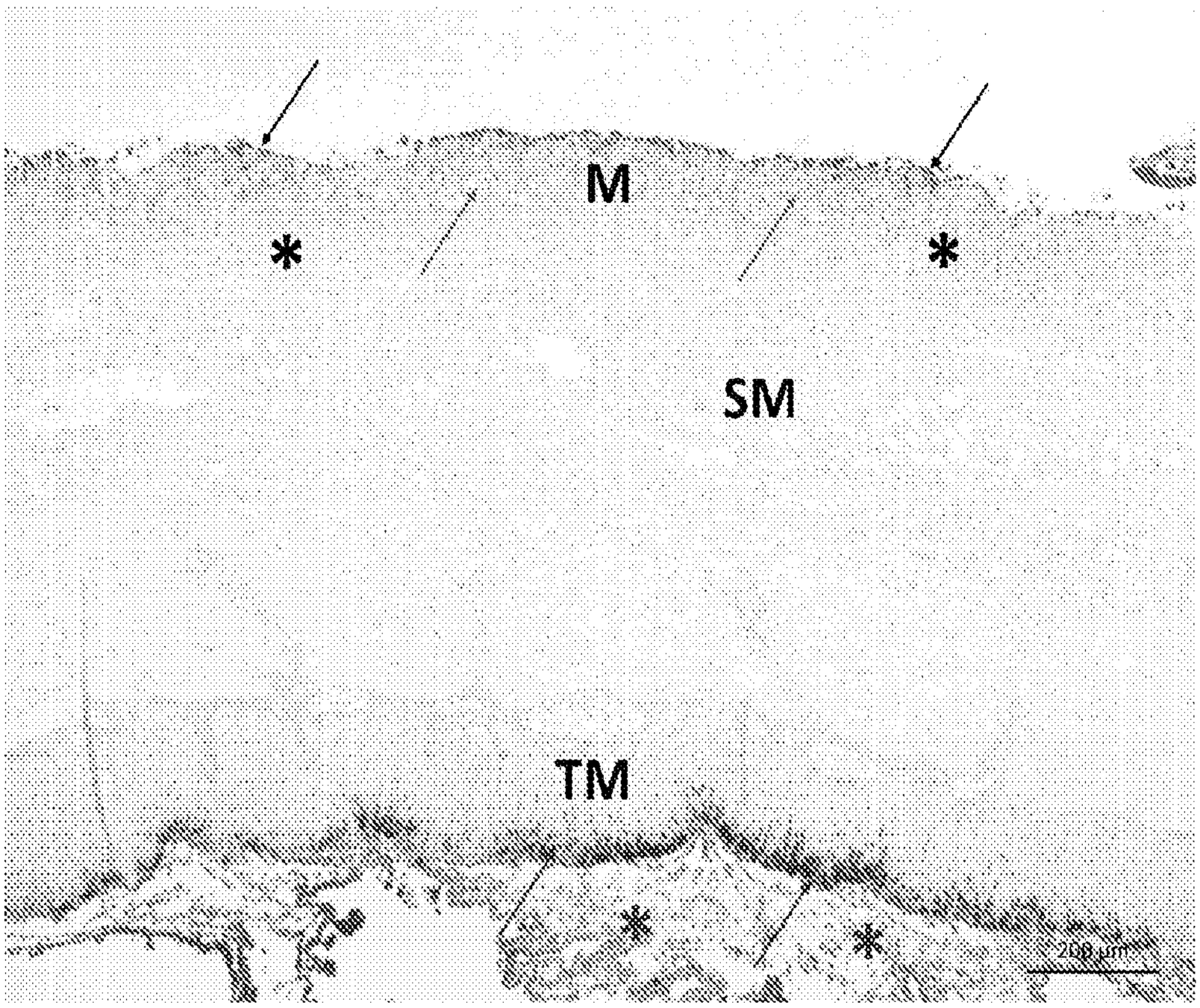


FIG. 99

