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(54) Title: DIAGNOSTIC SYSTEM

(57) Abstract: Methods and systems are provided for point-of-care nucleic acid amplification and detection. One embodiment of the point-of-care molecular diagnostic system includes a cartridge and an instrument. The cartridge can accept a biological sample, such as a urine or blood sample. The cartridge, which can comprise one or more of a loading module, lysis module, purification module and amplification module, is inserted into the instrument which acts upon the cartridge to facilitate various sample processing steps that occur in order to perform a molecular diagnostic test.

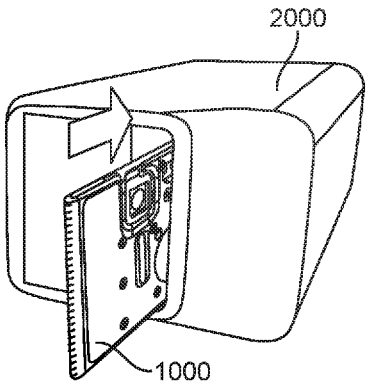

FIG. 3

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DIAGNOSTIC SYSTEM

I. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/887,469, filed August 15, 2019, titled "DIAGNOSTIC SYSTEM," which is herein incorporated by reference in its entirety.

[0002] This application also claims priority as a continuation of U.S. Patent Application No. 16/655,007, filed October 16, 2019, titled "DIAGNOSTIC SYSTEM," which is herein incorporated by reference in its entirety.

[0003] This application also claims priority as a continuation of U.S. Patent Application No. 16/655,028, filed October 16, 2019, titled "DIAGNOSTIC SYSTEM," which is herein incorporated by reference in its entirety.

II. STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0004] This invention was made with government support under contract number HR0011-11-2-0006 awarded by the Department of Defense (DARPA). The government has certain rights in the invention.

[0005] This invention was made with government support under contract number IDSEP160030-02 awarded by the Department of Health and Human Services (ASPR). The government has certain rights in the invention.

III. FIELD OF THE INVENTION

[0006] A molecular diagnostic instrument for performing tests on a sample contained in an integrated diagnostic cartridge.

IV. BACKGROUND OF THE INVENTION

[0007] In the U.S. alone, over one billion infections occur each year. To combat this, advancements in molecular diagnostic testing have enabled medical professionals to diagnose infectious diseases accurately. Nearly all molecular diagnostics testing currently is performed in centralized laboratories. While such tests performed in central laboratories are very accurate, results can be delayed several days or longer and they require expensive, high throughput instrumentation, regulated infrastructure, and trained personnel. For example, high throughput instrumentation generally processes many (e.g. 96 or 384 or more) samples at a time. Samples are collected during a time period, e.g., a day, and are then processed in one large batch. Additionally, requiring trained technician, responsible for operating laboratory equipment, adding reagents, and overseeing sample processing, e.g., moving samples from step to step, are too expensive or unavailable to practices in sparsely populated or economically challenged locations.

[0008] As an alternative to centralized laboratory testing, some testing can be performed at the point of care (POC) providing near patient rapid diagnosis outside of a laboratory environment.

However, there are limited POC testing options available and many known POC tests have poor sensitivity (30-70%), as compared to highly sensitive central-lab molecular diagnostic tests. Current POC testing options tend to be single analyte tests with low analytical quality. These tests are used alongside clinical algorithms to assist in diagnosis, but are frequently verified by higher quality, laboratory tests for the definitive diagnosis. Thus, neither consumers nor physicians achieve a rapid, accurate test result in the time frame required to "test and treat" a patient in one visit. As a result, doctors and patients often determine an empiric course of treatment before they know the diagnosis. This lack of knowledge has tremendous ramifications: either antibiotics are not prescribed when needed, leading to disease progress and/or transmission to another host; or antibiotics are prescribed when not needed, leading to new antibiotic-resistant strains in the community.

[0009] In one specific example, Gram-negative *Neisseria gonorrhoeae* has progressively developed resistance to the antibiotic drugs prescribed to treat it and is one of only three organisms on the CDC's list of urgent threats. Preventing the spread of gonorrhea relies on prompt diagnosis and treatment of infected persons and their partners. The turn-around time for centralized lab testing is 1-5 days. Therefore, physicians are faced with one of two choices: (1) wait days for test results before treating a patient and risk that a positive patient may continue to spread the infection through their partners, and their partners' partners or (2) treat empirically while the patient is in front of them. In a study of 1103 emergency room patients at Johns Hopkins, 440 patients who had a suspected CT or NG infection were treated with antibiotics though the vast majority, 323 patients, ultimately turned out to be negative. As a direct result of the overuse and misuse of antibiotics through empiric therapy, antibiotic resistance in gonorrhea is on the verge of becoming a public health crisis. To prevent the development of future antibiotic resistant strains, molecular diagnostic testing at the point of care can prevent unnecessary antibiotics from being prescribed and provide rapid diagnosis and treatment.

[0010] Highly trained personnel are required to perform molecular diagnostic tests because these sophisticated assays are powered by nucleic acid amplification methods, such as PCR, and carried out on biologic samples, which typically contain a variety of substances inhibitory to amplification. However, such trained personnel typically are not available at the locations where patients are being seen, i.e. at the point of care. Additional challenges associated with the point of care environment include fulfilling physician or clinical workflow compatibility coupled with an unknown skill level for system users. Accordingly, point of care molecular diagnostic systems must be designed for the ease of use by system users and be robust in performing sample preparation and amplification, with minimal user interaction, to generate reliable diagnostic results

[0011] Thus, despite the existence of some point of care diagnostic systems, a need exists for improved devices and methods for molecular diagnostic testing. In particular, an unmet need continues for an easy-to-use system enabling rapid molecular diagnostic capabilities in the point of care environment.

V. SUMMARY

[0012] In general, in one embodiment, a method of testing a sample suspected of containing one or more target pathogens includes: (1) accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens; (2) advancing the sample suspected of containing the one or more target pathogens to a lysis chamber having at least one lysis reagent therein; (3) mixing the sample with the at least one lysis agent to generate a lysed sample; (4) passing the lysed sample through a first porous solid support to capture a nucleic acid on the porous solid support; (5) releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid; (6) distributing the enriched nucleic acid to two or more assay chambers; (7) combining the enriched nucleic acid with one or more amplification reagents; (8) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers; and (9) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

[0013] This and other embodiments can include one or more of the following features. The sample can be a biological sample obtained from a mammal. The mammal can be a person providing a biological sample. The sample can be obtained from a food product, a natural non-growth hormone crop sample, a crop sample, a water sample, a non-biological fluid sample or a soil sample. The step of accepting a cartridge step can further include reading a bar code on the cartridge and determining to proceed with the method of testing. The method can further include obtaining and analyzing an image of a sample window of the sample port assembly and determining to proceed with the method of testing. The sample in the sample port assembly can be in fluid communication with a fill chamber, a metering chamber, and an overflow chamber. The sample window can be transparent and formed in at least a portion of a wall of a metering chamber. Obtaining an image can further include obtaining an image of the transparent viewing window. Analyzing an image can further include assessing a height of a sample liquid in the metering chamber via the transparent viewing window. The step of obtaining and analyzing an image can further include obtaining an image of the metering chamber including a buoyant ball and analyzing the image can include identifying a location of the ball within the metering chamber and determining to proceed with the method based on the location of the ball. The method can further include obtaining and analyzing an image of a patient ID label and determining to proceed with the method of testing. The method can further include confirming a rotary valve on the cartridge is in a shipping configuration before proceeding to the advancing the sample step. The method can further include obtaining a reading from an interference sensor on a valve drive assembly and confirming based on the reading that a rotary valve on the cartridge is not in an operational configuration prematurely. The method can further include engaging a rotary valve on

the cartridge with a valve drive assembly and rotating the rotary valve into an operational configuration. Rotating the rotary valve in an operational configuration can place a rotary valve gasket into contact with a stator on the cartridge. The method can further include moving a clamping block for engaging the cartridge with a door support assembly, a pneumatic interface assembly, and a thermal clamp assembly. The moving step can be a single continuous movement. The method can further include moving a frangible seal block having a plurality of frangible seal pins into position to engage one or more frangible seals on the cartridge. Moving the frangible seal block simultaneously can engage the plurality of frangible seal pins with the one or more frangible seals on the cartridge. Moving the frangible seal block sequentially can engage the plurality of frangible seal pins with the one or more frangible seals on the cartridge. The step of moving a frangible seal block can be performed after performing the step of moving a clamp block. The step of moving a frangible seal block can be performed initially with the clamp block and ends in a position separate from the clamp block. The method can further include moving a clamp block and a frangible seal block together for engaging the cartridge. The method can further include moving the clamp block together with the frangible seal block until the cartridge is engaged with a door support assembly, a pneumatic interface assembly, and a thermal clamp assembly. The method can further include only driving the frangible seal block assembly to engage one of more frangible seals on the cartridge simultaneously or sequentially. In mixing the sample with the at least one lysis agent, the lysis agent can be a mechanical agent. The mechanical agent can be ceramic beads, glass beads or steel beads, and the mixing the sample step can include rotating the stir bar at at least 1000 rpm. Mixing the sample can include rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent. The suspected pathogen can be a gram-positive bacterium, a fungus or a plant cell. In the mixing the sample with the at least one lysis agent step, the at least one lysis agent can be a chemical lysis agent. The one or more target pathogens can be a virus or a gram-negative bacterium and the lysis reagent can be a chaotropic agent. Prior to passing the lysed sample through the porous solid support, the method can further include passing the lysed sample through a size-exclusion filter, wherein nucleic acid can pass through the filter. The enriched nucleic acid can be combined with one or more amplification reagents before the distributing step. The one or more amplification reagents can be selected from the group consisting of a DNA polymerase, a reverse transcriptase, a helicase, nucleotide triphosphates (NTPs), a magnesium salt, a potassium salt, an ammonium salt, and a buffer. The one or more amplification reagents can further include a primer. Isothermal amplification can be initiated prior to distributing the enriched nucleic acid to the two or more assay chambers. After the distributing step, but prior to perform the isothermal amplification reaction, the method can further include combining the enriched nucleic acid with a primer set specific to one of the one or more target pathogens. A first assay chamber can contain a primer set specific to a first nucleic acid sequence. The first nucleic acid sequence can be present in one of the one or more target pathogens. Prior to mixing the sample with at least one lysis agent, a process control can be

added to the sample and the first nucleic acid sequence is present in the process control. Prior to passing lysed sample through the porous solid support, a process control can be added to the lysed sample and the first nucleic acid sequence can be present in the process control. A second assay chamber can contain a primer set specific to a second nucleic acid sequent. The second nucleic acid sequence can be present in one of the one or more target pathogens. The performing an isothermal amplification reaction step can be completed in less than 20 minutes. The performing an isothermal amplification reaction step can be completed in less than 15 minutes. The performing an isothermal amplification reaction step can be completed in less than 10 minutes. The method of testing a sample can further include providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction. The sample can be sputum and the chemical reaction can be incubation with a mucolytic agent. The mucolytic agent can be dithiothreitol or n-acetylcysteine. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction. The enzymatic reaction can be incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase. Pretreating can include incubating the sample with a DNase. Pretreating can include incubating the sample with a protease. The protease can be selected from pronase, chymotrypsin, trypsin and pepsin. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment. The physical treatment can include passing the sample through a size-exclusion filter in a first direction. The target pathogen can pass through the filter. The target pathogen may or may not pass through the filter and can thereby capture on a fill port side of the size-exclusion filter. The method can further include passing a volume of suspension buffer through the size-exclusion filter in a second direction, wherein second direction can be opposite the first direction, thereby releasing the target pathogen from the fill port side of the filter. The volume of suspension buffer can be less than the volume of the sample, and the target pathogen can be more concentrated than in the loaded sample. The physical treatment can include exposing the sample to a capture agent immobilized on a solid substrate. The method can further include, after exposure, separating the solid substrate from the sample. The capture agent can be a capture antibody. The capture agent can be an antibody with affinity for red blood cells. The solid substrate can be a magnetic bead, the capture agents can have affinity for a class of cells including the one or more target pathogens and the method can further include: (1) incubating the magnetic beads with the sample; (2) engaging a magnet to draw the magnetic beads to a location within the sample loading structure; (3) washing away unbound sample; (4) releasing the magnet; and (5) resuspending the magnetic beads and passing the suspension, including target pathogen bound to the magnetic beads, to the lysis chamber. The sample can be sputum and the method can further include, prior to mixing the sample with the at least one lysis reagent, bead beating the

sputum to liquify the sample. The bead beating can include mixing the sputum with ceramic, glass, or steel beads. The bead beating can include mixing the sputum with ceramic, glass, or steel beads and dithiothreitol. Prior to distributing the enriched nucleic acid to the assay wells, the method can further include passing the enriched nucleic acid through a second porous solid support. The second porous solid support can be the same as the first porous solid support. The enriched nucleic acid can be mixed with a matrix binding agent prior to passing through the second solid support. Matrix binding agent can be an alcohol or a salt solution. The second porous solid support can be different than the first porous solid support, and the second solid support can have an affinity for nucleic acid and the method can further include releasing the captured nucleic acid from the second solid support to generate a twice-enriched nucleic acid. The second porous solid support can be different than the first porous solid support. Prior to passing the lysed sample through a first porous solid support, the method can further include passing the lysed sample through a second porous solid support, wherein the second solid support may or may not bind nucleic acid and can have affinity for one or more contaminants, thereby removing contaminant from the lysed sample.

[0014] The method can further include releasing the cartridge from engagement with a clamp block and a frangible seal block after completing the performing an isothermal amplification reaction step. The method can further include displaying a result produced after the step of performing an isothermal amplification reaction step. The method can further include storing in a computer memory a result produced after the step of performing an isothermal amplification reaction step. The method can further include maintaining the cartridge in a vertical orientation while performing the steps of testing a sample. The cartridge can be inclined no more than 30 degrees while in the vertical orientation. The cartridge can be inclined no more than 15 degrees while in the vertical orientation.

[0015] In some embodiments, during the combining the enriched nucleic acid step in each of the two or more assay chambers, the enriched nucleic acid can combine with a dried reagent contained in each one of the two or more assay chambers. The dried reagent can be on a surface of a plug in each one of the two or more assay chambers. The dried reagent can be on a surface of the plug formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum used during the performing step.

[0016] The method can further include distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve further wherein the pneumatic signal continues to be introduced while the performing step is performed. Performing the isolation step can temporarily isolate each one of the two or more assay chambers are from each one of all the other two or more assay chambers. The isolation step can be performed using a pneumatic signal, a mechanical system to occlude one or more fluid channels to occlude one or more passages or channels of a cartridge. The mechanical

system can be one of a single pinch valve, a plurality of pinch valves, and a non-heated staker bar. Performing the isolation step can permanently isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers. After performing the isolation step, a portion of the cartridge can be melted or can be plastically deformed. After completing the performing step, each one of the two or more assay chambers can be isolated from each one of all the other two or more assay chambers. The method can further include distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve. The pneumatic signal can continue to be introduced while performing the isolating step by moving a heat staker into contact with the cartridge to isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers. After performing the isolating step, a single heat stake can isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers. The single heat stake can isolate a waste chamber on the cartridge. The method can further include moving a heat staker into contact with the cartridge to seal each one of the two or more assay chambers from each one of all the other two or more assay chambers. The method can further include providing a pneumatic pressure in the cartridge while moving the heat staker into contact with the cartridge. The method can further include forming a heat stake region in the cartridge to isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers. The method can further include obtaining a first image of a level of fluid in each of the one or more assay chambers after the step of distributing the enriched nucleic acid to each one of the two or more assay chambers. The method can further include obtaining a second image of a level of fluid in each of the one or more assay chambers after the isolating step. The method can further include determining the quality of the heat stake by comparing the level of fluid in the first image to the level of fluid in the second image. The method can further include rotating a rotary valve on the cartridge prior to performing the advancing the sample step. The method can further include advancing the sample to the lysis chamber using a pneumatic signal introduced into a cartridge pneumatic interface. The method of testing a sample can further include rotating a rotary valve on the cartridge prior to performing the step of passing the lysed sample through a first porous solid support to capture a nucleic acid on the porous solid support. The method of testing a sample can further include passing the lysed sample through the first porous solid support using a pneumatic signal introduced into the rotary valve. The method of testing a sample can further include distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve.

[0017] In general, in one embodiment, an apparatus includes an enclosure, a fixed support bracket within the enclosure, a first imaging system mounted on the fixed support bracket within the enclosure adjacent to an opening, a second imaging system mounted on the fixed support bracket within the enclosure configured to collect images from a second imaging area within the

enclosure, a moving support bracket within the enclosure and moveable relative to the fixed support bracket, the first imaging system and the second imaging system, a drive system on the fixed support bracket configured to position the moving support bracket relative to the fixed support bracket, and an opening positioned in the enclosure to provide access to an interior portion of the enclosure between the fixed support bracket and the moving support bracket. The first imaging system is configured to collect images from a first imaging area within the enclosure. The second imaging area is in non-overlapping relation to the first imaging area.

[0018] This and other embodiments can include one or more of the following features. The moving support bracket can be positioned between the first imaging system and the second imaging system. A rotary connector, a pneumatic connector and a multiple pin block can be connected to and move with the moving support bracket. The multiple pin block can be directly connected to the drive system. The multiple pin block can be configured to move together with the rotary connector and the pneumatic connector and independent of the rotary connector and the pneumatic connector. The opening can be a slot. The slot can be aligned to access an upper rail within the enclosure aligned to an upper portion of the slot and a lower rail within the enclosure aligned to a lower portion of the slot. The apparatus can further include a loading and ejection mechanism within the enclosure in sliding relation to the lower rail. The loading and ejection mechanism can move between a loading position and a loaded position. When in the loading position, the loading and ejection mechanism can be positioned in a forward most position towards the slot and when in the loaded position the loading and ejection mechanism can be engaged with a load position sensor. The load position sensor can provide an electronic indication when the loading and ejection mechanism has translated into the loaded position. The apparatus can further include a first heater and a second heater mounted on the fixed support bracket. The first heater can be positioned to heat a portion of the fixed support bracket between the first imaging area and the second imaging area. The second heater can be positioned to heat a portion of the fixed support bracket only within the second imaging area. The apparatus can further include a channel in the fixed support bracket and a heat stake assembly positioned to move a heating element through the channel. The channel can be positioned on the fixed support bracket to allow the heating element to interact within the enclosure between the first imaging area and the second imaging area. The channel can be positioned within the fixed support bracket such that the heating element may perform a heat staking operation directly adjacent to but outside of the second imaging area. The moving support bracket can partially block the channel when the moving support bracket is positioned at a closest position to the fixed support bracket.

[0019] In general, in one embodiment, an apparatus includes an enclosure, a fixed support bracket within the enclosure, a moving support bracket within the enclosure and moveable relative to the fixed support bracket, a drive system configured to position the moving support bracket relative to the fixed support bracket, an opening positioned in the enclosure to provide access to an interior portion of the enclosure between the fixed support bracket and the moving support

bracket; and an upper rail and a lower rail in the enclosure positioned adjacent to the opening wherein a cartridge positioned between the upper rail and the lower rail remains in a vertical position between the fixed support bracket and the moving support bracket.

[0020] This and other embodiments can include one or more of the following features. The apparatus can further include a feature within the upper rail or the lower rail positioned to interfere with the movement of a cartridge improperly aligned with respect to the upper rail and the lower rail. The apparatus can further include a loading and ejection assembly within the enclosure positioned to engage with a cartridge moving along the upper rail and the lower rail. The apparatus can further include a latch and pin assembly positioned adjacent to the upper rail adapted to engage a pin with a cartridge moving along the upper rail. The apparatus can further include a touch screen display on an exterior of the enclosure. The apparatus can further include a cellular communications module within the enclosure. The cellular communication module can be adjacent to the opening. The apparatus can further include a cartridge heater, a driving magnet system, a chemistry heater, a rehydration motor, a reaction camera and a heat stake assembly coupled to the fixed support bracket and positioned to interact with a corresponding portion of a cartridge positioned between the upper rail and the lower rail. The apparatus can further include a first imaging system mounted on the fixed support bracket within the enclosure adjacent to the opening. The first imaging system can be configured to collect images from a first imaging area within the enclosure and a second imaging system can be mounted on the fixed support bracket within the enclosure configured to collect images from a second imaging area within the enclosure. The second imaging area may be in non-overlapping relation to the first imaging area. The first imaging area can include a label of a cartridge positioned within the enclosure between the upper rail and the lower rail. The second imaging area can include one or more assay chambers of a cartridge positioned within the enclosure between the upper rail and the lower rail. The apparatus can further include a clamp block, a frangible seal block, a valve driver, a pneumatic interface, a thermal clamp, and a driven magnet system coupled to move along with the moving support bracket during operation of the drive system. The apparatus can further include a plenum adjacent to the chemistry heater and a fan in fluid communication with the plenum. The apparatus can further include a staker blade positioned to move relative to a depth stop frame. The staker blade can be coupled to a linear actuator motor and a spring with pivot washer.

[0021] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module. The loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation.

[0022] This and other embodiments can include one or more of the following features. The integrated diagnostic cartridge can further include one or more fluid filling conduits arranged to

flow into an upper portion of a chamber within a fluidic card of the integrated diagnostics cartridge and one or more fluid outlet conduits arranged to flow out of a lower portion of the chamber within the fluidic card of the integrated diagnostics cartridge. The chamber can be one or more of a lysis chamber, a metering chamber, a wash buffer chamber or an elution buffer chambers. The chamber can further include a filter assembly in fluid communication with a fluid outlet conduit of the chamber. The lysis module can include a mixing assembly having a vertically oriented lysis chamber containing a lysis agent and a non-magnetized stir bar. The non-magnetized stir bar can be made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system. The non-magnetized stir bar can be coated with an impermeable material to prevent corrosion by a chemical lysis buffer in the vertically oriented lysis chamber. When in use within a diagnostic instrument, the non-magnetized stir bar can be disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument. The driving magnet system can be configured to rotate the non-magnetized stir bar within the vertically oriented lysis chamber at least 1000 rpm. The integrated diagnostic cartridge can further include a fluid inlet to the vertically oriented lysis chamber and a fluid outlet to lysis chamber wherein the vertically oriented lysis chamber can be isolated from the other modules on the cartridge by a first frangible seal in fluid communication with the fluid inlet to the vertically oriented lysis chamber and a second frangible seal in fluid communication with the fluid outlet to the vertically oriented lysis chamber. The integrated diagnostic cartridge can further include a fluidic card and a cover. The fluidic card can further include a first film adhered to a surface of at least a portion of the fluidic card. The first film can form one surface of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module. The integrated diagnostic cartridge can further include an interference feature on the cover. The interference feature can be sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument. A thickness of the fluidic card can be selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument. A total sample process volume of the integrated diagnostic cartridge can be related to a thickness of the cartridge corresponding to a spacing between the one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module formed in the fluidic card and the first film. A diagnostic instrument can be adapted and configured to accommodate a variation of the thickness of the cartridge by increasing a width of an opening of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument is adapted to accommodate the increased thickness of the cartridge. The integrated diagnostic cartridge can further include a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing. Each of the upper spacing and the lower spacing can be sized and positioned to engage with the upper rail and lower rail of

the diagnostic instrument. The integrated diagnostic cartridge can further include an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation. The integrated diagnostic cartridge can further include a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module. The integrated diagnostic cartridge can further include a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or an image of a patient identification marking.

[0023] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module including a sample port assembly having a fill chamber, a metering chamber, and an overflow chamber arranged in fluid communication, a lysis module, a purification module, and a reaction module. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0024] This and other embodiments can include one or more of the following features. The metering chamber can include a transparent viewing window for observing the height of a sample within the metering chamber. The integrated diagnostic cartridge can further include a ball float in the metering chamber adapted for use with the transparent viewing window. The fill chamber can include a cap operable to provide access to the fill chamber. The cap can be positioned for interaction with a closing apparatus of a diagnostic instrument. The cartridge can be in a vertical orientation when in use within a diagnostic instrument and a fluid channel connects an outlet at a lower portion of the fill chamber with an inlet to the metering chamber located in an upper portion of the metering chamber. The metering chamber can include a transparent viewing window. The integrated diagnostic cartridge can further include a buoyant ball within the metering chamber. Said buoyant ball can adapt to appear adjacent to the transparent viewing window permitting an assessment of the height of the sample liquid in the metering chamber. The metering chamber can include a buoyant ball for assessing a height of a sample liquid in the metering chamber.

[0025] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module including a mixing assembly having a lysis chamber containing a lysis agent and a non-magnetized stir bar, a purification module, and a reaction module. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0026] This and other embodiments can include one or more of the following features. The non-magnetized stir bar can be made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system. The metal can include a ferritic stainless steel or a duplex stainless steel. The non-magnetized stir bar can be made from a metal selected from the group consisting of a carbon steel, a mild carbon steel, a low alloy steel, a tool steel, a metal alloy contain nickel, a metal alloy containing cobalt, a non-austenitic stainless steel, a ferritic grade of

stainless steel including 430 steel, Atlas CR12 steel, 444 steel, F20S steel, a duplex grade of steel including 2205 steel, 2304 steel, 2101 steel, 2507 steel and a martensitic grade of steel such as 431 steel, 416 steel, 420 steel and 440C steel. The metal can have a magnetic permeability to be responsive to a rotating magnetic field produced within the mixing chamber. The metal can have a magnetic permeability between 500-1,000,000. The non-magnetized stir bar can be coated with an impermeable material to prevent corrosion by a chemical lysis buffer in lysis chamber. The impermeable material can be PTFE, parylene C, parylene D, a functionalized perfluoropolyether (PFPE), Xylan Fluoropolymer, epoxy, or urethane. When in use within a diagnostic instrument, the non-magnetized stir bar can be disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument. The driving magnet system can be configured to rotate the non-magnetized stir bar within the lysis chamber at at least 1000 rpm. The lysis agent can be a mechanical agent. The mechanical agent can be ceramic beads, glass beads or steel beads. The lysis agent can be a chemical agent. The chemical agent can be an anionic detergent, a cationic detergent, a non-ionic detergent or a chaotropic agent. The cartridge can be configured for testing of one or more target pathogens that is a virus or a gram-negative bacterium. The integrated diagnostic cartridge can further include a fluid inlet in fluid communication with the lysis chamber and a fluid outlet in fluid communication with the lysis chamber and a filter assembly in fluid communication with the fluid outlet of the lysis chamber. The integrated diagnostic cartridge can further include a fluid inlet to the lysis chamber and a fluid outlet to lysis chamber wherein the lysis chamber can be isolated from the other modules on the cartridge by a first frangible seal in fluid communication with the fluid inlet to the lysis chamber and a second frangible seal in fluid communication with the fluid outlet of the lysis chamber. The integrated diagnostic cartridge can further include a process control chamber having an inlet, an outlet and a plug including a process control wherein the process control chamber is in fluid communication with the lysis chamber inlet.

[0027] In general, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module including a rotary valve including (a.) a stator including a stator face and a plurality of passages, each passage including a port at the stator face; (b.) a rotor operably connected to the stator and including a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel includes a porous solid support; and (c.) a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal, and a reaction module. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0028] This and other embodiments can include one or more of the following features. The rotary valve can further include a gasket between the stator face and the rotor valving face. The stator can include a displaceable spacer for preventing the gasket from sealing against at least one of the rotor and stator. When the spacer is displaced, the gasket can seal the rotor and stator

together in a fluid-tight manner. When the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument can displace the spacer and can seal the rotor and stator together in a fluid-tight manner. A rotation movement performed by the rotor driver of the diagnostic instrument can displace the spacer and can seal the rotor and the stator together in a fluid-tight manner. The integrated diagnostic cartridge can further include at least one pair of ridges and spaces on a retention ring and at least one pair of ridges and spaces on the rotor. While the at least one pair of ridges and spaces of the retention ring is engaged with the at least one pair of ridges and spaces of the rotor sealing of the rotor and stator can be prevented. Relative movement between the at least one pair of ridges and spaces on the retention ring and the at least one pair of ridges and spaces on the rotor can seal the rotor and stator together in a fluid-tight manner. When the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument can produce the relative movement between the at least two pairs of ridges and spaces on the retention ring and the rotor that can seal the rotor and stator together in a fluid-tight manner. A rotation movement of less than one full rotation of the rotor performed by the rotor driver of the diagnostic instrument can seal the rotor and stator together in a fluid-tight manner. The integrated diagnostic cartridge can further include a gasket interposed at the rotor-stator interface. The rotary valve can be maintained in a storage condition while a threaded portion of a retention ring is engaged with a threaded portion of the rotor. Relative motion between the threaded portion of the retention ring and the threaded portion of the rotor can seal the rotor and stator together in a fluid-tight manner. When the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument can produce the relative movement between the threaded portion of the retention ring and the threaded portion of the rotor. A rotation movement of less than one full rotation of the rotor performed by the rotor driver of the diagnostic instrument can seal the rotor and stator together in a fluid-tight manner. The integrated diagnostic cartridge can further include a gasket interposed at the rotor-stator interface. The integrated diagnostic cartridge can further include a waste collection element, a wash buffer reservoir and an elution buffer reservoir. The integrated diagnostic cartridge can further include a pneumatic interface in fluidic communication with at least the purification module. The porous solid support can be polymeric. The porous solid support can be selected from the group consisting of alumina, silica, celite, ceramics, metal oxides, porous glass, controlled pore glass, carbohydrate polymers, polysaccharides, agarose, Sepharose™, Sephadex™, dextran, cellulose, starch, chitin, zeolites, synthetic polymers, polyvinyl ether, polyethylene, polypropylene, polystyrene, nylons, polyacrylates, polymethacrylates, polyacrylamides, polymaleic anhydride, membranes, hollow fibers and fibers, and any combination thereof. The rotor valving face can include a gasket interposed at the rotor-stator interface. The integrated diagnostic cartridge can further include a fluid connector or a fluid selector including a volume dimensioned to provide an aliquot of liquid when filled. The rotor can include a plurality of flow channels, each flow channel can include an inlet, an outlet, and a porous

solid support. The integrated diagnostic cartridge can further include a fluid connector or a fluid selector including a volume dimensioned to provide an aliquot of liquid when filled. The integrated diagnostic cartridge can further include a waste collection element, a wash buffer reservoir and an elution buffer reservoir.

[0029] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module including a plurality of individual assay chambers. At least one wall in each one of the plurality of individual assay chambers is provided by a plug include a body with a bottom surface; a central opening in the body; and a dried reagent on the bottom surface, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0030] This and other embodiments of an assay chamber of an integrated diagnostic cartridge can include a plug having one or more or a combination of the following features. The bottom surface of the plug body can include a cavity in the bottom surface with the dried reagent within the cavity. The plug can have a plug thickness between a central opening bottom and the plug body bottom, and further wherein a depth of the cavity is less than 90% of the plug thickness, is less than 70% of the plug thickness or is less than 50% of the plug thickness. The plug can have a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths. The plug may have a dried reagent that can be selected from the group consisting of nucleic acid synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, monomers, detection reagents, catalysts or combinations thereof. The dried reagent can be a continuous film adhered to the plug bottom surface. The dried reagent can be a lyophilized reagent. The body of the plug can protrude into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber. In some embodiments, during the combining the enriched nucleic acid step in each of the two or more assay chambers, the enriched nucleic acid can combine with a dried reagent contained in each one of the two or more assay chambers. The dried reagent can be on a surface of a plug in each one of the two or more assay chambers. The dried reagent can be on a surface of the plug formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum used during the performing step. In one aspect, the surface of the plug having the dried reagent is also used during the performing an isothermal amplification reaction step. Images collected through the plug surface that contained the dried reagent are processed as part of the detection of an amplification product within an assay chamber.

[0031] In still additional embodiments, the integrated diagnostic cartridge can further include a cartridge perimeter. Each one of the plurality of individual assay chambers can be in communication with an air chamber and each air chamber is closer to the cartridge perimeter than the plug in each one of the plurality of individual assay chambers. The integrated diagnostic cartridge can further include a reaction area perimeter. Each one of the plurality of individual assay chambers can be in communication with an air chamber and further wherein each plug in each one of the plurality of individual assay chambers can be within the reaction area perimeter and each air chamber is outside of the reaction area perimeter. The integrated diagnostic cartridge can further include a cartridge perimeter and a reaction area perimeter wherein each one of the plurality of individual assay chambers can be in communication with an air chamber and each air chamber is closer to the cartridge perimeter than the plug in each one of the plurality of individual assay chambers and is located outside of the reaction area perimeter and each one of the plurality of individual assay chambers is within the reaction area perimeter. The integrated diagnostic cartridge can further include at least one fluid inlet conduit to each one of the plurality of individual assay chambers of the reaction module. Each one of the at least one fluid inlet conduits can further include a heat staked region. A heat stake in the heat staked region can fluidically isolate the reaction module from the loading module, the lysis module, and the purification module.

[0032] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module including one or more assay chambers. Each assay chamber includes: (1) a tapered inlet; (2) a tapered outlet; (3) a plug including a bottom surface and a central opening in the body, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of an ultraviolet spectrum, a blue spectrum, a green spectrum and a red spectrum; (4) two curved boundaries, wherein each curved boundary extends from the tapered inlet to the tapered outlet such that together, the two curved boundaries and the plug enclose a volume of the assay chamber; and (5) a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved boundaries, the shoulders extending from each of the curved boundaries and the plug.

[0033] In general, in one embodiment, an integrated diagnostic cartridge, includes a loading module; a lysis module; a purification module; and a reaction module. Additionally or optionally, the reaction module may also include a common fluid pathway, and a plurality of independent, continuous fluidic pathways connected to the common fluid pathway. Still further, each independent, continuous fluidic pathway also is in fluidic communication with an assay chamber, and a pneumatic compartment, and wherein the assay chamber is connected to the common fluid pathway, the assay chamber having a fluid volume defined in part by a plug having a dried reagent. In additional aspects, the pneumatic compartment, including a pneumatic volume, is connected to the common fluid pathway via the assay chamber. Still further, each fluidic pathway

of the plurality of independent, continuous fluidic pathways is a closed system excluding the connection between the assay chamber and common fluid source. In additional aspects, each assay chamber includes a double tapered chamber that includes a tapered inlet in fluidic communication with a terminus of the entry conduit of the fluidic pathway, a tapered outlet in fluidic communication with a terminus of the pneumatic compartment, and two curved boundaries, wherein each curved boundary extends from the tapered inlet to the tapered outlet such that together, the two curved boundaries enclose the volume of the assay chamber. There is also a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved boundaries, the shoulders extending from each of the curved boundaries and the plug. Additionally, the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0034] This and other embodiments can include one or more of the following features. The two curved boundaries can be formed in a monolithic substrate or a fluidic card of the cartridge. The body of the plug can protrude into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber.

[0035] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module including a reagent storage component including a capsule capable of holding a liquid or solid sample, said capsule including an opening, a closed end and a wall extending from the closed end to the opening, wherein the capsule is oval-shaped and the wall is rounded, and wherein the closed end and wall define an interior volume having a substantially smooth surface. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0036] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module including a capsule capable of holding a liquid or a solid sample. Said capsule includes an inner surface extending from the bottom of said capsule to an oval-shaped opening at the top of the capsule and a planar layer affixed around the oval-shaped opening of said capsule and oriented in the same plane as the oval-shaped opening of said capsule. Said inner surface is substantially smooth and includes a concave shape extending from the bottom of the capsule. Said planar layer includes a top surface and a bottom surface. Said top surface aligned with the inner surface of said capsule at said oval-shaped opening to provide a continuous surface. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

[0037] These and other embodiments can include one or more of the following features. Said capsule can be capable of holding a volume from approximately 50 µL to approximately 200 µL

or said oval-shaped opening can contained within an area of 9 mm x 9 mm. Said capsule can include a dried reagent. The integrated diagnostic cartridge can further include a fluidic card and a cover. At least two of the loading module, the lysis module, the purification module and the reaction module can be formed in or supported by the fluidic card. At least two of the loading module, the lysis module, the purification module and the reaction module can be formed in or supported by the cover. The integrated diagnostic cartridge can further include a slot positioned to engage with a latch and pin assembly of a diagnostic instrument to secure the integrated diagnostic cartridge in a testing position within the diagnostic instrument. The integrated diagnostic cartridge can further include an interference feature on the cover. The interference feature can be sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument. A thickness of the fluidic card can be selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument. A total sample process volume of the integrated diagnostic cartridge can be provided by increasing the thickness of the cartridge. A diagnostic instrument can be adapted and configured to accommodate the increased thickness of the cartridge by increasing a width of an opening of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument is adapted to accommodate the increased thickness of the cartridge. The integrated diagnostic cartridge can further include a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing. Each of the upper spacing and the lower spacing can be sized and positioned to engage with an upper rail and a lower rail of the instrument. The integrated diagnostic cartridge can further include an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation. The integrated diagnostic cartridge can further include a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module. The integrated diagnostic cartridge can further include a label section. The integrated diagnostic cartridge can further include one or more machine readable marking indicating the sample type to be used in the cartridge or target pathogen to be detected. The integrated diagnostic cartridge can further include a pneumatic interface. Prior to loading the cartridge into a diagnostic instrument, a lysis chamber in the cartridge can contain a lysis buffer. The integrated diagnostic cartridge can further include a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or a patient identification marking. The integrated diagnostic cartridge can further include a film adhered to a surface of the monolithic substrate, wherein the film forms one wall of the assay chamber. The integrated diagnostic cartridge can further include a first film adhered to a surface of at least a portion of the cartridge. The first film can form one wall of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module. The integrated diagnostic cartridge can further include a second film

adhered to the first film. The second film can have a higher melting temperature than the first film. The integrated diagnostic cartridge can further include a heat staked region formed in each of the fluidic pathways using the first film or the second film wherein the heat staked region seals off the common fluid pathway from the assay chamber and the pneumatic chamber. The integrated diagnostic cartridge can further include a raised platform within each of the plurality of independent, continuous fluidic pathways the raised platform positioned between an inlet to the assay chamber and the common fluid pathway wherein the heat staked region is formed using a portion of the raised platform.

[0038] In general, in one embodiment, an integrated diagnostic cartridge includes a loading module having a fill chamber within the cartridge having a volume sufficient to hold a sample, a fluid inlet in fluid communication with the fill chamber, a fluid outlet in fluid communication with fill chamber; a lysis module; a purification module, and a reaction module. The loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module. Further, the loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation. Further, when the cartridge is in a horizontal sample loading orientation the fluid inlet accesses the fill chamber via an upper surface of the cartridge and when the cartridge is in a vertical sample processing orientation the fluid inlet is positioned adjacent to an upper portion of the fill chamber and the fluid outlet is arranged for the sample to flow out of a lower portion of the fill chamber.

[0039] This and other embodiments can include one or more of the following features. The integrated diagnostic cartridge can further include one or more fluid filling conduits arranged to flow into an upper portion of a vertically oriented chamber within a fluidic card of the integrated diagnostics cartridge and one or more fluid outlet conduits arranged to flow out of a lower portion of the vertically oriented chamber within the fluidic card of the integrated diagnostics cartridge. The vertically oriented chamber can further include a filter assembly in fluid communication with a fluid outlet conduit of the vertically oriented chamber. The lysis module can include a mixing assembly having a vertically oriented lysis chamber containing a lysis agent and a non-magnetized stir bar. The non-magnetized stir bar can be made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system. The non-magnetized stir bar can be coated with an impermeable material to prevent corrosion by a chemical lysis buffer in the vertically oriented lysis chamber. When in use within a diagnostic instrument, the non-magnetized stir bar can be disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument. The driving magnet system can be configured to rotate the non-magnetized stir bar within the vertically oriented lysis chamber at least 1000 rpm. The integrated diagnostic cartridge can further include a fluid inlet to the vertically oriented lysis chamber and a fluid outlet to lysis chamber. The vertically oriented lysis chamber can be isolated from the other modules on the cartridge by a first frangible seal in fluid

communication with the fluid inlet to the vertically oriented lysis chamber and a second frangible seal in fluid communication with the fluid outlet to the vertically oriented lysis chamber. The integrated diagnostic cartridge can further include a fluidic card and a cover. The fluidic card can further include a first film adhered to a surface of at least a portion of the fluidic card, wherein the first film forms one surface of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module. The integrated diagnostic cartridge can further include an interference feature on the cover. The interference feature can be sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument. A thickness of the fluidic card can be selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument. A total sample process volume of the integrated diagnostic cartridge can be related to a thickness of the cartridge corresponding to a spacing between the one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module formed in the fluidic card and the first film. A diagnostic instrument is adapted and configured to accommodate a variation of the thickness of the cartridge by increasing a width of a loading slot of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument can be adapted to accommodate the increased thickness of the cartridge. The integrated diagnostic cartridge can further include a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing. Each of the upper spacing and the lower spacing can be sized and positioned to engage with the upper rail and lower rail of the diagnostic instrument. The integrated diagnostic cartridge can further include an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation. The integrated diagnostic cartridge can further include a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module. The integrated diagnostic cartridge can further include a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or an image of a patient identification marking.

[0040] In still another alternative implementation, there is integrated diagnostic cartridge with a loading module, a lysis module, and a purification module. The purification module also includes a purification module comprising a rotary valve. The rotary valve may also include a stator comprising a stator face and a plurality of passages, each passage comprising a port at the stator face; a rotor operably connected to the stator and comprising a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel comprises a porous solid support, and a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal. The integrated cartridge also includes a reaction module. The reaction module includes a plurality of individual assay chambers, wherein at least one surface in each one of the plurality of individual assay chambers

is provided by a plug. Each plug includes, for example, a body with a bottom surface, a central opening in the body and a dried reagent on the bottom surface. Still further, the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum. Additionally, the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module. Still further, the loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation.

[0041] This and other embodiments can include one or more of the following features. The bottom surface of the plug body can include a cavity in the bottom surface with the dried reagent within the cavity. The plug can have a plug thickness between a central opening bottom and the plug body bottom, and further, a depth of the cavity can be less than 90% of the plug thickness, can be less than 70% of the plug thickness or can be less than 50% of the plug thickness. The plug can have a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths. The dried reagent can be selected from the group consisting of nucleic acid synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, monomers, detection reagents, catalysts or combinations thereof. The body of the plug can protrude into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber. The integrated diagnostic cartridge can further include at least one fluid inlet conduit to each one of the plurality of individual assay chambers of the reaction module. Each one of the at least one fluid inlet conduits can further include a heat staked region. A heat stake in the heat staked region can fluidically isolate the reaction module from the loading module, the lysis module, and the purification module. The purification module can further include a rotary valve including: (a) a stator including a stator face and a plurality of passages, each passage comprising a port at the stator face; (b) a rotor operably connected to the stator and including a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel can include a porous solid support; and (c.) a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal. The rotary valve can further include a gasket between the stator face and the rotor valving face. The stator can include a displaceable spacer for preventing the gasket from sealing against at least one of the rotor and stator, and when the spacer is displaced the gasket can seal the rotor and stator together in a fluid-tight manner. When the cartridge is positioned within a diagnostic instrument, engagement with a valve drive assembly of the diagnostic instrument can displace the spacer and seal the rotor and stator together in a fluid-tight manner. The purification module can further include a waste collection element, a wash buffer reservoir and an elution buffer reservoir. The integrated diagnostic cartridge can further include a pneumatic interface in fluidic communication with at least the purification module. The loading module can further include a dried antifoam agent. The method of testing a sample can

further include combining the sample with a dried antifoam agent in the sample port assembly before the accepting step.

[0042] In general, in one embodiment, a method of testing a sample suspected of containing one or more target pathogens includes: (1) accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens; (2) advancing the sample suspected of containing the one or more target pathogens to a lysis chamber within the cartridge having at least one lysis reagent therein; (3) mixing the sample with the at least one lysis agent to generate a lysed sample; (4) passing the lysed sample through a porous solid support within the cartridge to capture a nucleic acid on the porous solid support; (5) releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid; (6) introducing the enriched nucleic into a rehydration chamber within the cartridge containing one or more dried reagents; (7) after introducing the analyte/reagent solution into a metering channel, mixing the contents of the rehydration chamber to produce an analyte/reagent solution; (8) distributing the analyte/reagent solution to two or more assay chambers within the cartridge after performing the mixing step; (9) combining the analyte/reagent solution with one or more amplification reagents after performing the distributing step; (10) sealing each one of the two or more assay chambers within the cartridge containing analyte/reagent solution from each one of all the other two or more assay chambers within the cartridge containing analyte/reagent solution and a waste chamber; and (11) performing an isothermal amplification reaction within each one of the two or more assay chambers in the cartridge while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

[0043] This and other embodiments can include one or more of the following features. In mixing the sample with the at least one lysis agent, the lysis agent can be a mechanical agent. The mechanical agent can be ceramic beads, glass beads or steel beads, and the mixing the sample step can include rotating a stir bar within the lysis chamber at at least 1000 rpm. Mixing the sample can include rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent. The at least one lysis agent can be a chemical lysis agent. The one or more target pathogens can be a virus or a gram-negative bacterium and the lysis reagent is a chaotropic agent. Prior to passing the lysed sample through the porous solid support, the method can further include passing the lysed sample through a size-exclusion filter, wherein nucleic acid can pass through the filter. The enriched nucleic acid can be combined with one or more amplification reagents before the distributing step and the one or more amplification reagents can include a primer. The performing of the isothermal amplification reaction step can be initiated prior to the distributing the enriched nucleic acid to the two or more assay chambers step. After the distributing step, but prior to performing the isothermal amplification reaction step, the method can further include combining the enriched nucleic acid with a primer set specific to one of the one or more target pathogens. A first assay chamber can contain a primer set specific to a first

nucleic acid sequence. The first nucleic acid sequence can be present in one of the one or more target pathogens. Prior to mixing the sample with at least one lysis agent, a process control can be added to the sample and the first nucleic acid sequence can be present in the process control. Prior to passing lysed sample through the porous solid support, a process control can be added to the lysed sample and the first nucleic acid sequence can be present in the process control. A second assay chamber can contain a primer set specific to a second nucleic acid sequence. The second nucleic acid sequence can be present in one of the one or more target pathogens. The performing an isothermal amplification reaction step can be completed in less than 15 minutes. The method of testing a sample can further include providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen. The method can further include prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction. The sample can be sputum and the chemical reaction can be incubation with a mucolytic agent. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction. The enzymatic reaction can be incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment. The physical treatment can include passing the sample through a size-exclusion filter in a first direction. The physical treatment can include exposing the sample to a capture agent immobilized on a solid substrate. The method of testing a sample can further include, after exposure, separating the solid substrate from the sample. The capture agent can be an antibody with affinity for red blood cells. The sample can be sputum and the method can further include, prior to mixing the sample with the at least one lysis reagent, bead beating the sputum to liquify the sample. The bead beating can include mixing the sputum with ceramic, glass, or steel beads. Prior to distributing the enriched nucleic acid to the assay chambers, the method can further include passing the enriched nucleic acid through a second porous solid support.

[0044] In general, in one embodiment, a method of testing a sample suspected of containing one or more target pathogens include: (1) accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens; (2) advancing the sample suspected of containing the one or more target pathogens to a lysis chamber within the cartridge having at least one lysis reagent therein; (3) mixing the sample with the at least one lysis agent to generate a lysed sample; (4) passing the lysed sample through a porous solid support within the cartridge to capture a nucleic acid on the porous solid support; (5) releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid; (6) introducing the enriched nucleic into a rehydration chamber within the cartridge containing one or more dried reagents to generate an analyte/reagent solution; (7) after introducing the analyte/reagent solution into a metering channel, mixing the contents of the rehydration chamber to homogenize an analyte/reagent solution; (8) distributing the analyte/reagent solution to two or

more assay chambers within the cartridge after performing the mixing step; (9) combining the analyte/reagent solution with one or more amplification reagents after performing the distributing step to generate an amplification solution; (10) sealing each one of the two or more assay chambers within the cartridge containing amplification solution from each one of all the other two or more assay chambers within the cartridge containing amplification solution and a waste chamber; and (11) performing an isothermal amplification reaction within each one of the two or more assay chambers in the cartridge while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

[0045] This and other embodiments can include one or more of the following features. In mixing the sample with the at least one lysis agent, the lysis agent can be a mechanical agent. The mechanical agent can be ceramic beads, glass beads or steel beads, and the mixing the sample step can include rotating a stir bar within the lysis chamber at at least 1000 rpm. Mixing the sample can include rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent. The at least one lysis agent can be a chemical lysis agent. The one or more target pathogens can be a virus or a gram-negative bacterium and the lysis reagent can be a chaotropic agent. Prior to passing the lysed sample through the porous solid support, the method can further include passing the lysed sample through a size-exclusion filter, wherein nucleic acid can pass through the filter. A first assay chamber can contain a primer set specific to a first nucleic acid sequence. The first nucleic acid sequence can be present in one of the one or more target pathogens. Prior to mixing the sample with at least one lysis agent, a process control can be added to the sample and the first nucleic acid sequence can be present in the process control. Prior to passing lysed sample through the porous solid support, a process control can be added to the lysed sample and the first nucleic acid sequence can be present in the process control. A second assay chamber can contain a primer set specific to a second nucleic acid sequence. The second nucleic acid sequence can be present in one of the one or more target pathogens. The performing an isothermal amplification reaction step can be completed in less than 15 minutes. The method of testing a sample can further include providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction. The sample can be sputum and the chemical reaction can be incubation with a mucolytic agent. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction. The enzymatic reaction can be incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase. The method can further include, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment. The physical treatment can include passing the sample through a size-exclusion filter in a first direction. The physical treatment can include exposing the sample to a

capture agent immobilized on a solid substrate. The method of testing a sample can further include, after exposure, separating the solid substrate from the sample. The capture agent can be an antibody with affinity for red blood cells. The sample can be sputum and the method can further include, prior to mixing the sample with the at least one lysis reagent, bead beating the sputum to liquify the sample. The bead beating can include mixing the sputum with ceramic, glass, or steel beads. Prior to distributing the analyte/reagent solution to the two or more assay chambers, the method can further include passing the analyte/reagent solution through a second porous solid support.

VI. DESCRIPTION OF THE DRAWINGS

[0046] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead placed upon illustrating the principles of various embodiments of the invention.

[0047] FIG. 1 is an illustration of a diagnostic instrument for conducting a molecular diagnostic test, in accordance to with an embodiment.

[0048] FIG. 2A and FIG. 2B depict an integrated diagnostic cartridge, configured to be used in conjunction with a diagnostic instrument, during filling by a user, in accordance with an embodiment.

[0049] FIG. 2C depicts an integrated diagnostic cartridge with a loading module sealed after filling is completed and prior to being inserted into a diagnostic instrument, in accordance with an embodiment.

[0050] FIG. 3 depicts an integrated diagnostic cartridge being inserted into a diagnostic instrument to perform a diagnostic test, in accordance with an embodiment.

[0051] FIG. 4A depicts a diagnostic instrument after an integrated diagnostic cartridge is inserted during initialization of a diagnostic test. The integrated diagnostic instrument is shown having a display configured to show information associated with a diagnostic test run, in accordance with an embodiment.

[0052] FIG. 4B depicts a diagnostic instrument when running a diagnostic test on an integrated diagnostic cartridge, in accordance with an embodiment.

[0053] FIG. 5 depicts a diagnostic instrument ejecting an integrated diagnostic cartridge upon completion of a diagnostic test, in accordance with an embodiment.

[0054] FIG. 6 depicts a frontal exploded illustration of a diagnostic instrument, in accordance with an embodiment.

[0055] FIG. 7 depicts a rear exploded illustration of a diagnostic instrument, in accordance with an embodiment.

[0056] FIG. 8 and FIG. 9 are frontal perspective views of a diagnostic instrument clamping subsystem during clamping of an integrated diagnostic cartridge.

[0057] FIG. 10 and FIG 11 are rear perspective views of a diagnostic instrument clamping subsystem during clamping of an integrated diagnostic cartridge.

[0058] FIG. 12 is a frontal exploded view of a diagnostic instrument clamping subsystem with an integrated diagnostic cartridge disposed between a fixed bracket assembly and a moving bracket assembly.

[0059] FIG. 13 is a rear exploded view of a diagnostic instrument clamping subsystem with an integrated diagnostic cartridge disposed between a fixed bracket assembly and a moving bracket assembly.