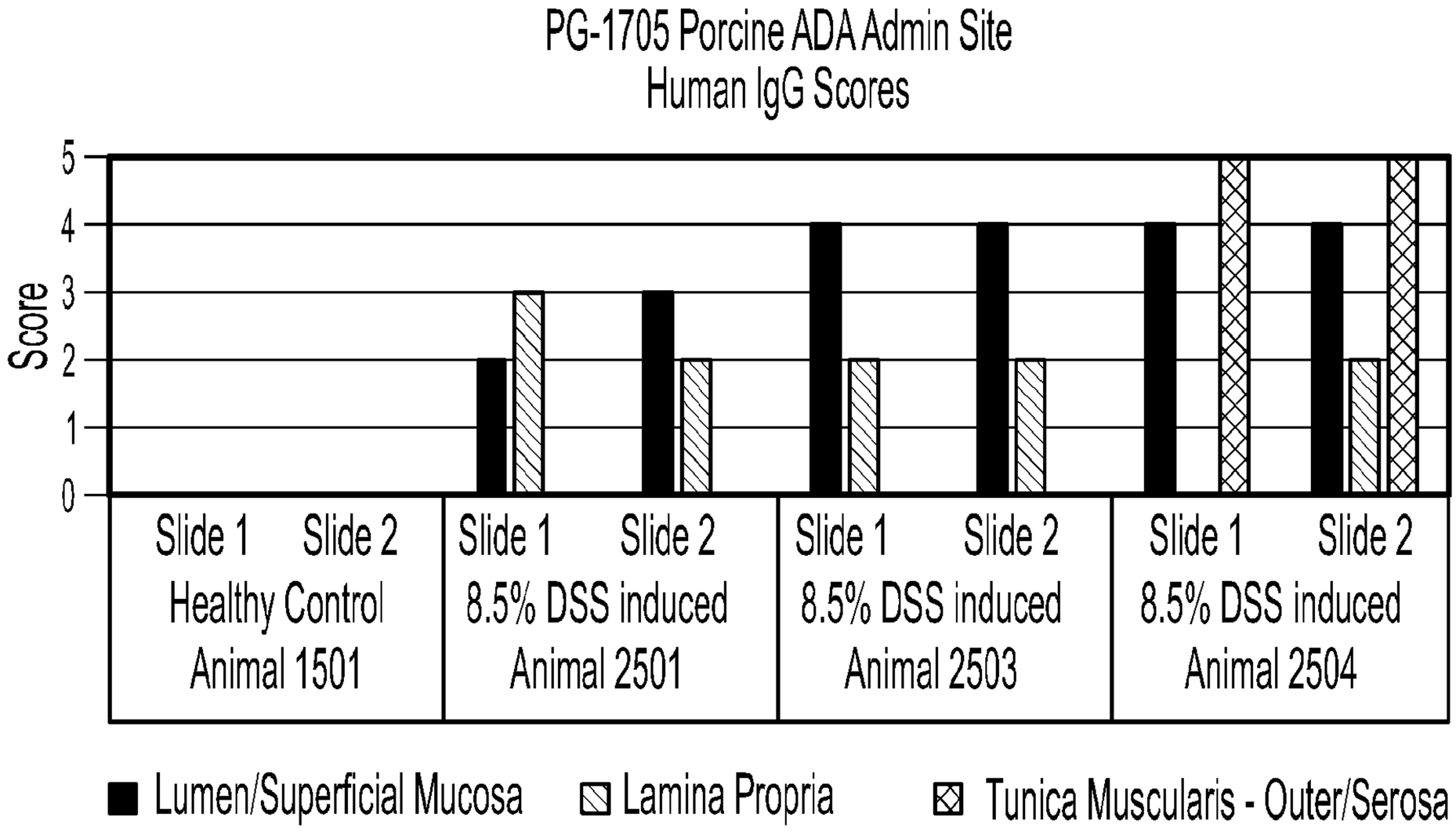


FIG. 100