[0060] FIG. 14 is a perspective view of a moving bracket assembly of a clamping subsystem. The view of the moving bracket assembly is shown from a first surface of a clamp block.

[0061] FIG. 15A is a frontal exploded view of a moving bracket assembly of a clamping subsystem.

[0062] FIG. 15B is a rear exploded view of a moving bracket assembly of a clamping subsystem.

[0063] FIG. 16A is a view of a clamping subsystem with an integrated diagnostic cartridge inserted between a fixed bracket assembly and moving bracket assembly taken from the front of a diagnostic instrument, as seen in FIG. 4A. The clamping subsystem is in a zero clamping position.

[0064] FIG. 16B is a view of a clamping subsystem with an integrated diagnostic cartridge inserted between a fixed bracket assembly and a moving bracket assembly taken from the front of a diagnostic instrument, as seen in FIG. 4A. The clamping subsystem is in a first clamping position with a valve drive assembly and a thermal clamp assembly of the moving bracket assembly contacting the integrated diagnostic cartridge.

[0065] FIG. 16C is a view of a clamping subsystem with an integrated diagnostic cartridge inserted between a fixed bracket assembly and a moving bracket assembly taken from the front of a diagnostic instrument, as seen in FIG. 4A. The clamping subsystem is in a second clamping position to clamp the integrated diagnostic cartridge. A valve drive assembly, a thermal clamp assembly, a door support assembly, and a pneumatic interface of the moving bracket assembly contact the integrated diagnostic cartridge.

[0066] FIG. 16D is a view of a clamping subsystem with an integrated diagnostic cartridge inserted between a fixed bracket assembly and a moving bracket assembly taken from the front of a diagnostic instrument, as seen in FIG. 4A. The clamping subsystem is in a third clamping position to render the integrated diagnostic cartridge fluidically active with a frangible seal block.

[0067] FIG. 16E is a view of a clamping subsystem with an integrated diagnostic cartridge inserted between a fixed bracket assembly and a moving bracket assembly taken from the front of a diagnostic instrument, as seen in FIG. 4A. The clamping subsystem is in a fourth clamping position to unclamp the integrated diagnostic cartridge and eject the integrated diagnostic cartridge from the clamping subsystem.

[0068] FIG. 17A is a frontal perspective view of a fixed support bracket of a clamping subsystem. The fixed support bracket is shown with a loading assembly for accepting and ejecting an integrated diagnostic cartridge. An integrated diagnostic cartridge is seen in a loading position.

[0069] FIG. 17B is an enlarged view of a loading assembly, as shown in FIG. 17A, in a loading position.

[0070] FIG. 17C is an enlarged partial view of a loading assembly, as shown in FIGs. 17A and 17B, depicting a spring which provides a motive force for ejecting an integrated diagnostic cartridge.

[0071] FIG. 18A is another frontal perspective view of a fixed support bracket of a clamping subsystem. The fixed support bracket is shown with a loading assembly from FIG. 17A for accepting and ejecting an integrated diagnostic cartridge. The loading assembly is now shown in a loaded position.

[0072] FIG. 18B is an enlarged view of a loading assembly in a loaded position. A load position sensor on the loading assembly is triggered by a flag.

[0073] FIG. 19A is a perspective frontal view of a fixed support bracket of a clamping subsystem with an integrated diagnostic cartridge inserted into a loading assembly from FIGs. 17A and 18A. The integrated diagnostic cartridge is in a loaded position.

[0074] FIG. 19B is an additional enlarged view of a loading assembly in a loaded position similar to FIG. 18B. A load position sensor on the loading assembly is triggered by a flag.

[0075] FIG. 19C is an additional frontal view of FIG. 19A with a fixed bracket assembly of a clamping subsystem and an integrated diagnostic cartridge inserted into a loading assembly. The integrated diagnostic cartridge and loaded assembly is shown in a loaded position.

[0076] FIG. 20 is a perspective view of loading assembly rails showing guide features extending along the rails.

[0077] FIG. 21 is an illustration of a loading assembly with rails viewed from the front of a diagnostic instrument, as seen in FIG. 4A and 16A-16E.

[0078] FIG. 22A is a top view of an integrated diagnostic cartridge prior to being loaded into a loading assembly. The integrated diagnostic cartridge is shown with a gap formed between a fluidics card and a cover configured to align with a guide feature on a top rail, as shown in FIGs. 20 and 21.

[0079] FIG. 22B is a top view of an integrated diagnostic cartridge during loading into a loading assembly. A guide feature on a top rail is shown inserted between a gap formed between a fluidics card and a cover.

[0080] FIG. 23A is a bottom view of an integrated diagnostic cartridge prior to being loaded into a loading assembly. The integrated diagnostic cartridge is shown with a gap formed between a fluidics card and a cover configured to align with a guide feature on a bottom rail, as shown in FIGs. 20 and 21.

[0081] FIG. 23B is a bottom view of an integrated diagnostic cartridge during loading into a loading assembly. A guide feature on a bottom rail is shown inserted between a gap formed between a fluidics card and a cover.

[0082] FIG. 24 is a rear perspective view of a latch and pin assembly of a clamping subsystem. An integrated diagnostic cartridge is inserted between a fixed bracket assembly and a moving bracket assembly of the clamping subsystem as shown in FIGs. 10 and 11.

[0083] FIG. 25A is a frontal perspective view of a latch and pin assembly of FIG. 24. An integrated diagnostic cartridge is seen inserted and latched. A latch from the latch and pin assembly is shown disposed within a notch of the integrated diagnostic cartridge to prevent the integrated diagnostic cartridge from being ejected.

[0084] FIG. 25B is an enlarged view of the latch and pin assembly of FIG. 24.

[0085] FIG. 25C is an additional view of a latch and pin assembly with a pin positioned within a narrow portion of a latch release arm. A latch from the latch and pin assembly is shown dropped into a notch of an integrated diagnostic cartridge to prevent the integrated diagnostic cartridge from being ejected.

[0086] FIG. 25D is an illustration of a latch and pin assembly after an integrated diagnostic cartridge is latched to prevent the integrated diagnostic cartridge from ejection. The integrated diagnostic cartridge is seen in an unclamped position and is viewed from a front of a diagnostic instrument shown in FIG. 4A.

[0087] FIG. 26A is an illustration of a latch and pin assembly after an integrated diagnostic cartridge is latched and clamped. The integrated diagnostic cartridge is seen in a clamped position and is viewed from a front of a diagnostic instrument shown in FIG. 4A.

[0088] FIG. 26B is a top view of a latch and pin assembly from FIG. 26A. A pin is shown in a wide portion of a latch arm slot when an integrated diagnostic cartridge is in a clamped position.

[0089] FIG. 27 is an illustration of a latch and pin assembly when an integrated diagnostic cartridge is ejected. The end of a latch release arm is shown contacting the end of a pin to lift the latch and is viewed from a front of a diagnostic instrument shown in FIG. 4A.

[0090] FIG. 28 is an illustration of a latch and pin assembly after an integrated diagnostic cartridge is ejected. The end of a latch release arm is shown not in contact with an end of a pin and is viewed from a front of a diagnostic instrument shown in FIG. 4A.

[0091] FIG. 29 is a perspective view of a valve drive assembly engaging with a rotary valve on an integrated diagnostic cartridge. The integrated diagnostic cartridge is shown inserted into a loading assembly and is in a loaded position, as demonstrated by FIGs. 18A and 19A.

[0092] FIG. 30 is an enlarged view of a valve drive assembly from FIG. 29. A valve drive and valve drive pins engage with a rotary valve on an integrated diagnostic cartridge.

[0093] FIG. 31 is an isometric view of a frangible seal block from a moving bracket assembly shown in FIG. 14, 15A, and 15B, in accordance with an embodiment.

[0094] FIG. 32 is an isometric view of a pocket formed within a fixed support bracket of a fixed bracket assembly. The pocket configured to receive portions of a frangible seal block.

[0095] FIG. 33 is a frontal view moving bracket assembly, in accordance with an alternative embodiment. A first frangible seal pin on a frangible seal block is shown to be longer than the remainder of a plurality of frangible seal pins.

[0096] FIG. 34 is an isolated perspective view of a frangible seal block, shown in FIG. 31, engaging with frangible seals on an integrated diagnostic cartridge. The integrated diagnostic cartridge is shown inserted into a loading assembly and is in a loaded position, similarly shown in FIGs. 18A-18B, 19A-19C, and 29.

[0097] FIG. 35 is a perspective view of a diagnostic instrument pneumatic interface engaging with an integrated diagnostic cartridge pneumatic interface. The integrated diagnostic cartridge is shown inserted into a loading assembly and is in a loaded position, similarly shown in FIGs. 18A-18B, 19A-19C, 29, and FIG. 34.

[0098] FIG. 36A is a frontal perspective view of a diagnostic instrument pneumatic interface, according to one embodiment. The pneumatic interface is shown having a flat plunger surface.

[0099] FIG. 36B is a cross-sectional view of FIG. 35. A diagnostic instrument pneumatic interface with a flat plunger surface is engaged with an integrated diagnostic cartridge pneumatic interface cover adaptor. The pneumatic interface is shown with a gimbaling mechanism active.

[0100] FIG. 36C is a cross-sectional view of a diagnostic instrument pneumatic interface of with a flat plunger surface retracted from an integrated diagnostic cartridge pneumatic interface cover adaptor during unclamping. The pneumatic interface is shown with a gimbaling mechanism locked.

[0101] FIG. 37A is a frontal perspective view of a diagnostic instrument pneumatic interface, according to another embodiment. The pneumatic interface is shown having an angled plunger surface.

[0102] FIG. 37B is an additional cross-sectional view of FIG. 35. A diagnostic instrument pneumatic interface with an angled plunger surface is engaged with an integrated diagnostic cartridge pneumatic interface cover adaptor. The pneumatic interface is shown with a gimbaling mechanism active.

[0103] FIG. 37C is a cross-sectional view of a diagnostic instrument pneumatic interface with an angled plunger surface retracted from an integrated diagnostic cartridge pneumatic interface cover adaptor during unclamping. The pneumatic interface is shown with a gimbaling mechanism locked.

[0104] FIG. 38 is a top down view of a thermal clamp assembly in a zero clamping position.

[0105] FIG. 39 is a top down view of a thermal clamp assembly in a first clamping position.

[0106] FIG. 40 is a top down view of a thermal clamp assembly in a second clamping position.

[0107] FIG. 41 is a top down view of a thermal clamp assembly in a fourth clamping position.

[0108] FIG. 42 is a perspective view of a thermal clamp assembly from FIGs. 38-41, engaged with a reaction area of an integrated diagnostic cartridge. An optical block of a reaction imaging assembly is shown enclosing the thermal clamp assembly and reaction area.

[0109] FIG. 43 is an enlarged perspective view of a clamping subsystem from and a reaction imaging assembly of a diagnostic instrument optical subsystem. A thermal clamp assembly from FIGs. 38-42 is shown disposed within the reaction imaging assembly. The clamping subsystem is viewed in a similar perspective in FIGs. 8 and 9.

[0110] FIG. 44 is a broadened perspective view of FIG. 43. A reaction imaging assembly of a diagnostic instrument optical assembly is shown attached to a fixed support bracket of the clamping subsystem. A clamping subsystem clamps an integrated diagnostic cartridge, as shown in FIGs. 8-13, 16A-16E, 26A, and 38-41. Furthermore, A frangible seal block is shown contained within a clamp block of a moving bracket assembly.

[0111] FIG. 45 is an isometric view of a diagnostic instrument optical subsystem comprising a label imaging assembly and a reaction imaging assembly. An integrated diagnostic cartridge is in a loading position, as shown in FIG. 17A and 17B. A reaction area of the integrated diagnostic cartridge is outside of an optical block from a reaction imaging assembly in a loading position.

[0112] FIG. 46 is an additional isometric view of a diagnostic instrument optical subsystem of FIG. 45. An integrated diagnostic cartridge is in a loaded position, as shown by FIGs. 18A-18B, 19A-19C, 29, 34, and 35. A reaction area of the integrated diagnostic cartridge is disposed below an optical block from a reaction imaging assembly in a loaded position.

[0113] FIG. 47A is an exploded view of a magnetic mixing assembly of a clamping subsystem.

[0114] FIG. 47B is a perspective view of a driving magnet system and a driven magnet system of a magnetic mixing assembly.

[0115] FIG. 48 is a perspective view of a diagnostic instrument pneumatic subsystem shown in FIGs. 6 and 7.

[0116] FIG. 49 is an enlarged perspective internal view of FIGs. 6 and 7. An arrangement of a clamping subsystem, an optical subsystem, and a pneumatic subsystem is readily apparent in this view. A valve drive assembly is removed from a moving bracket assembly to show a pneumatic interface, shown in FIGs. 35-37C, is connected to the pneumatic subsystem, shown in FIG. 48, via tubing.

[0117] FIG. 50 is a perspective frontal view of a cartridge heating area of a cartridge heater zone and a reaction well zone of a diagnostic instrument thermal subsystem.

[0118] FIG. 51 is an isometric enlarged view of FIG. 50 showing a reaction well zone comprising grooves to form a machined pocket geometry.

[0119] FIG. 52 is a perspective rear view of a cartridge heater assembly of a diagnostic instrument thermal subsystem.

[0120] FIG. 53 is an exploded view of FIG. 52. A cartridge heater assembly is shown comprising a chemistry heater, an insulator, a plurality of perforations and a plurality of cutouts.

[0121] FIG. 54 is a cross-sectional view of a chemistry heater assembly of a diagnostic instrument thermal subsystem.

[0122] FIG. 55 is an exploded rear view of FIG. 54. A chemistry heater assembly of a diagnostic instrument thermal subsystem is shown comprising a chemistry heater, a chemistry heater plate, a chemistry heater fan, a fan plenum, a flow vane, a flow guide frame, and a heater plenum.

[0123] FIG. 56 is a perspective view of a heat staking assembly of a diagnostic instrument thermal subsystem.

[0124] FIG. 57A is an isometric view of a heat staker bar assembly within a heat staking assembly of FIG. 56.

[0125] FIG. 57B is a cross-sectional view of FIG. 57A showing a heat staker bar assembly within a heat staking assembly.

[0126] FIG. 58 is a rear perspective view of FIG. 49. An integrated diagnostic cartridge is clamped, as shown in FIGs. 8-11, and is in a loaded position, as described regarding FIGs. 18A-B and 19A-C. A cellular antenna is shown mounted to an antenna ground plate, wherein the ground plate is attached to a fixed support bracket of the fixed bracket assembly.

[0127] FIG. 59 is an enlarged view of a cellular antenna and label imaging assembly of FIG. 58. The label imaging assembly of a diagnostic instrument is shown fixed to an antenna ground plate. A patient label area of the cartridge label and a loading module are disposed within the field of view of a label imaging assembly camera.

[0128] FIG. 60 is a perspective view of a label imaging assembly from FIG. 59. The field of view of the label imaging assembly includes a patient label area of a cartridge label and a loading module of an integrated diagnostic cartridge.

[0129] FIG. 61 is a cross-sectional view of a label imaging assembly from FIGs. 59 and 60.

[0130] FIG. 62 is a top down cross-sectional view of a reaction imaging assembly of a diagnostic instrument optical subsystem. Excitation wavelengths are shown contacting an image plane of an integrated diagnostic cartridge reaction area. Emission paths are shown emanating from an image plane of an integrated diagnostic cartridge reaction area to a reaction camera.

[0131] FIG. 63 is a cross-sectional view of an excitation lens cell of a reaction imaging assembly from FIG. 62.

[0132] FIG. 64 is an additional enlarged cross-sectional view of a bottom of an excitation lens cell.

[0133] FIG. 65 is an enlarged top down cross-sectional view FIG. 62. A reaction imaging assembly of a diagnostic instrument optical subsystem is shown with emission wavelengths reflected off a fold mirror, through a dichroic beam splitter and into a reaction camera.

[0134] FIG. 66 is an isometric view of a diagnostic instrument optical subsystem, as shown by FIGs. 45 and 46. A label imaging assembly and a reaction imaging assembly of the optical subsystem are attached to a fixed support bracket. An integrated diagnostic cartridge is inserted

into a loading assembly and is in a loaded position, as described with regard to FIGs. 18A-18B and 19A-19C.

[0135] FIG. 67A is a schematic diagram of an exemplary instrument computer control system

[0136] FIG. 67B is a schematic diagram of the optical cartridge label subsystem of the exemplary computer control system of FIG. 67A.

[0137] FIG. 67C is a schematic diagram of the optical reaction or assay well subsystem of the exemplary computer control system of FIG. 67A.

[0138] FIG. 67D is a schematic diagram of the thermal subsystem of the exemplary computer control system of FIG. 67A.

[0139] FIG. 67E is a schematic diagram of the lysing drive subsystem of the exemplary computer control system of FIG. 67A.

[0140] FIG. 67F is a schematic diagram of the loading cartridge subsystem of the exemplary computer control system of FIG. 67A.

[0141] FIG. 67G is a schematic diagram of the pneumatic subsystem of the exemplary computer control system of FIG. 67A.

[0142] FIG. 67H is a schematic diagram of the valve drive subsystem of the exemplary computer control system of FIG. 67A.

[0143] FIG. 67I is a schematic diagram of the rehydration mixing subsystem of the exemplary computer control system of FIG. 67A.

[0144] FIG. 68 is a schematic layout of an integrated diagnostic cartridge according to an embodiment described herein.

[0145] FIG. 69A is an illustration of an integrated diagnostic cartridge, according to an embodiment described herein, viewed from a feature side.

[0146] FIG. 69B is an illustration of an exemplary cartridge label for supplying a user and a diagnostic instrument with information associated with a given diagnostic test for use with a cartridge of FIG. 69A.

[0147] FIG. 70A is an illustration of an integrated diagnostic cartridge, according to an embodiment described herein, viewed from a fluidics side.

[0148] FIG. 70B is an enlarged view of the waste collection element 1470 of FIG. 70A.

[0149] FIG. 70C is an enlarged view of the upper proximal portion and lower proximal portion of the cartridge of FIG. 70A.

[0150] FIG. 70D is an exemplary chamber with a chamber reference line used to indicate an upper chamber portion and a lower chamber portion.

[0151] FIG. 70E is the exemplary chamber of FIG. 70E with chamber reference line with an inlet a top most position in the upper chamber portion and an outlet in a lower chamber portion in a lower most position.

[0152] FIG. 70F is the exemplary chamber of FIG. 70D with chamber reference line indicating a top zone for locating an inlet in the upper chamber portion and a bottom zone for locating an outlet.

[0153] FIG. 71 is an isometric view of a loading module in accordance with an embodiment shown in FIGs. 69A and 70. A fill chamber, a metering chamber, and an overflow chamber are shown in fluidic communication.

[0154] FIG. 72 is a view of an integrated diagnostic cartridge and a cartridge heating zone provided by a diagnostic instrument thermal subsystem.

[0155] FIG. 73 is a top view of a filter assembly on an integrated diagnostic cartridge, as described herein.

[0156] FIG. 74 is a cross-sectional view of a filter assembly shown in FIG. 73.

[0157] FIG. 75A is a cross-sectional view of an integrated diagnostic cartridge pneumatic interface and a filter assembly illustrated in FIGs. 73 and 74.

[0158] FIG. 75B is an enlarged cross section view of a portion of the filter in FIG. 75A when under pressure.

[0159] FIG. 76A is a cross-sectional perspective view of a rotary valve illustrating an interface between a rotor and a stator, according to an embodiment of the invention.

[0160] FIGs. 76B and 76C are bottom of views of exemplary gaskets for use with a rotor as in FIG. 76A.

[0161] FIG. 77 is a perspective drawing of a rotor comprising a plurality of flow channels. A magnified view of a single solid support chamber within one of the flow channels is shown.

[0162] FIGs. 78 and 79 are perspective cross-sectional views of a rotary valve with a threaded rotor in a shipping configuration.

[0163] FIG. 80 and 81 are perspective cross-sectional views of the rotary valve of FIGs. 78 and 79 with a threaded rotor in an operational configuration with a gasket forming a fluid tight seal with the stator.

[0164] FIG. 82 is a three-dimensional, cross-sectional illustration of a rehydration chamber, in accordance with an embodiment.

[0165] FIG. 83A is a cross-sectional view of an assay chamber taken through an inlet and an outlet.

[0166] FIG. 83B is a cross-sectional view of an assay chamber taken through the midpoint of an assay chamber.

[0167] FIG. 84 is a top down illustration of a reaction area with a plurality of assay chambers of FIGs. 83A and 83B showing a signal indicative of the presence of target nucleic acids from a target pathogen viewed through a transparent plug.

[0168] FIG. 85 is a cross-sectional illustration of a raised platform within each of the loading channels used to form a portion of a heat staked region. FIG. 85 additionally shows an illustration of a reaction area with a main loading channel configured with a u-bend.

[0169] FIG. 86 is a cross-sectional illustration of a raised platform within a main loading channel used to form a portion of a heat staked region.

[0170] FIG. 87 is a cross-sectional illustration of an assay chamber taken through an inlet and a loading channel with a raised platform of FIGs. 85 and 86 within.

[0171] FIG. 88 is an illustration of a waste collection element of an integrated diagnostic cartridge. A channel for filling the waste chamber and a vent channel are shown in proximity to loading channels forming a shared heat staking portion of the integrated diagnostic cartridge.

[0172] FIG. 89 is an exploded view of a cartridge, according to an exemplified embodiment described herein with regard to FIGs. 69A and 70, comprising a loading module, a lysing module, a purification module, and an amplification module.

[0173] FIG. 90 is an illustration of an exemplary cartridge label for supplying a user and a diagnostic instrument with information associated with a given diagnostic test.

[0174] FIG. 91 is an illustration of a cartridge label with one or more perforated areas configured to break when a diagnostic instrument contacts an integrated diagnostic cartridge.

[0175] FIG. 92 is an illustration of an alternate cartridge, according to an embodiment, comprising a loading module, a lysing module, and a purification module.

[0176] FIG. 93 illustrates the state of an integrated diagnostic cartridge after a biological sample is loaded into the sample port assembly, prior to insertion into a diagnostic instrument and/or prior to actuation of any cartridge features by the diagnostic instrument.

[0177] FIG. 94 illustrates the status of the integrated diagnostic cartridge features after cartridge preparation steps are completed and frangible seals are broken. All of the fluids remain in their original positions, as no motive force has yet been applied to the cartridge features.

[0178] FIG. 95 illustrates the status of the integrated diagnostic cartridge features after the lysis steps are performed.

[0179] FIG. 96 illustrates the status of the integrated diagnostic cartridge features after the filtration and binding steps – the lysis chamber is empty, and fluid has passed to a waste collection element.

[0180] FIG. 97 illustrates the status of the integrated diagnostic cartridge features after completion of the wash step.

[0181] FIG. 98 illustrates the status of the integrated diagnostic cartridge features after completion of the air dry step.

[0182] FIG. 99 illustrates the status of the integrated diagnostic cartridge features after the elution and metering step.

[0183] FIG. 100 illustrates the status of the integrated diagnostic cartridge features after loading the assay chambers.

[0184] FIG. 101 illustrates the status of the integrated diagnostic cartridge features after heat staking.

[0185] FIG. 102 illustrates the status of the integrated diagnostic cartridge features after release of pressure and during the assay step.

[0186] FIG. 103-105 depict a table of reference numbers used herein.

[0187] FIG. 106A-106E depict an exemplary sequence of operations executed by a diagnostic instrument to perform a molecular diagnostic test on an integrated diagnostic cartridge, as described in FIGs. 93-102.

[0188] FIG. 107 depicts a workflow diagram of an assay method of testing a sample suspected of containing a target pathogen.

[0189] FIG. 108 depicts a workflow diagram of a minimal assay method of testing a sample suspected of containing a target pathogen.

[0190] FIG. 109 depicts a workflow diagram of a blood assay method of testing a sample suspected of containing a target pathogen.

[0191] FIG. 110 depicts a workflow diagram of a vaginitis assay method of testing a sample suspected of containing a target pathogen.

[0192] FIG. 111 depicts a workflow diagram of a sputum assay method of testing a sample suspected of containing a target pathogen.

[0193] FIG. 112 depicts a workflow diagram of a stool assay method of testing a sample suspected of containing a target pathogen.

[0194] FIG. 113 depicts a workflow diagram of a solid tissue assay method of testing a sample suspected of containing a target pathogen.

VII. DETAILED DESCRIPTION

[0195] Described herein is a diagnostic system for performing rapid molecular diagnostic testing at the point of care. The diagnostic system comprises a diagnostic instrument and an integrated diagnostic cartridge as described in greater detail below. FIGs. 1-5 depict an exemplary workflow of using an integrated diagnostic cartridge in conjunction with a diagnostic instrument to conduct a molecular diagnostic test at the point of care. FIG. 1 illustrates an exemplary instrument configured to be used with this diagnostic system. As seen in FIGs. 2A and 2B, the first step of the workflow is depicted. A user is shown loading an integrated diagnostic cartridge with a sample loader, such as a bulb, syringe or pipette ***1060***. FIG. 2C illustrates the integrated diagnostic after sample loading is completed and the user seals the cartridge by closing a cap.

[0196] FIG. 3 illustrates the step of inserting a diagnostic cartridge ***1000*** into an opening, i.e. front slot ***2072,*** of the front ***2073*** of instrument ***2000***. The instrument includes features to ensure that a cartridge is loaded into the instrument only in the preferred orientation. Further description of the loading sequence is detailed below with reference to FIGs. 17A-23B.

[0197] Once a cartridge is properly loaded and verified by the instrument, the cartridge remains within the instrument slot as shown in FIGs. 4A and 4B. Regarding FIG. 4A, as part of the cartridge verification process, the display ***2820*** provides information regarding the patient information from the cartridge label and the type of test to be performed by the instrument. Additionally, the display

2820 may be configured to provide touch screen/GUI interactions with the instrument computer operating system. While running the diagnostic test, the instrument display may further provide information regarding the remaining time left for the diagnostic test. Once the automated testing sequence is completed, the cartridge is ejected from the instrument as shown in FIG. 5. Additional details and exemplary workflow for the use of embodiments of instruments and cartridges described herein may be appreciated with reference to commonly assigned U.S. Patent Application Serial Number 16/928,994, filed July 14, 2020 entitled, "Point-of-Care Diagnostic Instrument Workflow" (incorporated herein by reference for all purposes in its entirety).

[0198] By way of introduction, the diagnostic system will be described according instrument embodiments and cartridge embodiments presented herein. The diagnostic instrument ***2000*** will be described according to several subsystems and assemblies shown in FIGs. 6-66. The various subsystems and assemblies, as described herein, may operate under the control of a computer system shown in FIGs. 67A-67I. In one aspect, the instrument ***2000*** is configured to accept an integrated diagnostic cartridge of different configurations. The large number of different cartridge configurations are detailed below with regard to FIGs. 68-92. An exemplary method of using one embodiment of an integrated diagnostic cartridge ***1000*** is described in FIGs. 93-102. The exemplary method describes how a cartridge can be used to prepare a biological sample to amplify nucleic acid and detect the presence of a suspected pathogen in a diagnostic test. As a result of the modular and highly configurable design of the cartridge, a wide array of sample types may be analyzed by the instrument as described with regard to FIGs. 107-113.

A. Instrument General Overview

[0199] FIG. 1 is a front isometric view of a diagnostic instrument ***2000*** to be used with the diagnostic system described herein. The various embodiments of the instrument ***2000*** described herein are adapted and configured to accept and process samples using any of a wide array of different testing methodologies and sample types. The instrument ***2000*** includes a clamping subsystem, a pneumatic subsystem, a thermal subsystem and an optical subsystem. The various relationship between the various subsystems may be appreciated with reference to the exploded isometric views of instrument ***2000*** provided in FIGs. 6 and 7. The clamping subsystem is described with reference to FIGs. 8-47B. The pneumatic subsystem is described with regard to FIGs. 48 and 49. The thermal subsystem is described with reference to FIGs. 50-57B. Additionally, the optical subsystem is described with regard to FIGs. 58-66.

[0200] Returning to FIGs. 6 and 7, in these views, the subsystems are shown outside of the instrument enclosure ***2070*** with pneumatic subsystem ***2130*** shown in its position within the instrument enclosure. The major assemblies of the fixed bracket assembly ***2010*** and the moving bracket assembly ***2040*** are shown in these views. In FIG. 6, subsystems and assemblies of the diagnostic instrument are shown in a right side exploded view or from a first surface of a fixed support bracket. A reaction imaging assembly ***2700*** of the optical subsystem is viewed as detached from the fixed bracket assembly ***2010*** and valve drive assembly ***2400*** is similarly

detached from the moving bracket assembly ***2040***. Furthermore, a cellular assembly ***2800***, which provides communication to and from an instrument, and a label imaging assembly ***2770*** are readily apparent in this view. In FIG. 7, subsystems and assemblies of the diagnostic instrument are shown in a left side exploded view or from a second surface of a fixed support bracket. The fixed bracket assembly ***2010*** shows multiple components and assemblies supported from the second surface of the fixed support bracket ***2013***. Additionally, moving bracket assembly ***2040*** is viewed from a first surface of a clamp block ***2042*** and holds the remaining assemblies and components configured to interface with an integrated diagnostic cartridge to perform various processing steps.

[0201] Throughout the disclosure that follows, the term "vertical" position refers to the relationship of a testing cartridge to a vertical plane and a horizontal plane orientation provided by the design characteristics of a specific instrument embodiment. The vertical plane orientation is one allowing for the use of gravity for fluid movement for processing and handling steps performed during system operations. As such, terms of orientation such as higher and lower, upper and lower are understood in the context of gravitational flows of a generally vertical system orientation. In use, an instrument may be placed on a table or shelf that induces a tilt or incline to the instrument during use. Even though the instrument and cartridge are tilted during use this tilting up to and including +/- 30 degrees is considered vertical as used herein. Moreover, tilting may be within the range of +/- 15 degrees and also be considered vertical as used herein. Tilting within the above mentioned ranges would retain sufficient desired vertical orientation so as to maintain desired and expected gravity flow and characteristics.

[0202] The single use biologic test cartridge is received into and maintained within the instrument enclosure in a single orientation. This orientation is readily identified by the orientation of an opening of the instrument enclosure and along with the vertical and horizontal planes of the instrument. The instrument is adapted and configured to operate with cartridges configured to operate in such an orientation. Accordingly, the instrument receives a cartridge via an opening within the instrument enclosure when the cartridge is oriented with proper alignment. In various embodiments, the opening within the enclosure is a hole, gap, space, slot, window, drawer, cabinet or any other aperture for permitting limited access to the interior of the instrument. In one embodiment, the opening within the enclosure is a slot. In a preferred embodiment, the opening within the enclosure is a vertically oriented slot. As such, the meaning of upright is that positioning of the cartridge relative to the components of the instrument while maintaining an orientation of the cartridge so as to operate the cartridge within the designed cartridge orientation principals. In one embodiment, upright refers to an orientation of the cartridge within the instrument to being vertical within the instrument. This is the orientation that is illustrated in the several views of the instrument. In the views of FIGs. 68-72, and 89-92 an arrow ***1900*** indicates the vertical orientation and points towards UP. However, the operation and configurations of the instrument is not so limited. Based on variations in fluid flow characterizations of a specific single use cartridge, the

orientation of the cartridge to the components of the instrument may be modified while still enabling the upright fluid flow principals implemented in a specific cartridge design. As a result, in other configurations, upright may include a slightly inclined orientation where the cartridge may be inclined relative to a vertical plane of the instrument while still providing the needed discrete actions of having an up and a down within the cartridge fluid schemes.

B. Clamping Subsystem

[0203] The clamping subsystem disposed within the instrument orchestrates the various physical interactions between the instrument ***2000*** and cartridge ***1000*** to perform a molecular diagnostic test run on the cartridge. The coordinated operation of the clamping subsystem is under control of the instrument computer controller (see FIGs. 67A-67I). The clamping subsystem is configured to accept and align a cartridge once inserted into the instrument and maintain the cartridge in an operational orientation within the instrument until the completion of a testing protocol. The clamping process is used to sequentially initiate one or more interfaces between the instrument and specific cartridge components. Once diagnostic testing of a cartridge sample is completed, the clamping subsystem unclamps the cartridge and is ejected from the instrument. In one embodiment, the clamping subsystem includes a mechanism to break frangible seals within cartridge ***1000***, thus allowing fluid flow. In another embodiment, a magnetic mixing assembly ***2300*** is coupled to the clamping subsystem to provide mixing capabilities performed by the cartridge. In one implementation, a valve drive assembly ***2400*** actuates a rotary valve ***1400*** on the cartridge to move fluids and includes various sensors to monitor valving positions. In yet another implementation, the clamping subsystem supports an additional magnetic mixing motor to dissolve and rehydrate reagents within a cartridge to perform a diagnostic test.

1. Overview

[0204] As will be described further herein, a combination of instrument assemblies, subsystems, and an appropriate computer control system can be used to automate a plurality of steps in a testing protocol to perform rapid molecular diagnostic testing at the point of care. Upon cartridge insertion, an instrument computer control system may cause the instrument to automatically engage a clamping subsystem to immobilize the cartridge within the instrument enclosure in an operational orientation for conducting the testing sequence. Once engaged by the clamping subsystem, a cartridge is immobilized within the instrument during testing. The design of the clamping subsystem may vary based on the specific cartridge arrangement. Accordingly, instrument ***2000*** and the clamping subsystem may be configured with corresponding instrument-to-cartridge interfaces to accept and clamp an integrated diagnostic cartridge of varying different configurations. It is to be appreciated that the following embodiments and configurations are solely for the purposes of understanding and changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

[0205] In various aspects of the invention, the clamping subsystem is configured to clamp the integrated diagnostic cartridge in an operational orientation for performing the molecular

diagnostic test. In one implementation, the clamping subsystem clamps the cartridge in a vertical orientation. Such orientation may be directed and maintained by a clamping subsystem that comprises a fixed-bracket assembly ***2010*** and moving bracket assembly ***2040*** which provide the foundation from which all other subsystems and assemblies are mounted from. FIGs. 8 and 9 are two frontal views of the clamping subsystem at two angles with cartridge ***1000*** inserted. Additionally, FIGs. 10 and 11 are two rear views of the clamping subsystem at two angles with cartridge ***1000*** inserted. Door support assembly ***2280*** is seen pressing against a sample port assembly ***1100*** on cartridge ***1000.*** Linear actuator ***2014***, with lead screw ***2016***, mates with lead nut ***2044*** of the frangible seal block within the moving bracket assembly ***2040***. In FIG. 9 the valve drive assembly ***2400*** is readily visible. Furthermore, loading assembly ***2230*** is in a loaded cartridge position while thermal clamp assembly ***2680*** presses against the distal end of the cartridge. FIG. 10 and 11 illustrate the clamping subsystem, with cartridge ***1000*** inserted, from two angles of the second surface of the fixed support bracket ***2013***. Linear actuator ***2014***, latch and pin assembly ***2210***, drive motor ***2330*** of the driving magnet system ***2310***, rehydration motor ***2510*** and thermal subsystem are illustrated in these views.

[0206] An exploded view of the clamping subsystem with cartridge ***1000*** is seen from two angles in FIG. 12 and 13. Notch ***2015*** on the bottom of the first surface of fixed support bracket ***2012*** and linear slide ***2043*** on the moving bracket assembly ***2040*** define the direction the clamp block can move, such that the moving block assembly is configured to move toward the first surface of the fixed support bracket ***2012*** in a positive direction and away from the first surface of the fixed bracket support in a negative direction. In one embodiment, the linear actuator ***2014*** uses lead screw ***2016*** coupled to lead nut ***2044*** to move the moving bracket assembly ***2040*** to clamp and unclamp a cartridge. Lead nut ***2044*** is bolted to frangible seal block ***2260*** to drive the moving bracket assembly ***2040*** along linear slide ***2043***. Further detail of the assemblies and operation of the fixed bracket assembly ***2010*** in conjunction with a moving bracket assembly ***2040*** is described in the following sections.

2. Fixed Bracket Assembly

[0207] Fixed bracket assembly ***2010*** is the stationary component of the clamping subsystem and is composed of the loading assembly ***2230***, pin and latch assembly ***2210***, a driving magnet system ***2310***, and rehydration motor ***2510***. Various views of the fixed bracket assembly are provided in FIGs. 9, 10, 11, 12 and 13. In one embodiment, the fixed bracket assembly ***2010*** further supports the thermal subsystem responsible for generating the thermal requirements for executing a molecular diagnostic test and the optical subsystem for imaging separate distinctive areas of a cartridge. The optical subsystem comprises two assemblies: the label imaging assembly and the reaction imaging assembly. The label imaging assembly ***2770*** is attached to the bottom proximal end of the fixed support bracket, while the reaction imaging assembly ***2700*** is fixed to the distal end of the fixed support bracket. A frontal view of the fixed support bracket is viewed from a first surface ***2012*** or cartridge side in FIG. 12. Loading assembly ***2230***, which accepts and detects a

loaded cartridge within the instrument and ejects the cartridge upon completion of a diagnostic test, is attached to the first surface of the fixed support bracket. Notch ***2015*** on the bottom of the first surface of the fixed support bracket provides the area in which linear slide ***2043*** within moving bracket assembly ***2040*** resides. In some embodiments, a sensor ***2019*** is mounted to the fixed bracket assembly ***2010*** to detect when the cartridge is successfully clamped between the fixed bracket assembly ***2010*** and the moving bracket assembly ***2040***. The sensor ***2019*** can be viewed in FIGs. 12, 13, and 15A - 15E.

[0208] A rear view or a view from a second surface ***2013*** of fixed bracket assembly ***2010*** is depicted in FIGs. 10, 11 and 13. The fixed bracket assembly ***2010*** further comprises a linear actuator ***2014*** attached to a second surface of the fixed support bracket ***2013***. The linear actuator ***2014*** uses a lead nut ***2016***, coupled to lead nut ***2044*** on the frangible seal block ***2260***, to pull the moving bracket assembly toward the first surface of the fixed support bracket ***2012*** during clamping and push the frangible seal block ***2260*** and clamp block ***2041*** away from the fixed support bracket during unclamping. Further description of the clamping mechanism between the fixed bracket assembly ***2010*** and moving bracket assembly ***2040*** is discussed in greater detail with regard to the clamp block ***2041*** and frangible seal block ***2260***. The second surface of the fixed support bracket ***2013*** additionally serves as the surface responsible for carrying a driving magnet system ***2310***, rehydration motor ***2510***, and thermal subsystem of the instrument.

3. Moving Bracket Assembly

[0209] A front perspective view of the moving bracket assembly ***2040*** is viewed in FIG. 14. Exploded views of the moving bracket assembly ***2040*** from two different angles is viewed in FIGs. 15A and 15B. The moving bracket assembly is the dynamic component of the clamping subsystem and is configured to move linearly toward the fixed support bracket ***2011*** to clamp and contact the cartridge at numerous locations. The clamp block ***2041*** supports various systems interfacing with the cartridge and is configured to enable each system to perform respective tasks when running a diagnostic test. In one embodiment, assemblies supported by the clamp block ***2041*** include a frangible seal block ***2260***, a door support assembly ***2280***, a valve drive assembly ***2400***, a pneumatic interface ***2100***, a driven magnet system ***2350***, and a thermal clamp assembly ***2680***. As described in greater detail in the following sections, it is advantageous to separate the clamp block ***2041*** and the frangible seal block ***2260*** to separate the clamping action from the frangible seal actuation. In one implementation, the frangible seal block ***2260*** is configured to initially move with the moving bracket assembly ***2040*** and is capable of moving independently of the clamp block ***2041***. Additionally, the thermal clamp assembly ***2680*** is configured to move independently from the clamp block ***2041***. The door support assembly ***2280***, valve drive assembly ***2400***, pneumatic interface ***2100***, and driven magnet system ***2350*** are fixedly mounted to the clamp block ***2041***, such that movement of these assemblies entirely depends on the position of the clamp block ***2041***. The clamp block ***2041*** further comprises a first surface ***2042*** from which all

cartridge interfacing features extend out of. The first surface of the clamp block ***2042*** is seen in FIGs. 14 and 15A.

[0210] The clamp block sits along linear slide ***2043*** which corresponds to notch ***2015*** on the bottom of the first surface of the fixed support bracket ***2012*** to connect the fixed bracket assembly ***2010*** to the moving bracket assembly ***2040***. As described above, the linear actuator ***2014*** is coupled to lead nut ***2044*** on the frangible seal block ***2260***. The linear actuator ***2014*** rotates lead screw ***2016*** in a first direction within lead nut ***2044*** of the frangible seal block ***2260*** to pull the moving bracket assembly toward the first surface of the fixed bracket assembly ***2010*** during clamping. Clamping force applied to the cartridge by the moving bracket assembly is not a result of the clamp block contacting the fixed bracket. In one implementation, extension springs ***2045***, seen in FIGs. 18A and 24, provide the force needed to clamp all assemblies supported by the clamp block to interface with a cartridge. During unclamping the moving bracket assembly is driven away from the fixed support bracket as the lead screw ***2016*** of the linear actuator ***2014*** is rotated in a second direction, opposite to the first rotational direction.

[0211] The largest displacement the clamp block is configured to move, in the positive direction toward the fixed bracket, is constrained by hard stops ***2211*** at the top of the clamp block. This configuration separates the clamping action from the frangible seal action, allowing the clamp block to clamp and interface with the cartridge without actuating frangible seals and allowing fluid flow.

[0212] The moving bracket assembly includes a door support assembly ***2280*** comprising a door support ***2281*** and spring ***2282***. During clamping, spring ***2282*** contact pushes door support ***2281*** against a top of a cap ***1181***on a cartridge. Door support ***2281*** ensures the cap remains closed and sealed during pressurization of cartridge ***1000***.

4. Frangible Seal Block (Clamp Block)

[0213] Frangible seals keep fluids contained within cartridge ***1000*** and fluidic components isolated when the cartridge is not in use, such as during shipping and storage conditions. Accordingly, the diagnostic instrument includes a puncture mechanism for actuating frangible seals and allowing fluids within the cartridge to flow. The frangible seal block ***2260*** operates to break the frangible seals of the cartridge and is disposed within clamp block ***2041*** as a part of the moving bracket assembly ***2040***. The frangible seal block is a separate component from the clamp block ***2041***, wherein the frangible seal block and clamp block are coupled by linear slide ***2264*** to allow the frangible seal block **2260** to move independently from the clamp block ***2041*** during clamping. FIG. 34 illustrates the frangible seal block ***2260*** separated from the remaining moving bracket assembly ***2040.*** This configuration disconnects the clamping action from the actuation of frangible seals to enable a cartridge to be clamped but not fluidically active until commanded. Frangible seal block ***2260*** can be viewed in FIGs. 14, 15A, 15B, 31, 33, 34 and 44. The basic structure of the frangible seal block includes frangible seal pins ***2261*** and hard stop ***2263***. Lead nut ***2044*** is bolted to the front of the frangible seal block and is used to pull the frangible seal block

2260 and moving bracket assembly ***2040*** toward the fixed bracket assembly ***2010*** in the positive direction during clamping and drive the frangible seal block ***2260*** and moving bracket assembly ***2040*** away from the fixed bracket assembly ***2010*** in the negative direction during unclamping. The lead nut ***2044*** is coupled to the lead screw ***2016*** of linear actuator ***2014*** mounted on the second surface of the fixed support bracket ***2013***. The linear actuator ***2014*** rotates lead screw ***2016*** in a first rotational direction to pull the frangible seal block in a positive direction towards the fixed bracket assembly ***2010***. Extension springs ***2045*** housed within the top of the moving bracket assembly ***2040*** provide tension to pull the clamp block ***2041*** against the frangible seal block ***2260*** to move the frangible seal block and clamp block together along linear slide ***2043*** during clamping movement in the positive direction. In one implementation, extension springs ***2045*** are attached to pins ***2018*** which are fixed to portions of the fixed support bracket ***2011*** and clamp block ***2041***.

[0214] The moving bracket assembly ***2040*** is configured such that the frangible seal block ***2260*** and clamp block ***2041*** initially move together due to extension springs ***2045*** until hard stop ***2211*** on clamp block ***2041*** contacts the first surface of the fixed support bracket ***2012***. Hard stop ***2211*** prevents the clamp block ***2041*** from being displaced a further distance in the positive direction toward the fixed bracket assembly ***2010***. However, the separation between the frangible seal block ***2260*** and the clamp block ***2041*** enables the frangible seal block to be further displaced in the positive direction along linear slide ***2264*** toward the fixed support bracket to actuate frangible seals and render the cartridge fluidically active. To actuate frangible seals ***1201-1207*** on the cartridge, linear actuator ***2014*** continues to rotate the lead screw ***2016*** in a first rotational direction after the cartridge is clamped. While the clamp block ***2041***, remains stationary due to the contact between hard stop ***2211*** and the first surface of the fixed support bracket ***2012***, the frangible seal block ***2260*** is pulled along linear slide ***2264,*** seen in FIG. 31. Frangible seal pins ***2261*** on the frangible seal block ***2260*** press against frangible seals ***1201-1207*** and into pocket ***2262***, shown in FIG. 32, formed in the first surface of the fixed support bracket ***2012*** to actuate the seals. Movement of the frangible seal block ***2260*** is configured to move in the positive direction until hard stop ***2263*** contacts upper rail ***2231a*** of the loading assembly ***2230***. Hard stop ***2263*** prevents the frangible seal pins from over puncturing the frangible seals, which may result in the breaking of the one or more backing films on the cartridge and produce a leak. Additionally, hard stop ***2263*** prevents damaging the pins.

[0215] In one implementation, frangible seal pins ***2261*** are cylindrical in shape. Other pin shapes are possible included rounded tips or other shapes suited to produce the desired opening in a frangible seal or to have a complementary shape with a preferred seal rupture pattern or design.

[0216] One aspect of the invention provides the frangible seal pins ***2261*** of substantially equivalent length. When frangible seals are of substantially equal length, frangible seal pins on the frangible seal block ***2260*** will actuate all frangible seals on cartridge ***1000*** with one linear motion in a positive direction. Furthermore, when the frangible seal block ***2260*** is moved in a negative direction, all frangible seal pins are retracted from pocket ***2262*** in the first surface of the

fixed support bracket ***2012*** to release the cartridge during unclamping and ejection. In an alternative embodiment, one or more frangible seal pins ***2261*** may be of varying lengths, such that different frangible seals on the cartridge may be actuated at different times. In this configuration, the frangible seal block may actuate frangible seals in a sequence to convert one or more frangible seals fluidically active while one or more frangible seals remain fluidically inactive. Sequential actuation of one or more frangible seals depends on the position of the frangible seal block ***2260***, such that in a first actuation position frangible seal pins ***2261*** longer in length will actuate frangible seals before frangible seal pins smaller in length. Subsequently, the frangible seal block must be moved in the positive direction to a second actuation position to actuate frangible seals with smaller frangible seal pins to render respective seals fluidically active. As described in the previous sections, the clamp block ***2041*** remains stationary, clamping the cartridge, due to hard stop ***2211*** as frangible seal pins are actuated either all at once or in a sequence. This alternative embodiment is illustrated in FIG. 33 with first frangible seal pin ***2261a*** being shown longer than remaining pins ***2261b-g***.

[0217] When a diagnostic test is complete and a cartridge is ready to be unclamped and ejected, linear actuator ***2014*** rotates the lead screw ***2016*** in a second rotational direction. Rotating the lead screw ***2016*** in a second rotational direction initially pushes the frangible seal block in the negative direction along linear slide ***2264*** away from frangible seals ***1201-1207***. As an integrated part of the moving bracket assembly ***2040***, the frangible seal block continues to move in a negative direction along linear slide ***2264*** until the frangible seal block contacts ledge ***2046*** of clamp block ***2041***. The frangible seal block presses against ledge ***2046*** to subsequently move the entire moving bracket assembly ***2040*** in the negative direction away from the fixed bracket assembly ***2010*** to unclamp the cartridge.

5. Clamping Sequence

[0218] As described herein, the clamping subsystem can clamp a cartridge ***1000*** using a sequence of clamping positions to engage different interfaces of the moving bracket assembly ***2040*** with the cartridge either simultaneously or sequentially. A representative clamping sequence will be described with reference to FIGs. 16A – 16E for clamping an integrated diagnostic cartridge in a preferred vertical orientation. In the preferred orientation, the instrument is configured to maintain the cartridge in the vertical orientation during the duration of the testing sequence to determine the presence of a target pathogen The exemplary clamping sequence begins at FIG. 16A with the moving bracket assembly ***2040*** described above in a zero clamping position. Specifically, hard stop ***2211*** located at the top of the clamp block ***2041*** does not contact the first surface of the fixed support bracket ***2012*** and the moving bracket assembly ***2040*** is spaced apart from the fixed bracket assembly ***2010*** to allow a cartridge ***1000*** to be inserted into the instrument ***2000***. Engagement between each interface, e.g., a frangible seal block ***2260***, a door support assembly ***2280***, a valve drive assembly ***2400***, a pneumatic interface ***2100***, a driven magnet

system ***2350***, and a thermal clamp assembly ***2680,*** on the moving bracket assembly ***2040*** and the cartridge has yet to be established.

[0219] FIG. 16B shows the moving bracket assembly ***2040*** after it is moved in the positive direction from the zero clamping position to a first clamping position when linear actuator ***2014*** rotates lead screw ***2016*** in a first rotational direction. In the first position, the valve drive assembly ***2400,*** mounted within clamp block ***2041***, is engaged with rotary valve ***1400*** on the cartridge. Hard stop ***2211*** has yet to contact the first surface of the fixed bracket ***2012*** and sensor ***2019*** is untriggered. Additionally, the thermal clamp assembly contacts the distal end of the cartridge but is not engaged to seal the reaction area ***1600***. This position enables the instrument to execute multiple rotary valve verification tests on rotary valve ***1400*** of the cartridge, described in the sections below, before executing the remainder of the clamping sequence. Rotary valve verification tests ensure a cartridge rotary valve ***1400*** is in a shipping configuration to ensure an inserted cartridge is unused and can perform a diagnostic test.

[0220] FIG. 16C shows the moving bracket assembly ***2040*** after it is moved in the positive direction from the first clamping position to a second clamping position when linear actuator ***2014*** rotates lead screw ***2016*** again in a first rotational direction. In the second position, hard stops ***2211*** contact the first surface of the fixed support bracket ***2012*** and sensor ***2019***, which now hidden from view, is triggered. The door support assembly ***2280***, pneumatic interface ***2100***, valve drive assembly ***2400***, and thermal clamp assembly ***2680*** are actively engaged with each respective location on the cartridge. In this view, the cartridge is clamped, but not fluidically active. Furthermore, this position is the greatest distance the clamp block ***2041*** and all assemblies fixedly attached to the clamp block (i.e. door support assembly ***2280***, valve drive assembly ***2400***, pneumatic interface ***2100***, thermal clamp assembly ***2680,*** and driven magnet system ***2350***) are permitted to move in the direction of the fixed support bracket ***2011***.

[0221] FIG. 16D shows the frangible seal block ***2260*** after it moved in the positive direction from the second clamping position to a third clamping position. In the third position, the clamp block ***2041*** remains in the second clamping position and is prevented from moving as hard stops ***2211*** contact the first surface of the fixed support bracket ***2012***. All assemblies fixedly to the clamp block ***2041*** including door support assembly ***2280***, pneumatic interface ***2100***, valve drive assembly ***2400***, and driven magnet system ***2350*** remain in the second clamping position. Note, while the thermal clamp assembly ***2680*** is configured to move independently from the clamp block ***2041***, the thermal clamp assembly also remains in the second clamping position due to being in sealing contact with the distal end of the cartridge. As described herein, frangible seal block ***2260*** is configured to move independently of the clamp block ***2041*** along linear slide ***2264***. The frangible seal block ***2260*** moves to the third clamping position in the positive direction when linear actuator ***2014*** rotates lead screw ***2016*** in a first rotational direction, thus actuating frangible seals on a cartridge. This independent movement is observed by gap ***2265*** between the frangible seal block ***2260*** and clamp block ***2041***. The separation between the clamp block ***2041*** and frangible seal

block ***2260*** isolates the clamping action of the cartridge from the actuation of frangible seals on the cartridge. In the third clamping position, the cartridge is clamped, fluidically active, and ready to run a diagnostic test in the third position.

[0222] FIG. 16E shows the moving bracket assembly ***2040*** when it is moved in the negative direction away from the fixed bracket assembly ***2010*** to a fourth clamping position when linear actuator ***2014*** rotates lead screw ***2016*** in a second rotational direction. In the fourth position, the moving bracket assembly ***2040*** is located at a negative distance measured from the zero clamping position to unclamp a cartridge when the diagnostic test is completed. During unclamping, the frangible seal block ***2260*** is first driven away from the fixed support bracket until the frangible seal block contacts ledge ***2046*** of the clamp block ***2041***, thus eliminating gap ***2265*** seen in FIG. 16D. As the frangible seal block ***2260*** continues to move in a negative direction, the frangible seal block pushes against ledge ***2046*** to drive the entire moving bracket assembly ***2040*** away from the cartridge ***1000*** and fixed bracket assembly ***2010***.

6. Loading Assembly (Fixed Support Bracket)

a) Loading

[0223] In one aspect, the invention provides a loading assembly ***2230*** configured to accept a cartridge inserted into instrument ***2000*** and eject the cartridge upon completion of a diagnostic test. FIGs. 17A-17C, 18A-18B and 19A-19C illustrate various views of the operation of the loading assembly ***2230*** within instrument ***2000***. FIGs. 17A-17B illustrate the loading assembly ***2230*** in a loading position. FIG. 18A-18B illustrate the loading assembly ***2230*** in a loaded position. FIGs. 19A-19C illustrate a cartridge inserted into the loading assembly ***2230*** in a loaded position. The loading assembly comprises rails ***2231***, rack ***2232***, pinion ***2233***, pusher carriage ***2234***, spring ***2235*** and a load position sensor ***2236***.

[0224] A cartridge inserted into the loading assembly ***2230*** is viewed in a loading position in FIG. 17A. The cartridge is inserted along upper and lower rails ***2231*** until the distal end of the cartridge contacts pusher carriage ***2234***. In a loading position, pusher carriage ***2234*** is in a forward most position toward the front slot ***2072*** of the instrument such that load position sensor ***2236*** is not triggered by flag ***2237*** located on the pusher carriage. Further description of the load position sensor ***2236*** and flag ***2237*** is discussed in reference to the cartridge in a loaded position. An enlarged view of the pusher carriage ***2234***, rack ***2232***, and pinion ***2233*** is viewed in FIG. 17B when a cartridge is in a forward most loading position. FIG. 17C shows an enlarged view of spring ***2235*** which is fixed between post ***2239*** and pusher carriage ***2234***, such that when the cartridge and loading assembly is in a forward most loading position, spring ***2235*** is in a resting equilibrium position.

[0225] FIGs. 18A-18B illustrate the loading assembly ***2230*** in a loaded position without a cartridge. In the loaded position, pusher carriage ***2234*** is in a backward most position away from the front slot ***2071*** of the instrument. As viewed in FIGs. 18B and 19B, load position sensor ***2236*** is triggered by flag ***2237*** on the pusher carriage. FIG. 19A and 19C are perspective views of a

cartridge inserted into the loading assembly ***2230*** while in a loaded position. The cartridge transitions from the loading position, viewed in FIG. 17A, to a loaded position when the cartridge continues to move along rails ***2231,*** with the distal end of the cartridge pushing against the pusher carriage. The cartridge is permitted to move along rails ***2231*** until pinion ***2233*** reaches the end of rack ***2232*** and flag ***2237*** triggers load position sensor ***2236***, thus confirming the cartridge is inserted into the instrument. The latch and pin assembly ***2210***, described in the next section, obstructs the cartridge while in a loaded position to prevent the cartridge from being ejected by spring ***2235*** prior to the cartridge being clamped by the moving bracket assembly ***2040***. The cartridge remains in the loaded position for the duration of the diagnostic test until the cartridge is ejected upon completion of the test. A view of the cartridge in a loaded position from the outside of the instrument is seen in FIGs. 4A and 4B.

[0226] In one aspect of the invention, the loading assembly ***2230*** allows an inserted cartridge ***1000*** to ride along two rails ***2231*** until the distal end of the cartridge contacts pusher carriage ***2234***. Interaction between a cartridge and the upper and lower rails is shown in the various views of FIGs. 20-23B. Proper cartridge insertion orientation is ensured through the use of complementary features on both the cartridge and the rails. FIG. 20 and 21 illustrate an upper rail ***2231a*** and lower rail ***2231b***, of the loading assembly ***2230***, both comprising guide features ***2240***. A properly aligned cartridge is configured to align with guide features ***2240*** to maintain proper vertical orientation as described herein. In one embodiment, the width of the rail gap corresponds to the width or edge thickness of the fluidic card. It is to be appreciated that features used to ensure proper cartridge orientation may be used to interfere with one or both of the fluidic card, the cover or any designed gap or spacing formed or partially formed between the fluidic card and cover. In some embodiments, interference features may be included in one or both of a cartridge component or an upper rail or a lower rail to ensure proper cartridge insertion orientation.

[0227] A cartridge inserted with proper alignment is shown in a top down view in FIGs. 22A-B and shown in a bottom up view in 23A-B. FIG. 22A illustrates the distal end of the cartridge prior to being inserted into the loading assembly ***2230*** and prior to interacting with upper guide feature ***2240***. FIG. 22B shows a cartridge during loading with upper guide feature ***2240*** in alignment with the cartridge gap or spacing formed or partially formed between the fluidic card and cover. The gap or spacing formed between the fluidic card and cover is configured to interface with the upper guide feature ***2240*** to direct the cartridge along the upper rail ***2231a***. Additionally notch ***1021***, used to obstruct the cartridge from being ejected, is further viewed in FIGs. 22A and 22B. In one implementation, an interference feature ***1022*** is formed within the cartridge cover, as shown in FIGs. 23A and 23B. FIG. 23A illustrates the distal end of the cartridge, in a bottom up view, prior to being inserted into the loading assembly ***2230*** and prior to lower guide feature ***2240*** interacting with interference feature ***1022***. FIG. 23B shows a cartridge during loading with a lower guide feature ***2240*** in alignment with interference feature ***1022***. Alignment between the lower guide

2240 and interference feature ***1022*** prevents a user from inserting the cartridge with an incorrect orientation.

b) Ejection

[0228] When the diagnostic test is complete, the cartridge is unclamped by the moving bracket assembly ***2240*** and unlatched by the latch and pin assembly ***2210***. The loading assembly ***2230*** uses spring ***2235***, as shown in FIG. 17C, along the bottom rail ***2231*** to provide the force to eject the cartridge upon completion of the diagnostic test. Spring ***2235*** is fixed between post ***2239*** and pusher carriage ***2234*** such that when the cartridge is in a backward most loaded position (i.e. the load position sensor is triggered), spring ***2235*** is stretched out of equilibrium. During ejection, spring ***2235*** returns to its resting equilibrium position and pulls the pusher carriage and cartridge back to a forward most position toward the front slot ***2072***. The cartridge is returned to the loading position, as viewed in FIG. 17A, to eject the cartridge. An ejected cartridge is viewed from the outside of the instrument in FIG. 5.

7. Latch and Pin Assembly (Fixed Support Bracket)

[0229] In one embodiment, the instrument of the present invention comprises a latch and pin assembly ***2210*** to prevent a cartridge from being ejected by spring ***2235***. The latch and pin assembly ***2210*** keeps the cartridge stationary in the loaded position while the moving bracket assembly ***2240*** moves in a positive direction toward the first surface of the fixed support bracket ***2011*** to clamp the cartridge. Specifically, the latch and pin assembly ***2210*** is fixed to the second surface of the fixed support bracket ***2013*** and comprises latch ***2212***, spring ***2213***, latch arm ***2214***, arm slot ***2215***, and pin ***2216***. The latch and pin assembly ***2210*** illustrated in FIG. 24 is discussed in greater detail with regard to FIGS. 25A-28.

[0230] FIG. 25A is a perspective frontal view of the latch and pin assembly ***2210*** with cartridge ***1000*** fully inserted. In some embodiments, a latch release arm ***2214*** is attached to the moving bracket assembly ***2010*** and extends to the second surface of the fixed support bracket ***2013*** to interact with pin ***2216***, described in further detail with regards to FIG. 27. Latch ***2212*** is seen within notch ***1021*** at the top of the cartridge to prevent the cartridge from being ejected by the loading assembly ***2230***. It is to be appreciated features used to obstruct the cartridge from being ejected may be formed or partially formed in the fluidic card, such as the one depicted in FIG. 25A, and optionally extent through to the cover. FIG. 25B and 25C are additionally views illustrating the pin and latch assembly ***2210*** at two angles with spring ***2213*** configured to provide a downward force to drop the latch ***2212*** into notch ***1021*** when the cartridge is inserted into the loading assembly ***2230*** of the instrument. In FIGs. 25A-25C, pin ***2216*** resides within a narrow portion of a slot ***2215*** formed within the latch release arm ***2214***.

[0231] As described herein, the cartridge travels along upper and lower rails ***2231*** of the loading assembly ***2230*** when a user inserts a cartridge with proper alignment and orientation into the instrument. When implemented, the rounded distal end of the cartridge lifts latch ***2212*** up and spring ***2213*** drops latch ***2212*** into notch ***2210***. When the latch is trapped within the notch, the

cartridge is obstructed and remains in a loaded position (i.e. with load position sensor ***2236*** triggered). FIG. 25D shows the latch and pin assembly ***2210*** in a side view with cartridge ***1000*** in a loaded position and latched by the latch and pin assembly. However, the cartridge remains unclamped. Hard stops ***2211*** of the moving bracket assembly ***2040*** do not contact the first surface of the fixed support bracket ***2012*** and hard stop ***2263*** of the frangible seal block ***2260*** has yet to contact upper rail ***2231*** of the loading assembly ***2230***. Pin ***2216*** is constrained between the narrow portion of slot ***2215*** formed within the latch release arm and latch release arm ***2214*** does not contact the bottom of pin ***2216***.

[0232] FIG. 26A illustrates when a cartridge is in a loaded and latched position. Additionally, the cartridge is clamped and rendered fluidically active as denoted by hard stops ***2211*** contacting the first surface of the fixed support bracket ***2012*** and hard stop ***2263*** contacting upper rail ***2231***. As shown in FIG. 26B, pin ***2216*** resides in a widened section of slot ***2215*** formed within the latch release arm ***2214*** when a cartridge is clamped by the moving bracket assembly ***2040***. In some embodiments, latch and pin assembly ***2210*** uses latch release arm slot ***2215*** to constrain the movement of pin ***2216*** within the slot opening. The position of the pin, in relation to slot ***2215***, is free to gimbal during the clamping of the cartridge due to the widening of the slot opening at the vertical bend of the latch release arm. This feature addresses mild variation tolerances generated from the interaction between cartridge and the various interface features of the moving bracket assembly ***2240*** when the cartridge is clamped and ensures latch ***2212*** catches notch ***1021*** to prevent the cartridge from being ejected.

[0233] When the cartridge is ready to be ejected the moving bracket assembly ***2240*** travels in a negative direction away from the first surface of the fixed support bracket ***2012***, thus causing the latch release arm, fixedly attached to the moving bracket assembly, to simultaneously move in the negative direction. The unclamping motion of the moving bracket assembly causes tab ***2217*** on the latch release arm to contact the bottom of pin ***2216*** and urge latch ***2212*** upward, shown in FIG. 27. In this configuration, the cartridge is no longer obstructed by the latch ***2212*** and the cartridge ***1000*** is free to be ejected by the loading assembly ***2230***. FIG. 28 illustrates the latch and pin assembly ***2210*** when the used cartridge is removed from the instrument. Latch ***2212*** is returned to its resting position and the moving bracket assembly ***2040*** is separated from the first surface of the fixed support bracket ***2012***.

8. Valve Drive Assembly (Clamp Block)

[0234] As described herein, the moving bracket assembly ***2040*** comprises a valve drive assembly ***2400*** to facilitate the delivery and redirection of a sample and any of the necessary reagents through rotary valve ***1400*** on a cartridge ***1000***. FIGs. 29 and 30provide an enlarged view and a perspective view of the details and operation of a valve drive assembly. The valve drive assembly is configured to index the rotary valve ***1400*** to different valving positions in a sequence of steps for performing a diagnostic test. The valve drive assembly includes a valve drive ***2401***, a valve drive shaft ***2408***, a motor ***2403***, a pulley ***2406***, and various sensors to detect the valve drive

position. As seen in FIG. 29, valve drive ***2401*** is connected to the valve drive shaft ***2408*** wherein the end of the valve drive shaft ***2408*** is coupled to pulley ***2406***. Motor ***2430*** supplies the motive force to rotate the valve drive to index the rotary valve to different valving positions. The motor is mechanically coupled to the valve drive using valve drive shaft ***2408*** and pulley ***2406***. Specifically, as the motor rotates, belt ***2407*** translates rotational motion to the pulley thereby causing the valve drive shaft ***2408*** to rotate the valve drive. In some embodiments, the valve drive assembly may incorporate the use of various sensors to perform multiple verification checks on the rotary valve to ensure an inserted cartridge is suitable to run a diagnostic test (i.e. the cartridge is unused and untampered). In one implementation, the valve drive assembly ***2400*** uses an interference sensor ***2404*** to track the linear displacement of the valve drive assembly. In a further implementation, valve drive assembly ***2400*** includes a homing sensor ***2409*** to monitor the rotational position of the valve drive ***2401***.

[0235] Valve drive ***2401*** defines the operational coupling between the valve drive assembly and rotary valve ***1400*** on a cartridge. In some implementations, the valve drive may further include a plurality, e.g., two, three, four, or more, valve drive pins ***4202***, shown in FIG.30, which extend from an outermost peripheral wall or edge of a rotary valve. Valve drive pins ***2402*** are associated with engagement openings on the rotary valve to interface between the valve drive assembly and rotary valve ***1400*** when indexing. In various embodiments, the configuration is reversed and the valve drive may include a series of receptacles for receiving projections. In some versions, a rotor portion forms a gear that interlocks with a propulsion element, or a portion thereof and the gear interaction drives the indexing of the rotor. Typically, valve drive pins are arranged concentrically about the rotational axis of the rotary valve. In one embodiment, the valve drive pins may be of cylindrical shape. In a further embodiment, the valve drive pins include a chamfered edge to guide the valve drive pins into engagement openings when the valve drive engages with the rotary valve.

[0236] The valve drive assembly ***2400*** is configured to move linearly in the positive and negative directions, depending on the moving bracket assembly position during clamping and unclamping. In this manner, the valve drive ***2401***, valve drive shaft ***2408***, pulley ***2406***, and belt ***2407*** are capable of both linear and rotational motion. Interference sensor ***2404*** tracks the linear position of the valve drive with respect to the cartridge while homing sensor ***2405*** monitors the rotational position of the valve drive shaft. Both sensors are used to ascertain information about rotary valve ***1400*** and enable the instrument to perform a series of verification checks to ensure the rotary valve is satisfactory for running the diagnostic test.

[0237] Cartridge ***1000*** is configured for long-term storage and includes a rotary valve ***1400*** configured for a shipping configuration and an operational configuration when actuated on command. Accordingly, the valve drive assembly is configured to perform a series of verification tests on rotary valve ***1400*** to verify cartridge ***1000*** can support a diagnostic test and subsequently actuate the rotary valve into an operational configuration to deliver and direct fluids. In one

implementation, the shipping configuration of a rotary valve is determined using interference sensor ***2404***. When the moving bracket assembly is moved to the first position, the valve drive assembly is the first interface to contact the cartridge. In this position, valve drive pins ***2404*** are inserted into engagement openings of the rotary valve. The engagement between the valve drive and the rotary valve causes the valve drive shaft to be located at a linear distance away from the cartridge. The interference sensor uses the end of the valve drive shaft to determine the status of the rotary valve. For example, when the valve drive ***2401*** correctly engages with the rotary valve ***1400*** the interference sensor ***2404*** is triggered by the end of the valve drive shaft ***2405***, thus confirming the rotary valve is in a shipping configuration. Alternatively, the rotary valve ***1400*** may be defected and not be in a shipping configuration. In such case, the valve drive must move a larger distance in the positive direction to mate valve drive pins ***2404***, seen in FIG. 30, with the rotary valve. This results in the end of the valve drive shaft being located at a different linear distance from the cartridge. The interference sensor is not triggered by the interference sensor and notifies the instrument that the rotary valve in not in a shipping configuration and is unfit to run a diagnostic test. Upon successful confirmation of rotary valve ***1400*** in a shipping configuration, the valve drive assembly rotates to transition the rotary valve from a shipping configuration and into an operational configuration, as described herein with in greater detail with regard to the cartridge.

[0238] In a further embodiment, the valve drive assembly ***2400*** is configured to conduct a second rotary valve verification check prior to the moving bracket assembly ***2010*** moving in the positive direction to a second clamping position. The second rotary valve verification check confirms a valve drop into an operational configuration is successful. In a similar manner as the first rotary valve verification check, the valve drive assembly uses the interference sensor ***2404*** and end of the valve drive shaft ***2405*** to verify the operational configuration. In one implementation, the valve drive shaft will not trigger the interference sensor, indicating a successful rotary valve drop and proceed onto commanding the moving bracket assembly to a second clamping position. When the interference sensor is triggered, the valve drive assembly ***2400*** detects a failed valve drop and ejects the cartridge due to an unusable rotary valve. After a successful valve drop into operational configuration, the instrument proceeds to clamp the cartridge to a second clamping position. When all subsequent verification checks are performed and the cartridge is rendered fluidically active, the valve drive assembly may begin the valving sequence to direct the sample and reagents throughout the cartridge to different processing modules. In one embodiment, the valve drive assembly ***2400*** uses a sensor (i.e. homing sensor ***2405***) to monitor the valve drive rotational position during rotation.

9. Pneumatic Interface (Clamp Block)

a) General Description

[0239] In one embodiment of the present invention, fluids (i.e., a sample, reagents, air) are advanced through the cartridge using a pneumatic source. A pneumatic interface ***2100*** is included

within the moving bracket assembly ***2040*** and is appreciated with respect to the various views in FIGs. 14, 15A-B, 33, and 35-37C. The pneumatic interface is configured to provide pressurized air from the pneumatic subsystem ***2130*** to the cartridge to motivate fluids though various locations of a cartridge for different sample processing steps. Shown in FIGs. 14, 15A, and 15B, the pneumatic interface is fixed to the clamp block ***2041*** such that movement of the pneumatic interface is dictated by the movement of the moving bracket assembly ***2040***. The pneumatic interface ***2100*** engages with the cartridge to form a pneumatic seal when the moving bracket assembly is moved in the positive direction to the second clamping position. Plunger ***2104*** breaks the pneumatic interface perforations on the cartridge label and the plunger surface grips the pneumatic interface cover adaptor. Spring *2102* is urges plunger *2104* into the pneumatic interface cover adaptor by pushing against shim *2105* and housing *2106*. FIG. 35 illustrates the pneumatic interface ***2100*** engaged with the cartridge pneumatic interface.

[0240] Referring to FIGs. 36A and 37A, the basic design employs a spring ***2102*** loaded plunger ***2101***, with a plunger surface ***2104***, a shim ***2105***, and housing ***2106***. In one implementation, the housing ***2106*** is fixed to the clamp block and includes plunger ***2101*** further configured to be moveable within the inner surface of the housing ***2108***. In one embodiment, housing ***2108*** has a central opening wherein the central opening has a smaller portion of the central opening and a larger portion of the central opening. Plunger ***2101*** has a long cylindrical shape and includes a proximal end with a plunger surface ***2104***, a central portion housed within the smaller portion of the central opening, and a distal end housed within the larger portion of the central opening. Additionally, the plunger further comprises an outer plunger surface ***2107***. The body of the plunger can be made from any material with appropriate rigidity such as plastics or metals, but is preferentially made from steel. The central portion of the plunger is substantially equivalent in diameter to the proximal end of the plunger, such that the central portion and the proximal end of the plunger are both smaller in diameter than the diameter of the distal end of the plunger. A step-up feature ***2109*** links the central portion to the distal end of the plunger. The central portion of the plunger is disposed in the smaller portion of the housing central opening. Furthermore, the distal end of the plunger disposed within the larger portion of the housing central opening forms a gap between the outer surface of the plunger ***2107*** and inner surface of the housing ***2108***. The space formed between the outer surface of the plunger and inner surface of the housing constrains the movement of the plunger for properly engaging the pneumatic interface ***2100*** with the pneumatic interface adaptor ***1172*** on a cartridge. The proximal end of the steel plunger comprises a plunger surface ***2104*** that is responsible for gripping the pneumatic interface adaptor to form a pneumatic seal. In one embodiment the shape of the plunger surface ***2104*** is designed with an angled surface, shown in FIG. 37A, to minimize potential pneumatic leaks. In an alternative embodiment shown in FIG. 36A, the plunger surface flat. Engagement with a pneumatic cover interface ***1172*** on the cartridge is shown in FIGs. 36B, 36C, 37B and 37C and further discussed in greater detail with regard to a gimbaling mechanism described below.

[0241] In one embodiment, the pneumatic interface includes a gimbaling mechanism to account for any potential parallelism issues arising during engagement between the moving bracket assembly ***2040*** and cartridge ***1000***. FIG. 36B and 36C depict the gimbaling mechanism of the pneumatic interface with a flat plunger surface seen in FIG. 36A. FIG. 36B illustrates a pneumatic interface with the gimbaling mechanism active when the pneumatic interface engages with the pneumatic interface cover adaptor ***1172***. Housing ***2106*** is fixed to the clamp block ***2041***, such that when plunger ***2101*** contacts the pneumatic interface cover adaptor, the housing ***2106*** remains stationary while plunger ***2101*** is pushed back into the housing central opening to cause spring ***2102*** to compress between shim ***2105*** and housing ***2106***. The position of the plunger within the housing central opening creates a gap between the plunger step-up feature ***2109*** and the inner surface of the housing ***2108***. In this configuration, the plunger is permitted to pivot within the housing central opening to ensure a secure pneumatic seal is established when the plunger surface ***2104*** contacts the pneumatic interface cover adaptor ***1172***. The degree of pivoting is constrained by the inner surface of housing ***2106***, where the central portion of the plunger, step up feature, and distal end of the plunger can pivot until any one part of the plunger contacts the inner surface of the housing.

[0242] FIG. 36C shows the pneumatic interface with a flat plunger surface ***2104*** and the gimbaling mechanism locked when the moving bracket assembly ***2040*** moves to unclamp a cartridge. As the moving bracket assembly ***2040*** moves in the negative direction away from the pneumatic interface cover adaptor ***1172***, housing ***2106*** retracts the plunger ***2101*** from the pneumatic interface cover adaptor ***1172***. The larger portion of the central opening contacts the corner of the distal end of the plunger, adjacent to step-up feature ***2109***, to pull the plunger back as the moving bracket assembly ***2040*** is moved in the negative direction. The contact between the inner surface of the housing ***2108*** and the corner of the distal end of the plunger eliminates the gap seen in FIG. 36B when the gimbaling mechanism is active. In this configuration, the plunger is prevented from pivoting while the larger portion of the central opening remains in contact with the corner of the distal end of the plunger. FIG. 37B illustrates the pneumatic interface gimbaling mechanism active when the pneumatic interface ***2100*** contacts the pneumatic interface cover adaptor ***1172*** with an angled plunger surface ***2104***. FIG. 37C illustrates the pneumatic interface with an angled surface when the gimbaling mechanism is locked.

10. Thermal Clamp Assembly (Clamp Block)

[0243] The thermal clamp assembly ***2680*** is a component of the moving bracket assembly ***2040*** and is connected to the clamp block ***2041*** (see the various views of FIGs. 38-43). In some embodiments, the thermal clamp assembly is configured to move independently of the clamp block ***2041*** and is not fixedly attached to clamp block ***2040***, such that the position of the thermal clamp assembly ***2680*** does not solely depend on the position of clamp block ***2041***. The thermal clamp assembly ***2680*** comprises a clamp plate ***2681***, a light frame ***2686***, and a plurality of clamp posts ***2682***, wherein each clamp post ***2682*** further comprises a shoulder screw ***2684***, spring ***2683***,

and bushing ***2685***. The thermal clamp assembly ***2680*** is configured to presses against a cartridge ***1000*** to ensure the cartridge remains flat against the fixed support bracket ***2011*** during the heat staking process, as described herein, and additionally produces a light seal around the reaction area ***1600*** of the cartridge during imaging and detection by the reaction imaging assembly ***2700***. In implementations where the thermal clamp assembly ***2680*** is configured to move independently of the clamp block, the thermal clamp assembly is connected to the clamp block using a bushing ***2685*** for each of the plurality of clamp posts ***2682***, wherein the each bushing is operably coupled to a shoulder screw ***2684*** thus permitting independent movement of the thermal clamp assembly ***2680*** along shoulder screws ***2684***. In one embodiment, each of the plurality of clamp posts, ***2682*** comprises one or more springs ***2683*** along shoulder screws ***2684*** for constraining the maximum movement of the clamp block ***2041***, with respect to the thermal clamp assembly ***2680***, in the positive and negative direction during clamping and unclamping. Such configuration allows clamp block ***2041*** to move in the positive direction toward the fixed bracket assembly until contacted by spring ***2683a*** and allows clamp block ***2041*** to move in the negative direction until contacted by spring ***2683b***. In one implementation, a clamp plate ***2681*** is fixed to the plurality of clamp posts ***2682***. Furthermore, as shown by FIG. 38, a light frame ***2686*** is housed within clamp plate ***2681***, wherein the light frame ***2686*** is configured to contact the distal end of the cartridge during the clamping sequence, as described herein below. The light frame ***2686*** is shaped to correspond to a perimeter about the assay chambers within a specific reaction module configuration of a diagnostic cartridge embodiment.

[0244] The thermal clamp assembly ***2680*** is arranged between the optical block ***2710*** and cartridge as seen in FIG. 42, wherein the optical block is shown with dashed lines. As illustrated in FIGs. 38-41, the clamp plate ***2681*** resides in the space between the moving block assembly ***2040*** and a cartridge in a loaded position defined by loading assembly ***2230***. In one implementation illustrated in these views, the light frame ***2686*** is disposed within a pocket ***2710*** formed within optical block ***2710***. Additional views of the optical block is further shown in FIGs.45, 46, 62 and 66.The position of the light frame within the optical block enables the thermal clamp assembly ***2680*** to form a light seal around reaction area ***1600*** of a cartridge, viewed in FIG. 45. The light seal around the reaction area, as provided by the light frame ***2686***, helps ensure the darkest possible background is achieved for a reaction camera ***2701*** of a reaction imaging assembly ***2700*** to capture fluorescent images of assay chambers within the reaction area. When a cartridge is in a loaded position, the movement of clamp plate ***2681*** is constrained between the distal end of the cartridge and optical block ***2710***, such that the thermal clamp is permitted from moving in the negative direction until clamp block ***2041*** contacts spring ***2685b*** and light frame ***2686*** contacts the edge of pocket ***2710*** within the optical block. By way of example, further description of the movement of the thermal clamp assembly ***2680*** is depicted in a top down view of the thermal clamp assembly ***2680*** and optical block ***2710*** according to the clamping sequence in FIGs. 38-41.

[0245] FIG. 38 illustrates the thermal clamp assembly ***2680*** when the moving bracket assembly ***2040*** is in the zero clamping position. Cartridge ***1000*** is in a loaded position given by loading assembly ***2230*** and latched by the latch and pin assembly ***2210***. Note light frame ***2686*** is not in contact with cartridge ***1000***.

[0246] The moving bracket assembly ***2040*** is moved in the positive direction from the zero clamping position to a first clamping position when linear actuator ***2014*** rotates lead screw ***2016*** in a first rotational direction. The clamp block ***2041*** slides along shoulder screws ***2684*** and causes light frame ***2686*** to contact the cartridge. However, a seal between the light frame ***2686*** and the reaction area ***1600*** is not established in the first position. As described herein, the first clamping position only establishes an operational coupling between the valve drive assembly ***2400*** and cartridge rotary valve ***1400***. FIG. 39 illustrates the thermal clamp assembly ***2680*** after the moving bracket assembly ***2040*** is in the first clamping position with light frame ***2686*** lightly contacting the distal end of the cartridge. In the first clamping position, the movement of the moving bracket assembly ***2040*** and thermal clamp assembly ***2680*** causes light frame ***2686*** to move in the positive direction toward the cartridge and away from pocket ***2711*** of optical block ***2710***.

[0247] After rotary valve verification checks are performed on the rotary valve in the first clamping position, the moving bracket assembly ***2040*** is moved in the positive direction to a second clamping position when linear actuator ***2014*** rotates lead screw ***2016*** in a first rotational direction. Clamp block ***2041*** slides along shoulder screws ***2684*** until the clamp block ***2041*** compresses spring ***2683a*** to exert a force against the clamp plate ***2681***. Accordingly, the clamp plate ***2681*** urges the light frame ***2686*** into the cartridge and establishes a light seal around the cartridge imaging area ***1600***. FIG. 40 illustrates the thermal clamp assembly ***2680*** after the moving bracket assembly ***2040*** is in the second clamping position to clamp the cartridge. In the second position, the thermal clamp assembly is prevented from moving any further in the positive direction, such that when the frangible seal block ***2260*** is moved to the third clamping position to actuate frangible seals the position of the thermal clamp assembly ***2680*** remains unchanged due to light frame ***2686*** contacting the cartridge and clamp block ***2041*** contacting spring ***2683a***. The thermal clamp assembly ***2680*** will remain in the second clamping position until a diagnostic test run is completed on the cartridge.

[0248] The moving bracket assembly ***2040*** is moved in the negative direction to a fourth clamping position when linear actuator ***2014*** rotates lead screw ***2016*** in a second rotational direction to unclamp the cartridge. Clamp block ***2041*** is driven away from the cartridge by the coupling between the lead screw ***2016*** and frangible seal block ***2260*** contacting ledge ***2046*** of the clamp block, as described herein. This action causes clamp block ***2041*** to slide along shoulder screws ***2684*** until the clamp block ***2041*** contacts spring ***2683b***. Pocket ***2711*** formed within optical block ***2710*** of the reaction imaging assembly ***2700*** allows the light frame ***2686*** of the thermal clamp assembly to retract away from the cartridge to establish a clearance between the clamp plate

2681 and cartridge for ejection. FIG. 41 illustrates the thermal clamp assembly ***2680*** after the moving bracket assembly ***2040*** is in a fourth clamping position.

11. Magnetic Mixing (Fixed Support Bracket & Clamp Block)

a) Magnetic Mixing Assembly

[0249] The clamping subsystem supports two magnetic mixing systems that interface with elements within a cartridge to perform respective functions. The first magnetic mixing system of instrument 2000 is magnetic mixing assembly 2300, illustrated in an exploded view in FIG. 47A and a perspective assembly view in FIG. 47B. The various views illustrate the arrangement, spacing, orientation and operation of the magnetic mixing assembly for use with various vertically oriented diagnostic cartridge and instrument embodiments described herein. Magnetic mixing assembly 2300 provides the means to mix a sample in a vertically oriented lysis chamber using a stir bar alone or in combination with other lysis agents, while minimizing the amount of contact of the stir bar with the walls of said vertical lysis chamber. The driving magnet system 2310 and driven magnet system 2350, as seen in FIGs. 47A, 47B, are arranged to effectuate a magnetic coupling between the one or more driving magnets and the one or more driven magnets. Specifically, each driving magnet and driven magnet are arranged with respect to one another such that an alignment of the driving magnet magnetic axis and an alignment of the driven magnet magnetic axis effectuate a magnetic coupling between the driving magnetic and the driven magnet. Still further, the arrangement and operation of the magnetic mixing assembly is adapted for rotation of a stir bar within the magnetic field produced between the driving and driven magnets. In certain embodiments, to effectuate magnetic coupling between a driving magnet and driven magnet, the driven magnet magnetic axis is parallel to the driving magnet magnetic axis. In further, preferred embodiments, the driven magnet magnetic axis is substantially collinear with the corresponding driving magnet magnetic axis. As used herein, "substantially collinear" encompasses deviations from absolute collinearity of up to 10° and/or 3 mm at a plane bisecting the gap between the driving and driven magnet system.

[0250] The magnetic coupling between the driving magnet system and the driven magnet system comprises an attractive magnetic coupling. In such embodiments, the one or more driving magnets and the one or more driven magnets are arranged with respect to one another such that the alignment of the each driving magnet magnetic axis and the alignment of the each driven magnet magnetic axis effectuate an attractive magnetic coupling between the one or more driving magnets and the one or more driven magnets. In general, to effectuate an attractive magnetic coupling between a driving magnet and a driven magnet, the driving magnet magnetic axis and the driven magnet magnetic axis are aligned such that opposing poles of the driving magnet magnetic axis and the driven magnet magnetic axis are located in proximity to one another.

[0251] In certain embodiments, a strength of the magnetic coupling between the driving magnet system and driven magnet system is based on a distance of the gap located between the one or more driving magnets and the corresponding one or more driven magnets. Additionally, the

magnetic coupling is based on a magnet strength of the one or more driving magnets, as well as a magnet strength of the one or more driven magnets. In some embodiments, the gap separating the driving magnet system from a driven magnet system is between about 10mm and about 30mm. Furthermore, in a preferred embodiment, the magnetic strength of one or more driving magnets is the same as the strength of the one or more driven magnets.

[0252] FIG. 47A is an illustration of an exploded view of the magnetic mixing assembly 2300 of instrument 2000, in accordance with an embodiment. The exemplary mixing assembly shows a driving magnet system 2310 comprising a first driving magnet 2311 and a second driving magnet 2316 separated by a distance, and a driven magnet system 2350 comprising a first driven magnet 2353 and a second driven magnet 2356 separated by a distance. As shown in both FIGs. 47A, 47B, the drive motor is operably/mechanically coupled to the drive belt 2332, wherein the drive belt is operably/mechanically coupled to the driving magnet spindle 2361, and further wherein the spindle is operably coupled to the driving magnet holder 2325. In some embodiments, the driving magnet holder is configured to house one or more driving magnets. The illustrated embodiment shows the driving magnet holder contains the first driving magnet 2311 and a second driving magnet 2316. In a preferred embodiment, the driving magnet holder 2325 is positioned in proximity and aligned to a first face, e.g. fluidic side 1006, of a cartridge containing a vertically oriented lysis chamber 1371.

[0253] Analogous to the driving magnet system is a driven magnet system 2350. In some embodiments, the driven magnet system comprises at least one driven magnet, a driven magnet holder, and a driven magnet spindle. In some embodiments, the driven magnet holder is configured to house one or more driven magnets. FIG. 47A and 47B show an embodiment where the driven magnet holder 2365 contains the first driven magnet 2351 and the second driven magnet 2356, further wherein the driven magnet holder is operably coupled to a driven magnet spindle 2361. In a preferred embodiment, the driven magnet holder 2365 is similarly positioned in proximity and aligned to a second face, e.g. feature side 1007, of a cartridge containing a vertically oriented lysis chamber 1371. FIGs. 12 and 13 illustrate the complementary arrangement of the driving magnet system 2310 and driven magnet system 2350 with respect to a vertically oriented cartridge containing a vertically oriented lysis chamber position therebetween.

[0254] As further detailed in the sections below, in embodiments where a magnetic stir bar is provided within the lysis chamber, the operation of the magnetic mixing assembly induces a magnetic field to rotate the stir bar substantially within the vertical plane of the diagnostic cartridge, i.e. rotating within a plane collinear with the cartridge width axis 1025, when clamped in an operational orientation within the instrument. Further, in certain implementations, a first driving magnet field focuser 2312 can be coupled to the first driving magnet 2311 and/or a first driven magnet field focuser 2352 can be coupled to the first driven magnet 2351 to concentrate magnetic fields generated toward the center of the vertically oriented lysis chamber.

[0255] In certain embodiments, the magnetic mixing assembly can further comprise an acoustic mechanism for detecting magnetic decoupling of the stir bar 1390 from one or more of the driving magnet system 2310 and the driven magnet system 2350. In such embodiments, the acoustic mechanism is configured to detect a change in one or more of an amplitude and a frequency of vibrations produced by the stir bar during rotation of the driving magnet system, the change indicating the magnetic decoupling of the stir bar. In some embodiments, the change comprises a sudden decrease in one or more of the amplitude and the frequency of the vibrations produced by the stir bar. In some embodiments, the acoustic mechanism comprises a microphone 2380 (see FIG. 11).

b) Rehydration

[0256] The second magnetic mixing system supported by the clamping subsystem is the mechanism for rehydrating dried reagents contained within a cartridge. In one implementation, motor ***2500*** contains a magnet to gyrate a magnetic element contained within a reservoir of a cartridge. The motor is mounted to the fixed support bracket and is best seen in the views of FIGs. 10 and 11. In one embodiment, the cartridge reservoir containing a magnetic element holds dried reagents, such that gyration of the magnetic element facilitates rehydration and mixing of dried reagents with fluids.

C. Pneumatic Subsystem

1. Overview

[0257] In one embodiment, the instrument includes a pneumatic subsystem that is configured to generate pneumatic pressure to advance fluids to various locations within the cartridge that are responsible for sample preparation, nucleic acid amplification, and detection. FIGs. 48 and 49 illustrate a pneumatic subsystem ***2130*** in isolation and in position within the instrument enclosure, respectfully. The pneumatic subsystem comprises at least a pump ***2131***, a pressure regulator ***2132***, a proportional valve ***2133***, an accumulator ***2135***, and a pressure sensor ***2134***. In some implementations, the pneumatic subsystem includes an output selector valve ***2136***. The pneumatic pump compresses air to convey fluids through the cartridge, wherein pump ***2131*** is connected to pressure regulator ***2132*** to down regulate the pressure to a desired value. An accumulator ***2135***, in line with a proportional valve ***2133***, acts as a pressure storage reservoir until pressure is needed on demand.

[0258] In one aspect, the pneumatic subsystem includes environmental sensors and additional hardware contained within the instrument to monitor various instrument measurements including internal temperature, atmospheric pressure, and humidity of the instrument. As described herein, the firmware of the pneumatic subsystem allows the instrument to control the time spent varying increasing or decreasing pressure set points and control steady state pressure with varying flow resistances from the cartridge. In some embodiments, the pneumatic subsystem includes a flow sensor to monitor the flow rates of various fluids within the cartridge for sample preparation and amplification. In a preferred embodiment, the pneumatic subsystem contains no flow sensors to

monitor flow rates of fluids in the cartridge. In a further preferred embodiment, indirect measurements are used to determine when the pneumatic subsystem completes the act of pushing a fluid or substance through the porous solid support chamber prior to moving on to the next processing step. A feedback control system uses a pressure feedback sensor ***2134*** and a proportional valve ***2133*** to push finite amounts of fluid through the porous solid support of the cartridge and indicate when all the fluid has exited the channel. The feedback system, as described herein, replaces a flow sensor by using an actuation signal to indicate when the system is ready for the next fluid sequence.

[0259] In one embodiment, the pneumatic subsystem provides pressurized atmospheric air to the cartridge via the pneumatic interface ***2100*** shown in FIG. 35, 36A-36C and 37A-37C. The pneumatic interface ***2100*** punctures a perforated area ***1052*** on the label of the cartridge to access the cartridge pneumatic interface ***1170*** located on the cartridge. As described herein, spring ***2102*** establishes a connection between the cartridge pneumatic interface and pneumatic interface ***2100*** to deliver the pressurized atmospheric air. In yet another aspect of the invention as described herein, a gimbaling mechanism is used to account for small degrees of misalignment between the cartridge and instrument. FIG. 49 is a perspective view of the clamping subsystem and optical system engaged with a cartridge in a loaded position with the valve drive assembly ***2400*** removed from the moving block assembly ***2040*** to demonstrate the connection between the pneumatic subsystem ***2130*** and the pneumatic interface ***2100***. In this view, the instrument pneumatic interface ***2100*** is shown connected to the pneumatic subsystem via tubing ***2190***. Furthermore, FIG. 49 demonstrates the relationship of the pneumatic subsystem position in reference to the clamping subsystem and the reaction imaging assembly and label imaging assembly of the instrument optical subsystem. In one implementation, the pneumatic subsystem is fixed to the bottom of the instrument, unlike all other subsystems and assemblies which are fixed to either the fixed bracket assembly ***2010*** or the moving bracket assembly ***2040***. As a result, the pneumatic subsystem remains stationary during the clamping and unclamping sequence in a similar manner to the fixed bracket assembly.

[0260] In one implementation, each pneumatic pressure control component or aspects thereof, such as the pump ***2131***, pressure regulator ***2132***, proportional valve ***2133***, accumulator ***2135***, output selector valve ***2136***, and various sensors are mounted in the manifold block ***2137***. In a further implementation, a control board ***2138*** contains the proportional valve ***2133***, pressure sensor ***2134***, and various environmental sensors, wherein the control board ***2138*** is mounted within manifold block ***2137***, as shown in FIG. 48. The manifold block can, in various aspects, be made of one or more rigid materials, such as a polymeric material, like plastic. In some implementations, the manifold block is machined from acrylic. In a further embodiment, the acrylic manifold block is vapor polished. In one aspect, pneumatic routing channels and mounting ports are fabricated in the manifold block for all components of the pneumatic subsystem. Additionally, due to the thermodynamics of the compression of air in the pump, humidity in the air can be

condensed. In one embodiment, the instrument manages condensation control with the regulator's manifold entry geometry. Advantageously, the implemented geometry vents moisture/condensation within the instrument enclosure through the use of one or more bleed orifices ***2191*** so that it does not enter the regulator inlet.

[0261] Given the pressurization of the pneumatic subsystem, in one implementation filters are installed on the pump's intake, inlet, and outlet to eliminate the possibility of external particulates from reaching the manifold or cartridge to control the risk of contamination within the instrument. In an exemplified implementation, the pneumatic subsystem is shown in FIG. 48 comprising a pump filter ***2160*** and outlet filter ***2162***.

[0262] In some embodiments, the pneumatic subsystem optionally comprises several components to minimize noise due to vibration. In one implementation, the assembly uses pump isolation mounts. In another implementation the assembly includes silicone foam damping pads reduce noise of pump components vibrating against manifold. In an alternate implementation, the assembly uses isolation grommets ***2194*** to reduce the vibration of the pneumatic subsystem against the instrument's enclosure. In a preferred embodiment, the pneumatic subsystem uses pump isolation mounts, silicone foam damping pads, and isolation grommets to provide noise damping.

D. Thermal Subsystem

[0263] In one aspect, a cartridge configured to perform sample preparation or both sample preparation and amplification requires the use of one or more heaters supported by the instrument ***2000***. In one implementation, the thermal subsystem is configured to provide a controlled steady state temperature to areas of the cartridge used to conduct sample preparation and enable controlled heating and cooling of assay chambers to permit isothermal amplification and detection of target nucleic acids during a diagnostic test. In implementations where amplification is performed, avoiding cross-contamination between assay chambers as well as isolating the reaction from the outside environment is imperative to prevent amplicon contamination. Containing amplified nucleic acids ensures false positive results are not obtained on all cartridge runs performed on the instrument thereafter. The diagnostic instrument includes one or more mechanisms to provide the desired containment by either temporary or permanent isolation between one or more components, modules or chambers within a cartridge. Temporary isolation refers to a mode of isolation present in the cartridge so long as the cartridge is clamped within the instrument. Once, unclamped and ejected from the instrument, isolation is not maintained. Examples of temporary isolation include the use of pneumatic pressure, or a mechanical system such as one or a number of pinch valves or non-heated stakes to occlude one or more passages or channels of a cartridge. In contrast, permanent isolation refers to a mode of isolation that once formed is present in the cartridge even after the cartridge is ejected from the instrument. Permanent isolation includes any suitable form of modification to produce a suitable fluid tight

constriction or occlusion, region of plastic deformation, or sealing between one or more components, modules or chambers within a cartridge.

[0264] In one specific implementation, the thermal subsystem further includes a heat staker assembly ***2640*** used as an isolation mechanism for permanent isolation by sealing a portion of a cartridge containing amplified nucleic acids. Additionally, the thermal subsystem includes a cartridge heater assembly ***2550*** and a chemistry heater assembly ***2600***for providing the thermal requirements of sample preparation and amplification when performing a diagnostic test. The various views of the components of the thermal subsystem are provided in FIGs. 50-58. The various components of the thermal subsystem operate under the control of the instrument computer control system as described in FIGs. 67A-67I. The thermal subsystem, as described herein, contains various embodiments used to precisely control the temperature of specific areas of the cartridge to prepare a sample and, if desired, amplify and detect target nucleic acids and prevent amplicon from escaping the cartridge.

1. Overview

[0265] A thermal subsystem of the present invention comprises a chemistry heater assembly ***2600***, a cartridge heater assembly ***2550***, and a heat staker assembly ***2640***, wherein the heat staker assembly further comprises a staker bar assembly ***2641***. All assemblies and components of the thermal subsystem are supported by the fixed bracket assembly ***2010***. In one implementation, more than one heater e.g., two or more, heaters are used to provide multiple controlled temperatures to different areas of the cartridge responsible for conducting sample preparation and amplification. In one embodiment, the cartridge heater assembly ***2550*** is configured to maintain an operational temperature within a cartridge heating zone ***2552*** which include portions of the integrated cartridge containing the wash buffer reservoir ***1475***, elution buffer reservoir ***1500***, rehydration chamber ***1520***, and lysis chamber ***1371***. In another embodiment, the chemistry heater ***2601*** is configured to maintain a reaction temperature to the reaction area ***1600*** of a cartridge to enable the amplification of target nucleic acids within assay chambers. In yet another embodiment, a third heater is used to seal a cartridge according to an embodiment described herein.

2. Chemistry Heater Assembly

[0266] In one embodiment, a chemistry heater assembly ***2600*** is configured to provide a reaction temperature for amplifying nucleic acids contained within a plurality of assay chambers in a cartridge. A cross sectional view of the chemistry heater assembly ***2600*** is illustrated in FIG. 54 and an exploded view of the chemistry heater assembly ***2600*** is seen in FIG. 55. In one embodiment, the chemistry heater assembly ***2600*** comprises a chemistry heater ***2601***, a flow guide frame ***2606***, a chemistry heater plate ***2602***, a chemistry heater fan ***2603***, a heater plenum ***2607,*** and a fan plenum ***2604*** with a flow vane ***2605***. The chemistry heater ***2601*** can be of any suitable design but is most preferably a resistance heater (e.g. a Kapton heater). In certain

aspects of the invention, the chemistry heater assembly further consists a thermistor integrated with the chemistry heater ***2601***.

[0267] In one embodiment, shown in FIGs. 54 and 55, the chemistry heater ***2601*** is in thermal contact and bonded to a second surface ***2622*** of chemistry heater plate ***2602*** using a pressure sensitive adhesive or other adhesive appropriate to the operating temperature range. When assembled, there is a reaction well zone ***2620*** formed in a first surface of the chemistry heater plate ***2621***, wherein the first surface of the chemistry heater plate is in thermal contact with the film side of a cartridge. The chemistry heater zone ***2620*** is viewed from the first surface of the chemistry heater plate ***2621*** in FIGs. 50 and 51. The cartridge heater plate ***2602*** is susceptible to thermal effects from the ambient environment. Thus, in one embodiment, the chemistry heater assembly addresses the thermal effects on the thermal gradient of the chemistry heater plate by bonding chemistry heater plate ***2602*** to a flow guide frame ***2606***. In a further embodiment, the flow guide frame ***2606*** is flush with the second surface of the fixed support bracket ***2013***. In another implementation the chemistry heater plate ***2602*** is bonded to the flow guide frame ***2606*** to ensure proper thermal contact is maintained between the first surface of the chemistry heater plate ***2621*** and the film side of the cartridge regardless of mechanical tolerances.

[0268] In some implementations, a chemistry heater fan ***2603*** is fluidically coupled to a fan plenum ***2604*** with flow vane ***2605*** and a heater plenum ***2607*** to direct cooled air through a cutout disposed within the flow guide frame ***2606*** and directly over chemistry heater ***2601***. As shown in FIG. 54, arrows demonstrate the flow path of air from the chemistry heater fan ***2603*** and through the opening formed within the flow guide frame ***2606*** and heater plenum ***2607***. This configuration is advantageous when optionally thermally fluctuating the chemistry heater between two or more temperatures rapidly prior to setting the chemistry heater to a reaction temperature. As described according to an embodiment herein, thermally fluctuating the chemistry heater generates convection of the fluids within the assay chambers. Specifically, the convection generated within a plurality of assay chambers facilitates mixing of a sample with dried reagents within the assay chambers prior to beginning amplification. Chemistry heater fan ***2603***, fan plenum ***2604***, flow vane ***2605***, and heater plenum ***2607*** are fluidically coupled to facilitate a faster cooling ramp rate of the chemistry heater ***2601*** to a low temperature during the sequence of thermal fluctuations. In one implementation, the chemistry heater fan ***2603*** may be turned off after the sequence of thermally fluctuations and remains off for the remainder of the diagnostic test while the chemistry heater is set to the reaction temperature.

[0269] In various aspects, a flow guide frame ***2606*** is composed, e.g., entirely composed, of one or more polymeric materials (e.g., materials having one or more polymers including, for example, plastic). A flow guide frame ***2606*** can be composed of any of the elastic materials provided herein. Materials of interest for the flow guide frame include, but are not limited to, polymeric materials, e.g., plastics. In a preferred embodiment, the flow guide frame is polyether ether ketone (PEEK).

[0270] Thermal boundary conditions affect the temperature gradient of the chemistry heater plate ***2601*** in contact with the film side of a cartridge and can result in undesired temperature variation across assay chambers. Uniformity among assay chambers is critical to amplifying nucleic acids for accurate detection. According to various embodiments, the chemistry heater assembly ***2600*** includes a chemistry heater plate ***2602*** comprising a machine pocket geometry for thermal gradient reduction.

[0271] Returning to FIGs. 50 and 51, the chemistry heater plate ***2601*** is viewed from a first surface ***2621***. In one embodiment, the reaction well zone includes a machined pocket geometry ***2623*** comprising grooves ***2624***. The machined pocket geometry ***2623*** is a series of grooves arranged in a pattern to decrease heat flux through the center of the reaction well zone ***2620*** to compensate for edge heat loss due to the environment. The configuration, as described herein, provides precise isothermal control of the reaction well zone ***2620*** of the chemistry heater plate ***2602*** to supply a uniform temperature to the plurality of assay chambers conducing amplification.

[0272] The term "grooves," is used herein, refer to any hole, cutout, orifice, aperture, gap, or space machined into the chemistry heater plate ***2602*** to reduce heat flux variation between the chemistry heater ***2601*** and chemistry heater plate ***2602*** to supply a consistent temperature to the reaction well zone for amplification. In some embodiments, grooves extend entirely through the chemistry heater plate from a first surface of the chemistry heater plate ***2621*** to a second surface ***2622***. In other implementations, grooves extend partially at a depth measured from the first surface of the chemistry heater plate. Examples of geometries for cutouts include, but are not limited to, circles, rectangles, rounded rectangles, ovals, ellipses, or any combinations thereof.

3. Cartridge Heater Assembly

[0273] In one embodiment, a cartridge heater assembly ***2550*** provides controlled heating of sample preparation areas of the cartridge, i.e., a cartridge heater zone. FIG. 50 provides a perspective view of cartridge heater zone ***2552*** from the first surface of the fixed support bracket ***2012***, wherein the first surface of the fixed support bracket is in contact with the film side of the cartridge. In one embodiment, the cartridge heater zone ***2552*** is in thermal contact with a wash buffer reservoir ***1475***, elution buffer reservoir ***1500***, rehydration chamber ***1520***, and lysis chamber ***1371*** housed within an integrated diagnostic cartridge to provide a controlled steady state temperature to areas of the cartridge performing sample preparation. In one aspect, the cartridge heater assembly comprises a cartridge heater ***2551*** and insulator ***2553***. FIG. 52 illustrates the cartridge heater assembly and FIG. 53 illustrates the cartridge heater assembly in an exploded view. The cartridge heater ***2551*** can be of any suitable design but is most preferably a resistance heater (e.g. a Kapton heater). In one embodiment, the cartridge heater ***2551*** is in thermal contact and bonded to a second surface of the fixed support bracket ***2013*** with a pressure sensitive adhesive. The thermal contact between cartridge heater ***2551*** and the second surface of the fixed support bracket forms the cartridge heater zone ***2552*** in the first surface of the fixed support

bracket ***2012*** viewed in FIG. 50. In another embodiment, an insulator ***2553*** is in thermal contact with the cartridge heater ***2551*** to prevent thermal energy from escaping into the ambient environment.

[0274] Thermal boundary conditions affect the uniformity of heat transfer between the cartridge heater assembly zone ***2552*** and areas of the cartridge housing the wash buffer reservoir ***1475***, elution buffer reservoir ***1500***, rehydration chamber ***1520***, and lysis chamber ***1371***. In one implementation, as shown by FIG. 50, the cartridge heater assembly ***2550*** further comprises a series of cutouts ***2554*** around the perimeter of cartridge heater zone ***2552*** for thermal gradient reduction to control heat loss.

[0275] The term "cutouts," as used herein, refer to any hole, groove, orifice, aperture, gap, or space machined into the fixed support bracket ***2011*** to reduce heat flux variation between the cartridge heater ***2551*** and the cartridge heater zone ***2552*** to provide a consistent temperature to areas of the cartridge responsible for sample preparation. In some implementations, cutouts may extend through from the first surface of the fixed support bracket ***2012*** to the second surface of the fixed support bracket ***2013***. In other implementations, the cutouts may partially extend a depth measured from the first surface of the fixed support bracket ***2012.*** Examples of geometries for cutouts include, but are not limited to, circles, rectangles, rounded rectangles, ovals, ellipses, or any combinations thereof.

[0276] In a further embodiment, the cartridge heater zone may comprise a plurality of perforations ***2377***. In embodiments of the magnetic mixing assembly of the instrument, the driving magnet system ***2310*** and driven magnet system ***2350*** rotate in a circular pattern. As such, eddy currents are induced in the fixed support bracket ***2012*** radially from a center of the circular pattern. To limit the induction of eddy currents in the fixed support bracket, the plurality of perforations can be arranged in a concentric pattern around the center of the circular pattern of the magnetic mixing assembly ***2300***, as described herein. This concentric arrangement of the plurality of perforations causes the eddy currents induced in the fixed support bracket to follow a convoluted path along their radial induction pathways. This convoluted pathway limits the formation of the eddy currents in the fixed support bracket.

4. Heat Staker Assembly

[0277] The high sensitivity of nucleic acid amplification methods, in particular isothermal amplification methods, pose the threat of amplicon contamination. A cartridge unable to successfully contain amplified nucleic acids may cause cross contamination between assay chambers or in the case of leakage may contaminate the instrument. Cross contamination between assay chambers produces erroneous results in the cartridges while cartridge leakage within the instrument will lead to subsequent false positive results on all cartridges run thereafter. The instrument's thermal subsystem provides a heat staker assembly ***2640*** as appreciated with reference to the various views of FIGs. 51, 52, 55, 56, 57A, 57B and 58. The operation of the

heat staker assembly forms a heat stake across a number of individual fluid channels on the integrated diagnostic cartridge to seal off the channels one from another and prevent sample contamination. Advantageously, the heat stake is performed under sufficient pressure across the main loading channel leading to the assay chambers to prevent amplified nucleic acids from escaping the cartridge and mitigate the risk of amplicon contamination. In addition, the heat staker assembly is configured to heat stake the channel leading to and exiting from the waste collection element to stop fluids from exiting the waste chamber when the cartridge is removed from the instrument. In a preferred embodiment, the integrated diagnostic cartridge has a portion of the fluid channels arranged with planar portions intended to support this integrated heat stake operation. Still further, the fluid pathways of the integrated diagnostic cartridge are arranged so that they are adjacent in a spacing which permits the use of a single linear heat stake element.

[0278] The term "heat stake," as used herein, refers to an exemplary permanent isolation technique for performing the process of melting and rapidly cooling a portion of the cartridge to form a seal and prevent fluids from leaving the cartridge. In implementations where the cartridge comprises one or more polymeric films, the heat staker assembly ***2640*** provides the means to melt and fuse the stack of polymeric films attached to the fluidics side of the cartridge, wherein melting the one or more films into the cartridge forms a barrier across selected fluid channels to retain liquids within. The term "heat stake" may also refer to the seal or barrier formed as a result of the heat staking process. According to an embodiment described herein, the one or more thermoplastic films may be placed on the fluidic card or used as part of the cartridge as part of a heat stake compatible design. In one specific embodiment, there are two thermoplastic films used each having a different melting temperature wherein the first film has a substantially similar melting temperature as the cartridge and the second film has a higher melting temperature than the first film such that only the second film will melt during a heat stake operation to form the barrier. The two thermoplastic film approach described herein has the added benefit of protecting other components or the integrity of the fluidic card or cartridge during heat staking.

[0279] As previously described herein, the fixed bracket assembly ***2010*** is configured to support the thermal subsystem responsible for generating the thermal requirements for executing a molecular diagnostic test. In one embodiment, the thermal subsystem comprises a heat staker assembly ***2640*** for providing one means of permanently sealing each one of the cartridge assay chambers. In such implementation, the fixed support bracket ***2011*** may contain contains a channel ***2020*** integrally formed therein to accommodate such heat staker assembly. Channel ***2020*** permits a staker bar assembly to directly contact the cartridge to perform the sealing action and is most readily apparent in FIG. 51. In FIG. 56 depicts a heat staker assembly **2640** comprises a linear actuation motor ***2642***, a spring ***2643***, a staker bar assembly ***2641***, heat staker fan ***2644***, and an inductive linear sensor ***2645***. In one embodiment, a spring ***2643*** provides the force needed to perform heat staking. As used herein, the linear actuation motor is configured to move the heat staker bar assembly ***2641*** to make contact with the film side of a cartridge. The linear actuator

motor does not, however, provide the force or depth control necessary for heat staking. The linear actuation motor ***2642*** releases spring ***2643***, which supplies the force necessary to heat stake, to push the staker bar assembly ***2641*** into the film side of the cartridge. In one implementation, an inductive linear sensor ***2645*** enables the measurement of linear displacement heat staker assembly ***2640*** and provides a means for heat staking error detection.

[0280] FIGs. 57A and 57B provide perspective and cross section views of the staker bar assembly ***2641***. As shown in FIGs. 57A and 57B the staker bar assembly ***2461*** comprises a heater ***2661***, a staker blade ***2660***, and a depth stop ***2662***. The heater can be of any suitable design but is most preferably a resistance heater (e.g. a wire heater) and is in thermal contact with the staker blade ***2660***. In one implementation, the staker blade ***2660*** has a draft angle to form the heat stake when the blade contacts the polymeric films of the cartridge without tearing. In a further implementation, the draft angle of the staker blade is surrounded by a depth stop ***2662*** to control the depth of the heat stake to a desired displacement range. Linear actuation motor ***2642*** moves the staker bar assembly ***2641*** to the cartridge and then releases spring ***2643*** to apply the force needed to press the heated staker blade ***2660*** into the film side of the cartridge. The staker blade is permitted to melt into the cartridge until depth stop ***2662*** contacts the cartridge, thus preventing the staker blade from traveling further.

[0281] In various aspects, a depth stop is composed, e.g., entirely composed, of one or more polymeric materials (e.g., materials having one or more polymers including, for example, plastic). The polymeric depth stop can be composed of any of the elastic materials provided herein. Materials of interest include, but are not limited to, polymeric materials, e.g., plastics. In a preferred embodiment, the depth stop is polyether ether ketone (PEEK) suited to the operational temperature range of the heat stake assembly.

[0282] According to the subject embodiments, the staker blade can be composed of a variety of materials and can be composed of the same or different materials. Materials that the staker blade described herein can be composed of include, but are not limited to, metals, such as aluminum. In a preferred embodiment the staker blade is aluminum.

E. Optical Subsystem

[0283] Instrument ***2000*** includes an optical subsystem comprising two assemblies which separately interact with cartridge ***1000***. FIGs. 58, 59, 60 and 61 provide various views of a label imaging assembly ***2770***. The label imaging subsystem illuminates and captures an image of the cartridge label area. The label imaging assembly may further be configured to illuminate and capture a series of images of the loading module to aid in monitoring and verification that an adequate sample is loaded into the cartridge prior to running a diagnostic test. The reaction imaging assembly ***2700*** is illustrated in the various views of FIGs. 62-66. In implementations where one or more assay chambers are configured to produce a fluorescent signal indicative of the presence of a target pathogen, the reaction imaging assembly **2700** provides excitation wavelength illumination to the cartridge reaction area ***1600*** and captures images of fluorescence

resulting from the amplification of target nucleic acids. Both optical assemblies are supported by the fixed support bracket and remain stationary during the clamping and unclamping of a cartridge.

1. Label Imaging Assembly

[0284] Label imaging assembly ***2770*** is configured to illuminate and capture images of the patient label and loading module. As shown in FIGs. 58 and 59, the label imaging assembly is mounted to the antenna ground plate ***2810*** and comprises a camera ***2771***, LED ***2772***, aperture ***2773*** and diffuser ***2774***. The label imaging assembly ***2770*** will include at least one, but preferably more than one (e.g., two or three), LEDs ***2772*** for illuminating the patient label area ***1040*** and the loading module while minimizing shadows cast in the patient label area. The aperture ***2773*** defines an opening to transmit and reshape illumination by LEDs to reduce off axis light and stray light from affecting the patient label image quality. Once illumination from the LEDs passes through each respective aperture ***2773***, light travels through diffuser ***2774*** which generates a more uniform illumination intensity on the patient label and loading module. In one implementation, seen in FIGs. 60 and 61, the LEDs may be arranged in an oblique configuration to illuminate the patient label. This arrangement can be advantageous for increasing the contrast of images and improving the overall image quality of the cartridge.

[0285] In a preferred implementation, the label imaging assembly ***2770*** is further configured to image the sample port assembly ***1100*** to verify adequate sample is loaded into a cartridge prior to running a diagnostic test. Given the low concentrations of target pathogens in some samples, it is advantageous to determine a sufficient sample volume is present in the loading module. In a preferred implementation, the label imaging assembly is configured to capture an image of the sample port assembly ***1100*** and detect a mechanism (e.g., a ball disposed within the loading module) to determine the sample volume. Alternatively, the label imaging assembly may detect the meniscus of the sample fluid. Furthermore, the label imaging assembly may be configured to read the sample volume through a sample window ***1050*** provided by a cut out in the cartridge label.

2. Reaction Imaging Assembly

[0286] In some implementations of the present invention, a visual signal, e.g., fluorescent signal, is used to indicate the presence of nucleic acids of a target pathogen within a sample. Specifically, a plurality of target nucleic acids may be detected using one or more distinct excitation and emission wavelengths. A wide variety of fluorophores, with varying emission spectra, are known in the art and one of ordinary skill would be able to select an appropriate fluorophore for a given assay performance. A reaction imaging assembly ***2100*** permits the instrument ***2000*** to simultaneously detect nucleic acids from one or more target pathogens. The reaction imaging assembly ***2100*** may be configured to provide excitation wavelengths to excite one or more fluorophores. Additionally, various elements to filter and capture emitted wavelengths are

described herein to determine the presence or absence of target nucleic acids. The arrangement and operation of the reaction imaging assembly are shown in FIGs. 62-66.

[0287] In a preferred implementation, the reaction imaging assembly ***2700*** shown in FIG. 62 is designed with an epifluorescence arrangement, such that illumination and emitted wavelengths travel through the same objective lens. Unlike oblique illumination, the epifluorescence arrangement illuminates uniformly within the plug structures, described herein with regard to a cartridge amplification module, of the assay chambers to minimize or prevent shadows. Shadows casted on assay chambers hinder the likelihood of detecting a positive sample for an infectious disease. In one implementation, the reaction imaging assembly ***2700*** comprises a camera ***2701***, dichroic beam splitter ***2702***, excitation lens cell ***2730***, emission lens cell ***2750***, objective lens ***2706***, and a fold mirror ***2704***. In a preferred embodiment, all components of the reaction imaging assembly are either contained within or fixedly attached to an optical block ***2710*** or a beam splitter block ***2707.*** In one embodiment, the optical block and beam splitter block are joined to form the reaction imaging assembly. The optical block ***2710*** may be configured with a pocket ***2711*** which is an opening therein to permit the transmission of excitation wavelengths from the excitation lens cell to the cartridge imaging plane ***2760*** and emission wavelengths from the plurality of assay chambers to the reaction camera. Further the pocket ***2711*** surrounding the cartridge reaction area ***1600*** may prevent any potential stray light within the instrument enclosure from interfering with the detection of fluorophore emission spectra by generating the darkest reference background when capturing images of the reaction area. In a further preferred embodiment, the reaction imaging assembly is fixed to the first side of the fixed support bracket ***2012*** and therefore remains stationary during the clamping and unclamping of a cartridge.

[0288] In various embodiments, reaction camera ***2701*** captures images of the assay chambers within a cartridge reaction area ***1600*** for an instrument image processing to determine the presence of target nucleic acids and generate a result of the diagnostic test. In some implementations, the reaction camera ***2701*** is monochromatic. In such implementations, the reaction camera may be optically coupled to a corresponding cartridge configuration adapted to perform with said monochromatic reaction camera. For example, a cartridge may comprise a plurality of assay chambers, wherein each assay chamber contains a distinct primer set and fluorescent probe. When the reaction camera captures an image of the cartridge reaction area containing a plurality of assay chambers, image processing within the instrument computer system may determine the presence of target nucleic acids based on the visual signal and corresponding chamber position to determine a diagnostic result. In an alternative embodiment, the reaction camera ***2701*** is a multicolor camera. In such implementations, a cartridge configured to perform with said multicolor reaction camera may comprise assay chambers where multiple primer sets and probes are used. For example, the multicolor reaction camera may capture images a cartridge reaction area with assay chambers comprising a plurality of primer/probe sets within a single assay chamber. Further, the multicolor reaction camera may capture images of a

cartridge reaction area with assay chambers comprising a plurality of primer/probe sets within multiple assay chambers. Appropriate optical components, e.g. LEDS, filters, lenses, and sensors, may be selected such that imaging processing within the instrument computer system may determine the presence of target nucleic acids based one the plurality of emission wavelengths.

[0289] In a further embodiment, additionally included in the reaction imaging assembly is a dichroic beam splitter ***2702***, which separates excitation light from emitted light by reflecting shorter wavelengths of light from the excitation lens cell ***2703*** and passing longer wavelengths emitted from the fluorophore. in another embodiment, a fold mirror ***2704*** directs excitation wavelengths to the reaction well image plane ***2760*** and redirects emitted wavelengths from the reaction well image plane to the reaction camera ***2701***.

[0290] In one embodiment, an excitation lens cell generates excitation wavelengths for a fluorophore to absorb and is comprised of at least one or more excitation LED ***2731***, a plano-convex lens ***2733***, an aspheric lens ***2734***, an aperture ***2732***, and a bandpass filter ***2735***. The excitation lens cell is shown in FIGs. 63 and 64 as section views. In one embodiment, the at least one or more excitation LED ***2731*** illuminates the plurality of assay chambers. A person of ordinary skill may select the appropriate number and type of LEDs such that emission spectra corresponds to the excitation wavelengths of a chosen fluorophore. The optical path of the excitation light travels through aspheric lens ***2734*** to correct for spherical aberration, an optical effect commonly observed with plano-convex lenses, where incident light rays focus at different points resulting in a blurry image. Aspheric lens ***2734*** focuses incident light from the excitation LED ***2731*** to a small point, thus improving the image quality. In one implementation, an aperture is used to reshape excitation illumination. Focused excitation light is transmitted through the aspheric lens ***2734*** and enters aperture ***2732*** such that, aperture ***2732*** alters the illumination shape from the excitation LED to minimize off axis light and stray light from hindering fluorescent imaging. In one embodiment, one or more bandpass filters may be used within the excitation lens cell to selectively transmit light of specific wavelengths. Excitation light passes through bandpass filter ***2735*** to filter wavelengths outside the fluorophore excitation bandwidth and transmit wavelengths within the excitation bandwidth. Furthermore, the excitation bandpass filter substantially prevents light in the fluorophore emission band from entering the reaction well imaging plane due to the epifluorescence arrangement. Filtered excitation light travels through a plano-convex lens ***2733*** to diffuse the light prior to reaching dichroic beam splitter ***2702***. Filtered excitation light strikes the dichroic beam splitter ***2702*** and reflects shorter excitation wavelengths to the objective lens ***2706*** while longer wavelengths are transmitted through the dichroic beam splitter ***2702***. In one implementation, transmitted longer wavelengths from the dichroic beam splitter are directed to a light trap ***2703***.When implementing a light trap, the light trap prevents excitation light from entering the camera by reflecting the light off multiple angled surfaces substantially away from the camera. Excitation wavelengths reflected by the dichroic beam splitter ***2702*** are transmitted through the

objected lens ***2706*** where fold mirror ***2704*** redirects the light to the image plane ***2760*** of the reaction well area ***1600*** of the cartridge.

[0291] The excitation LED peak wavelength and intensity can vary with temperature, thus requiring precise thermal control of the LED temperature. The excitation lens cell further includes various elements to ensure the excitation cell ***2730*** functions properly. As shown in FIG. 64, a temperature sensor ***2738*** provides temperature feedback control while photodiode ***2739*** monitors the LED output to ensure the LED is on. A thermal isolation spacer ***2737*** isolates the excitation system from short ambient thermal transients and heat sink ***2736*** provides cooling.

[0292] In another embodiment, the reaction imaging assembly ***2700*** includes emission lens cell ***2750***, shown in FIG. 65, comprising an image lens ***2751***, long pass filter ***2752***, and objection lens ***2706***. The fluorophore absorbs excitation light from the excitation lens cell ***2730*** and almost instantaneously emits emission wavelengths to fold mirror ***2704***. The bent emitted light travels through objective lens ***2706*** where the longer emitted wavelengths are subsequently transmitted through the dichroic beam splitter ***2702***. In one implementation, the emission lens cell ***2750*** comprises one or more longpass filters to transmit emitted wavelengths from the fluorophore. A longpass filter ***2752*** ensures light in the emission band enters the reaction camera ***2701*** and substantially eliminates interfering wavelengths, outside of the emission band, from the excitation LED.

[0293] FIGs. 45, 46 and 66 illustrate the relationship between the label imaging assembly ***2700*** and the reaction imaging assembly ***2700*** of the instrument optical subsystem. Regarding FIG. 66, the label imaging assembly is shown fixed to the antenna ground plate ***2810*** at the proximal end of the instrument near front slot ***2072***, while the reaction imaging assembly is fixed to the distal end of the instrument in close proximity to the loading assembly ***2230***. In such a configuration, the label imaging assembly ***2770*** is advantageously separated from the reaction imaging assembly ***2700.*** In this way, the label imaging system 2770 may be used initially to detect an insufficient sample volume error and eject a cartridge prior to executing sample preparation steps for an assay to be imaged by the reaction imaging assembly ***2700***.

[0294] With regard to FIG. 45 and 46, a cartridge ***1000*** is shown with respect to the label imaging assembly ***2770*** and reaction imaging assembly ***2700*** in a loading and loaded position, as described herein. FIG. 45 depicts the cartridge in a forward most loading position within the loading assembly ***2230***. Patient label area ***1040*** is not within the field of view of the label imaging assembly ***2770***, as observed in FIGs. 59, 60, and 61. Furthermore, in the forward most loading position the reaction area ***1600*** of a cartridge containing a plurality of assay chambers ***1621*** is adjacent to the reaction imaging assembly ***2700*** and outside of pocket ***2711*** within optical block ***2710***. Note, the loading position of the loading assembly ***2230*** in FIG. 45 reiterates the loading position shown in FIGs. 17A and 17B. The cartridge is shown in a loaded position in FIG. 46. Patient label area ***1040*** is now hidden by the label imaging assembly ***2770*** and is within the field of view as shown in FIGs. 59, 60, and 61. Furthermore, the reaction area of the cartridge ***1600*** is

disposed within pocket ***2711*** of the optical block and is hidden from view. The loaded position of the loading assembly ***2230*** similarly reflects the loaded position in FIGs. 18A, 18B, 19A, 19B,and 19C. Additional details of the positioning of the cartridge and the movement of the thermal clamp assembly ***2680*** with respect to the reaction imaging system ***2700*** may be appreciated with reference to FIGs. 38-42. The position of the reaction imaging assembly relative to other components of the instrument, (e.g., the moving bracket assembly ***2040***) may be appreciated with reference to the various views provided in FIGs. 43 and 44.

F. Exemplary Computer System

[0295] FIGs 67A-67I represent various schematic views of a representative computer control system for use with a diagnostic instrument described herein. Generally, the instrument computer control system includes instructions in computer readable code used to coordinate the synchronous performance of the one or more of the operations described herein related to receiving, handling, processing and analyzing a suspected sample in a cartridge. Additional details of the various steps performed related to receiving, handling, processing and analyzing a suspected sample in a cartridge are provided with regard to FIGs. 93-102 and 106A-113. The computer system may comprise an exemplary client or server computer system. Computer system includes a number of communication channels or busses for communicating control signals, sensor information, or other information from a component or system within the instrument to a processor. These various communication pathways are indicated by the lines connecting each of the various components, systems and subsystems. The host processor 2900 is used for processing information and generating signals according to one or a number of programmed control sequences. Processor 2900 may be any suitable computer controller, processor with co-processor, microprocessor or suitable combination thereof.

[0296] Additionally or optionally, the instrument computer control system may include one or more of a random access memory (RAM), or other dynamic storage device (referred to as main memory) coupled to bus for storing information and instructions to be executed by processor. Main memory also may be used for storing temporary variables or other intermediate information during execution of instructions by processor.

[0297] Instrument computer system also includes a read only memory (ROM) and/or other static storage device coupled to bus for storing static information and instructions for processor, and a data storage device, such as a magnetic disk or optical disk and its corresponding disk drive. Data storage device is coupled to bus for storing information and instructions.

[0298] With reference to FIG. 67A, the host processor 2900 is in communication with a communications module 2905 which includes a cellular antenna 2800 located in the front panel 2073 of the instrument 2000 along with associated firmware and software. Additionally, the host processor 2900 is in communication with a USB and Ethernet port 2903 as well as any other external communication port. There is access provided to data storage including encrypted data

2901 along with calibration, firmware upgrade and test results data. There is also provided appropriate storage for de-identified patient results data. The host processor 2900 is also in communication with a display or graphical user interface 2902 such as the one on the instrument front panel 2073. The host processor 2900 is also in communication with various instrument application software 2904. This software and firmware corresponds, by way of example, to particular testing routines to be implemented by the diagnostic instrument 2000 based on the type of sample/integrated diagnostic cartridge 1000 that is loaded into and detected by the instrument 2000. Additionally, the instrument software and firmware 2904 includes computer readable instructions for an instrument operating system along with the various appropriate computer drivers for instrument components. The host processor 2900 is also configured to access and execute the camera operation and imaging firmware 2915 responsible for executing the specific imaging routines performed by the label imaging camera 2771 and the reaction chemistry or assay chamber camera 2701.

[0299] FIG. 67A also illustrates the communication busses to each of the different computer subsystems utilized in the instrument. Each one includes appropriate software, firmware and communication components adapted and configured to the functional and operational requirements of that specific instrument subsystem. As such, each instrument subsystem is provided appropriate communication channels for transmission and receipt of computer readable instructions from the one or more processors, co-processors or suitable microprocessor(s). Additionally, a number of specifically configured subsystem of the instrument control system are configured to deliver, receive or monitor signals from one or more actuators, components, switches or sensors as will now be described.

[0300] Advantageously, the instrument computer system may include a host processor and a co-processor 2900 in coordinated operation. In one configuration, the host processor 2900 includes instrument operating system and device drivers, specific instrument application software and firmware 2915 for operation of the label camera 2771 and reaction well camera 2701. A second processor may be configured as a slave processor to handle other commands such as the operation of various motors and actuator in the diagnostic instrument 2000. Additionally, the co-processor would be responsible for prioritization and execution of various control signals throughout the various instrument subsystems. The instrument computer system memory or computer readable storage may include stored or accessible computer records of various test methods, scripts, parameters, completed records storage, instrument calibration readings and results based on specific operations performed by the instrument 2000 for a specific cartridge diagnostic test or sample type.

[0301] In general, the instrument computer system includes the following functional subsystems adapted and configured to correspond to the steps performed in a wide variety of functions corresponding to a desired preprogrammed testing sequence. As shown in FIG. 67A, the functional subsystems are optical cartridge label subsystem 2910, the optical reaction well

subsystem 2990, the thermal subsystem 2970, the lysing drive subsystem 2950, the loading cartridge subsystem 2920, the cartridge seal rupturing subsystem 2930, the pneumatic-interface subsystem 2960, the valve drive subsystem 2940 and the rehydration mixing subsystem 2980. In one aspect, these functional groups may be functionally grouped more generally into an optical subsystem, a thermal subsystem and a clamping subsystem. One or more of these functional groups may be assigned to the co-processor.

[0302] The optical subsystem includes an optical cartridge label subsystem 2910 (FIG. 67B) and an optical reaction well subsystem 2990 (FIG. 67C).

[0303] As shown in FIG. 67B, the optical cartridge label subsystem 2910 includes software, firmware and communication components adapted and configured to for use with a cartridge label imaging camera 2771, a label illumination LED 2792 and a sample illumination LED 2775. Under control of instructions from the one or more processors 2900, the optical cartridge label subsystem 2910 interacts with the patient label area 1040 and sample port assembly 1100 of a cartridge 1000 undergoing processing within the instrument 2000.

[0304] As shown in FIG. 67C, the optical reaction well or assay imaging subsystem 2990 includes software, firmware and communication components 2980 adapted and configured for use with a reaction camera 2701. Additionally, the optical reaction well subsystem 2990 controls a bright field LED 2753, an excitation LED heater 2741, an excitation LED 2791, an LED excitation intensity sensor 2740, an assay well reaction camera 2701, and an LED excitation temperature sensor 2738. Under control of instructions from the one or more processors 2900, optical reaction well subsystem 2990 interacts with the assay chamber 1621 in the reaction area 1600 of a cartridge 1000 undergoing processing within the instrument 2000.

[0305] As shown in FIG. 67D, the thermal subsystem 2970 includes software, firmware and communication components 2960 adapted and configured to for use with an heat stake cooling fan 2644, a chemistry heater 2601, chemistry heater sensor 2608, heat staker heater 2661, cartridge heater temperature sensor 2555, heat staker motor 2642, cartridge heater 2551, heat stake temperature sensor 2646, staker linear displacement sensor 2645, and chemistry heater cooling fan 2603. Under control of instructions from the one or more processors 2900, the thermal subsystem 2970 interacts with the central cartridge portion, assay wells and heat stake zone portions of a cartridge undergoing processing within the instrument.

[0306] As shown in FIG. 67E, the lysing drive subsystem 2950 includes software, firmware and communication components adapted and configured for use with a lysing drive motor 2330 and an audible sensor/microphone 2380. Under control of instructions from the one or more processors 2900, the lysing drive subsystem 2950 interacts with the stir bar or other lysing agents within the lysis-lysis chamber 1371 of the cartridge while being monitored for magnetic uncoupling via the audible sensor 2380.

[0307] As shown in FIG. 67F, the loading cartridge subsystem 2920 includes software, firmware and communication components adapted and configured for coordinated operation of a linear

actuator 2014, hardstop clamping sensor 2019, cartridge door support assembly 2280, homing clamping sensor 2017, cartridge loading sensor 2236, and frangible seal switch 2266. Under control of instructions from the one or more processors 2900, the loading cartridge subsystem 2920 provides coordinated interactions with cartridge to ensure proper loading, positioning and clamping of the cartridge with respect to the instrument interior.

[0308] As shown in FIG. 67G, the pneumatic subsystem 2960 includes software, pneumatic control firmware and communication components adapted and configured to for use with a pneumatic pump 2131, proportional valve 2133, output selector valve 2136, altitude sensor 2140, pressure regulator 2132, and a humidity sensor 2142. Under control of instructions from the one or more processors 2900, the pneumatic subsystem 2960 interacts with a pneumatic interface 2100 on the cartridge to deliver pneumatic drive signals to the cartridge undergoing processing within the instrument.

[0309] As shown in FIG. 67H, the valve drive subsystem 2940 includes software, firmware and communication components adapted and configured to for use with a valve drive motor 2403, an interference sensor 2404 and a valve drive homing sensor 2409. Under control of instructions from the one or more processors 2900, the valve drive subsystem 2940 interacts with the rotary valve on the cartridge to index the rotary valve for alignment of a desired flow channel on the cartridge undergoing processing within the instrument.

[0310] As shown in FIG. 67I, the rehydration mixing subsystem 2980 includes software, firmware and communication components adapted and configured for use with a rehydration motor 2510 and rehydration motor rotation sensor 2530. Under control of instructions from the one or more processors 2900, the rehydration motor 2510 interacts with a stir ball or other component within the master mixing rehydration chamber of the cartridge while being monitored for rotation using the motor rotation sensor 2530.

[0311] Additional alternative computing environments and modifications to both user experience and user interaction are possible and within the scope of the various embodiments described herein, The instrument computer control system may further be coupled to a display device, such as a liquid crystal display (LCD) including touch screen or other functionality by direct connection or wirelessly. The display is also coupled to bus for displaying information to an instrument user. An alphanumeric input device, including alphanumeric and other keys, may also be provided via the touch display or coupled to bus for communicating information and command selections to processor. An additional user input device is cursor control, such as a mouse, trackball, trackpad, stylus, or cursor direction keys, voice or touch controllers coupled to bus for communicating direction information and command selections to processor, and/or for controlling cursor movement on display.

[0312] Another device that may be coupled to bus is hard copy device, which may be used for marking information on a medium such as paper, film, or similar types of media. Additionally, the computer system may include wired and wireless communication capabilities depending on

configuration. Remote communications using the communications module described above with the instrument computer system may be utilized for transferring information, calibration, service, maintenance or other system or patient information collected or produced by the instrument computer system.

[0313] Note that any or all of the components of system and associated hardware may be used in the present invention. However, it can be appreciated that other configurations of the instrument computer system may include some or all of the devices. Certain variations of system may include peripherals or components not illustrated in these various exemplary figures. Additional such components may be included and configured to receive different types of user input, such as audible input, or a touch sensor such as a touch screen.

[0314] Certain embodiments may be implemented as a computer program product that may include instructions stored on a machine-readable medium. These instructions may be used to program a general-purpose or special-purpose processor to perform the described operations. A machine-readable medium includes any mechanism for storing or transmitting information in a form (e.g., software, processing application) readable by a machine (e.g., a computer). The machine-readable medium may include, but is not limited to, magnetic storage medium (e.g., floppy diskette); optical storage medium (e.g., CD-ROM); magneto-optical storage medium; read-only memory (ROM); random-access memory (RAM); erasable programmable memory (e.g., EPROM and EEPROM); flash memory; electrical, optical, acoustical, or other form of propagated signal (e.g., carrier waves, infrared signals, digital signals, etc.); or another type of medium suitable for storing electronic instructions. The label imaging camera firmware or the optical cartridge label subsystem may be adapted and configured to recognize machine readable markings as part of a cartridge verification protocol as well as to aid in the identification of a particular sample type and/or diagnostic testing routine to be performed with that sample/cartridge.

[0315] Additionally, some embodiments may be practiced in distributed computing environments where the machine-readable medium is stored on and/or executed by more than one computer system. In addition, the information transferred between computer systems may either be pulled or pushed across the communication medium connecting the computer systems.

[0316] The digital processing device(s) described herein may include one or more general-purpose processing devices such as a microprocessor or central processing unit, a controller, or the like. Alternatively, the digital processing device may include one or more special-purpose processing devices such as a digital signal processor (DSP), an application specific integrated circuit (ASIC), a field programmable gate array (FPGA), or the like. In an alternative embodiment, for example, the digital processing device may be a network processor having multiple processors including a core unit and multiple micro engines. Additionally, the digital processing device may include any combination of general-purpose processing device(s) and special-purpose processing device(s).

G. Integrated Diagnostic Cartridge

[0317] The embodiments described herein relate to a disposable single use device (a "cartridge") used for molecular diagnostic testing. The cartridge can contain a plurality of modules for performing a variety of functions in order to effect the diagnostic test including, but not limited to, a loading module, a lysis module, a purification module, and an amplification module. The loading module is configured to receive a sample, minimize the spilling of the sample, and prepare the sample for lysis. The lysis module is configured to disrupt cells walls and cell membranes in order to release inter-cellular materials such as nucleic acids (DNA, RNA), protein or organelles from a cell, and, in some cases, clear debris from the lysate. The purification module is configured to isolate and/or enrich nucleic acid from a lysed sample. The amplification module is configured to generate and detect a signal from target amplicon, indicative of the presence of target pathogen in the sample.

[0318] Generally, cartridge dimensions are defined by its length, width, and height. Accordingly, each dimension has a respective associated axis, e.g. a cartridge length axis, a cartridge width axis, and a cartridge height axis. FIGs. 68-70, 89, and 92 are exemplary embodiments of an integrated diagnostic cartridge ***1000***. In FIGs. 68-70 and 92 the dimensions of the integrated diagnostic cartridge are arranged such that the cartridge length axis ***1035*** and cartridge width axis ***1025*** lie within the plane of the page. Further, the cartridge height axis, i.e. axis defining the thickness of the cartridge, is represented by a circle ***1030*** which is normal to the plane of the page. Additionally, FIGs. 68-72 and 89-92 depict an arrow ***1900*** adjacent to the illustrated cartridge embodiments. Arrow ***1900*** corresponds to one preferred embodiment of an operational cartridge orientation maintained within the instrument during the performance of a diagnostic test. As described herein and further detailed below, the preferred orientation is a vertical cartridge orientation.

[0319] FIGs. 3-5, 8, 11-13, and 16A-16E depict various views of the cartridge positioned between the clamping subsystem in the vertical orientation from outside and within the instrument enclosure. Accordingly, arrow ***1900,*** which indicates the preferred cartridge orientation, is collinear with the cartridge width axis ***1025*** and slot ***2072*** such that the cartridge width axis ***1025*** is substantially normal to a base of the instrument and the cartridge length axis ***1035*** is substantially normal to a rear wall of the instrument when inserted and clamped by the instrument. In many embodiments, one or more portions of the cartridge and/or instrument may comprise alignment features, e.g. rails, protrusions, indents, or keys, for inserting the cartridge into the instrument in the acceptable orientation. The following cartridge embodiments describing various modules for performing and housing the molecular diagnostic test will be described according to this orientation. A discussion of several vertical cartridge advantages will be made readily apparent throughout the remaining disclosure. However, it is to be appreciated that a person of ordinary skill may design a cartridge based on alternative orientations while achieving the same desired objective of detecting nucleic acids.

[0320] FIG. 68 is a side view of one cartridge interface schematic of an integrated diagnostic cartridge ***1000***. In this illustrative embodiment, the integrated cartridge ***1000*** includes a loading module, a lysis module, a purification module and an amplification module. In alternative embodiments, the diagnostic cartridge may optionally and/or additionally include a pre-amplification module, a distinct detection module, a plurality of any of the aforementioned modules, e.g. a second purification module, or any other module designed for the effect of performing a molecular assay, In the embodiment shown in FIG. 68, the interface region allocated to the loading module is on a proximal end of the cartridge ***1920*** adjacent to the patient label area ***1040***. Further, the interface region for the amplification module, containing reaction area ***1600,*** is on a distal end of the cartridge ***1915***. Further to the compact and modular design aspects of the various cartridge embodiments, the interstitial portion of the cartridge between respective interfaces for the loading module and amplification module can be occupied by remaining module interfaces. As shown in FIG. 68, the interface regions for the lysis module and the purification modules are arranged between the indicated proximal and distal modules... As such, the additional interface regions of the lysis and purification modules are occupied by the frangible seal area ***1200***, the rotary valve ***1400***, the rehydration chamber ***1520***, the cartridge pneumatic interface ***1170*** and the lysis chamber ***1371***.The placement of these interfaces, and others described herein, are in advantageous arrangement not only as to the loading module and the amplification module but also to take advantage of vertical orientation within the instrument for sample processing.

[0321] The inventive modular design of the cartridge embodiments described herein leverage the ability to easily modify cartridge configurations, modules, and/or their respective interfaces to process samples and detect specific target pathogens with a desired diagnostic assay. One or more distinct modules may be simply altered, redesigned, or substituted in whole, without significantly impacting the remaining cartridge modules, to change the diagnostic assay performed by the instrument. Such modifications may enable the processing of a different sample type, the lysing of various target pathogens, the purification of different analytes of interest, and/or the amplification and detection of one or more pathogens. Accordingly, the modular design of the cartridge embodiments presented herein advantageously permit simple substitution of individual elements within respective modules. In one specific example, described in greater detail below, assay chambers for amplifying and detecting purified analyte may be modified with reagent plugs comprising dried down reagents. The dried down reagents may comprise one or more primer sets and probes for the specific detection of a target pathogen, such that one may replace one or more reagent plugs in the amplification module to detect a different pathogen. Specifically, cartridge modifications driven by altering the sample type to be processed and target pathogen can be made with little to no impact on the design and functionality of analogous instrument subsystems. However, in any case, it is most advantageous and preferable to modify the cartridge configuration, modules, and/or interfaces within one or more fixed parameters of instrument-to-

cartridge interfaces established to minimize the redesign of the diagnostic instrument. Additionally or optionally, the plugs may be modified by shape, size or placement to provide for a range of assay chamber volumes as described herein.

[0322] In many embodiments, the instrument is configured to recognize and interact with the cartridge interfaces to perform the diagnostic assay. Accordingly, the instrument interfaces which interact with the cartridge may be one or more of those which are physically coupled or non-physically coupled. The aforementioned cartridge configuration of FIG. 68 illustrates a combination of physically and non-physically coupled interfaces. Physically coupled interfaces may include elements that directly contact the cartridge, such as interfaces which are in thermal contact or direct physical contact. For example, the door support assembly **2280** presses upon the fill port cap **1181**, the valve drive assembly ***2400*** inserts a valve drive into engagement openings in the rotary valve ***1400***, and the instrument pneumatic interface ***2100*** presses against the cartridge pneumatic interface ***1170***. Alternatively, non-physically coupled interfaces may include elements that still interact with the cartridge, but otherwise do not physically contact the cartridge. Such non-physical interfaces include, but are not limited to, magnetic, optic, acoustic, ultrasonic, and electromagnetic. For example, the magnetic mixing assembly ***2300*** acts upon the contents of the lysis chamber ***1371*** and a magnet associated with the rehydration motor ***2510*** acts upon a magnetic ball ***1524*** with the rehydration chamber ***1520***. The illustrated embodiment is further configured to interact with a camera ***2771*** within the label imaging assembly ***2770*** to capture an image of the patient label area ***1040***. Further, a reaction camera ***2701*** of the reaction imaging assembly ***2700*** may capture and image of the reaction area ***1600*** of the cartridge.

[0323] In certain implementations, the cartridge is comprised of a fluidics card, which comprises most of the functional structures of the cartridge, and a cover ***1004***, which protects the active areas of the cartridge. Figure 69A illustrates a cartridge from the feature side ***1007*** of the fluidics card ***1001***. The cover is substantially removed to permit visualization of the fluidics features hidden behind the cover. Similarly, Figure 70A illustrates the cartridge from the fluidics side ***1006***, which provides the fluidic network for transporting a sample and various substances to different modules of a cartridge. Typically the fluidics side comprises a plurality of fluidic channels, ducts, and pathways formed within the surface of the cartridge. In many embodiments, the channels, ducts and pathways are enclosed with a film applied against the fluidics side of the cartridge. In a preferred implementation, the channels, ducts and pathways are microfluidic features having a smallest dimension of 750 µm or less. In other implementations, the smallest dimension can be 600 µm or less, 500 µm or less, 400 µm or less, 200 µm or less, or 100 µm or less. In another aspect, the fluidics side 1006 can include multiple vias, e.g. openings, passages or ports configured for passing fluids therethrough from one side of the fluidics card to the other, e.g. from the fluidics side 1006 to a structure on the features side 1007. In another aspect, the fluidics side ***1006*** can include multiple vias, e.g. openings configured for passing fluids therethrough from one side of the fluidics card to the other, e.g. from the fluidics side ***1006*** to a structure on the features

side ***1007***. A via can have any of the dimensions, such as the cross-sectional diameter of any of the channels provided herein. In another embodiment, the feature side ***1007*** of a fluidics card ***1001*** defines the various structures to enable the loading, lysing, purifying, and amplification of a sample.

[0324] In certain implementations, the cartridge is comprised of a fluidics card, which comprises most of the functional structures of the cartridge, and a cover ***1004***, which protects the active areas of the cartridge. Figure 69A illustrates a cartridge from the feature side ***1007*** of the fluidics card ***1001***. The cover is substantially removed to permit visualization of the fluidics features hidden behind the cover. Similarly, Figure 70A illustrates the cartridge from the fluidics side ***1006***, which provides the fluidic network for transporting a sample and various substances to different modules of a cartridge. Typically the fluidics side comprises a plurality of fluidic channels, ducts, and pathways formed within the surface of the cartridge. In many embodiments, the channels, ducts and pathways are enclosed with a film applied against the fluidics side of the cartridge. In a preferred implementation, the channels, ducts and pathways are microfluidic features having a smallest dimension of 750 µm or less. In other implementations, the smallest dimension can be 600 µm or less, 500 µm or less, 400 µm or less, 200 µm or less, or 100 µm or less. In another aspect, the fluidics side 1006 can include multiple vias, e.g. openings, passages or ports configured for passing fluids therethrough from one side of the fluidics card to the other, e.g. from the fluidics side 1006 to a structure on the features side 1007. In another aspect, the fluidics side ***1006*** can include multiple vias, e.g. openings configured for passing fluids therethrough from one side of the fluidics card to the other, e.g. from the fluidics side ***1006*** to a structure on the features side ***1007***. A via can have any of the dimensions, such as the cross-sectional diameter of any of the channels provided herein. In another embodiment, the feature side ***1007*** of a fluidics card ***1001*** defines the various structures to enable the loading, lysing, purifying, and amplification of a sample.

[0325] In some implementations, one or more fluidic channels may specifically be pneumatic channels, wherein only pressurized air or gas is permitted to flow. The diameter of the pneumatic channels may be of similar dimensions of fluidic channels as described herein. Such pneumatic channels may be configured for venting and rerouting air or gas within the cartridge when a sample is loaded.

[0326] The terms “fluidic communication” as used herein, refers to any duct, channel, tube, pipe, or pathway through which a substance, such as a liquid, gas, or solid may pass substantially unrestricted when the pathway is open. When the pathway is closed, the substance is substantially restricted from passing through.

[0327] It is noted that, as used herein, the term “input” refers to vias or channels of a cartridge where active pressurization is applied to motivate a liquid (i.e. a sample or reagent) or a gas (i.e. air) residing within a channel. As used herein, the term “output” refers to the leading front of said motivated liquid or gas which is displaced as a result of active pressurization and which terminates

at a via or channel for venting. In one aspect, the input and/or output may comprise one or more filter plugs for filtering a fluid. In one embodiment, a filter plug is configured to capture pollutants and particles from pressurized air. In another embodiment, a filter plug is configured to be hydrophobic to vent gasses while retaining liquids.

[0328] As described above, fluids are motivated throughout the fluidic network of a cartridge using pressurized air. Thus, the cartridge is configured to receive pressurized air through one or more pneumatic vias. In the cartridge exemplified in Figure 69A, main pneumatic via ***1193*** is present on the feature side of the cartridge. Each pneumatic via is in fluidic communication with a pneumatic channel, such that pneumatic channels enable the motive force to transport a sample and liquids through various modules within the cartridge.

[0329] Given the pressurization of the device, as will become apparent in the sections to follow, in some embodiments, the cartridge, optionally, includes a liquid trap configured to capture liquids to prevent contamination of various structures of the cartridge. The liquid trap, preferably, is formed by a widening or depression in a pneumatic channel, wherein liquid droplets fall to bottom of the depression thereby captured outside the main pneumatic flow within the cartridge. Alternatively, the liquid trap can be a physical structure, such as a sintered vent plug placed within the pneumatic channel.

[0330] In some embodiments, the cartridge cover can further include a cartridge label to supply the user and instrument with information associated with a given diagnostic test. FIGs. 69B and 90 illustrate an exemplary cartridge labels ***1005***. In some embodiments, the cartridge label includes a cut out to provide visual access to a sample window ***1050*** formed within a metering chamber ***1110***, enabling the user and/or a system, such as instrument ***2000*** as described herein, to view and detect the sample volume loaded into the device. Additionally, one cut out within the cartridge label may be configured to exclude the reaction area ***1600*** enabling the amplification and detection of target nucleic acids from optically transparent plugs, as described herein. A portion of the cartridge label, i.e., the patient label area ***1040***, in some embodiments, is configured to be written on to enable a user to provide patient information relating to the diagnostic test. Such information can include, for example, the name of the patient, the date of birth of a patient, and the sample type gathered from the patient. In some embodiments, the cartridge label may include computer readable information. In some embodiments, the cartridge label provides a computer readable visual code ***1053*** to store computer readable information. Such information can include, for example, the type of test the cartridge is configured to run and general manufacturing information, e.g., a lot number, expiration date, and/or recalls associated with the cartridge. In some implementations, the computer readable information is configured to be encrypted. The computer readable information may be configured to be read by a system or instrument, such as instrument ***2000,*** as described herein. In a further implementation, illustrated in FIG. 91, the cartridge label ***1005*** can include one or more perforated areas within said cartridge label configured to be broken when contacted. In one implementation, a perforated area ***1051*** exists

around the frangible seal area. In another implementation, a perforated area ***1052*** is located around the cartridge pneumatic interface to enable a pneumatic interface, for example like pneumatic interface ***2100*** of instrument ***2000*** to break the perforated area to make contact with the device.

[0331] Returning to FIGs. 69A and 69B, there is provided a cartridge orientation reference. In these views the cartridges are in a vertical orientation as in indicated by the arrow which corresponds to the orientation during use when processing a sample while within the instrument 2000. Treating the rotary valve as an origin, there is one dashed line extending along the longitudinal axis of the cartridge and another extending along the vertical axis of the cartridge. As a result, any cartridge embodiment may be described by reference to the cartridge distal end 1915, the cartridge proximal end 1920, cartridge upper portion 1905 and cartridge lower portion 1910. Thus, by using the dashed lines and this convention, the cartridge may be described according to an upper proximal portion 1914, upper distal portion 1907, lower proximal portion 1917 and lower distal portion 1912. By way of illustrative example, FIG. 69A is a top down view of another cartridge embodiment. In this embodiment there is a reaction area ***1600*** in the cartridge upper distal region ***1907***, a waste collection element ***1470*** in the cartridge lower distal region ***1912***, a cap for the sample port assembly ***1181*** is in the cartridge upper proximal region ***1914*** and exemplary manufacturing bar codes are located in the cartridge lower proximal region ***1917,*** but may be provided in other locations depending upon various configurations.

[0332] Additionally, in an aspect which aids in the mitigation or elimination of bubble formation during process, many of the various chambers in the integrated cartridge have been designed so that in a general way once the cartridge is in a vertical orientation fluids will flow from the top of a chamber of enclosure to the bottom of the chamber or enclosure. Even if a chamber or enclosure employs reversible flows for mixing or other purposes, there may still be advantages to this general design guideline of upper inlet and lower out placement. FIG. 70A provides a number of examples of this design guidance. Fill/sample chamber 1101 has a pneumatic inlet 1176 in an upper portion and a fill chamber outlet 1102 in a lower portion. Wash buffer reservoir 1475 has a wash inlet 1476 in an upper portion and a wash outlet 1477 in a lower portion. Lysis chamber 1371 has a lysis chamber inlet 1371 located in an upper portion of the lysis chamber while the lysis outlet/bead filter channels 1387 are provided in a lower portion of the lysis chamber.

[0333] Also shown in FIG. 70A are dashed lines for the enlarged views of FIG. 70B and FIG. 70C. FIG. 70B is an enlarged view of the waste collection element 1470 of FIG. 70A. A chamber reference line 1310 has been added to the waste collection element to divide it into an upper portion and a lower portion. The waste collection element provides an exception to the general design rule of in from the top and out from the bottom described above. Because of the special handling of the various waste products generated by cartridge operations as well as for venting, the waste collection element includes the inlet 1471 and as illustrated a number of waste outlets

1471. Both the inlet 1471 and the several outlets 1474 are in the upper chamber portion – that is above the chamber reference line 1310.

[0334] FIG. 70C is an enlarged view of the upper proximal portion 1914 and lower proximal portion 1917 of the cartridge of FIG. 70A. In this view, a chamber reference line 1310 has been added to each of fill/sample chamber 1101, wash buffer reservoir 1475 and the metering chamber 1120. As is clear from a chamber reference line in the approximal mid vertical point in the fill/sample chamber 1101, it is clear that the pneumatic inlet 1176 in an upper chamber portion that is above the chamber reference line. Still further, the fill chamber outlet 1102 is below the chamber reference line 1310 and is such in a lower chamber portion. Similarly, the reference chamber line 1310 placed on wash buffer reservoir 1475 makes clear that the wash inlet 1476 is in an upper chamber portion above the chamber reference line. The wash outlet 1477 is below the chamber reference line and is in a lower chamber portion. A similar result is found when considering the chamber reference line 1310 with respect to the metering chamber 1120. The inlet 1111 is above the reference line 1310 and is therefore in an upper chamber portion. The outlet 1115 is below the chamber reference line 1310 and is therefore considered in a lower chamber portion.

[0335] This general design guidance is summarized in the example chamber views of FIGs. 70D, 70E and 70F. FIG. 70D is a front view of an exemplary chamber as positioned in a vertical processing orientation as in FIGs. 69A and 70A. FIG. 70D is an exemplary chamber with a chamber reference line 1310 used to indicate an upper chamber portion and a lower chamber portion. To the general case of FIG. 70D, FIG. 70E illustrates the exemplary chamber of FIG. 70D having an inlet in a top middle or upper most portion of the upper portion. Along the same lines, the outlet is shown in the middle bottom or bottom most portion of the chamber bottom portion. FIG. 70F illustrates that the inlet and the outlet may be provided in a more general way but still within the chamber upper portion and the chamber lower portion and still within the design guidance of in from the top and out from the bottom. By way of illustrative example, if one were to apply a clock face to the exemplary chamber, the (i) the chamber reference line 1310 would run from the 9 o'clock position to the 3 o'clock position (FIG. 70D); (ii) the top most and bottom most positions would be positioned, respectively, at the 12 o'clock position and the six o'clock position (FIG. 70E); and the top zone and bottom zones would be positioned, respectively, between the 10 o'clock to 2 o'clock position and between the 4 o'clock and the 8 o'clock position.

[0336] Another advantageous design guide of several embodiment of the integrated diagnostic cartridge are also shown with reference to FIGs. 69A and 69B. Each cartridge embodiment includes a cartridge perimeter 1011 within which are arranged the various components of a specific cartridge embodiment. Additionally, there is a reaction area perimeter 1601 which is shown in relation to the reaction area 1600. The reaction area includes plugs 1770 (FIG. 69A) and assay chambers 1621 (FIG. 70A). As described herein, with reference to FIGs. 69A and 70A, each one of the plurality of individual assay chambers 1621 is in communication with an air

chamber 1631 (FIGs. 69A and 70A). In one embodiment, each air chamber 1631 is closer to the cartridge perimeter 1601 than the plug 1770 in each one of the plurality of individual assay chambers. 1621. In another aspect, each one of the plurality of individual assay chambers 1621 is in communication with an air chamber 1631. Additionally, each plug 1770 in each one of the plurality of individual assay chambers 1621 is within the reaction area perimeter 1601 and each air chamber 1631 is outside of the reaction area perimeter 1601. In another aspect, there is an integrated diagnostic cartridge having a perimeter 1011 and a reaction area perimeter 1601. Each one of the plurality of individual assay chambers 1621 of the cartridge is in communication with an air chamber 1631. Still further, each air chamber 1631 is closer to the cartridge perimeter 1011 than the plug 1770 in each one of the plurality of individual assay chambers 1621. Each air chamber 1631 is located outside of the reaction area perimeter 1601 and each one of the plurality of individual assay chambers 1621 (and plug 1770) is within the reaction area perimeter 1601.

1. Loading Module

[0337] In one embodiment, a cartridge of the present invention comprises a loading module configured to, e.g., accept a sample, prevent the sample from spilling liquids outside the cartridge, and optionally prepare the sample for lysis. The loading module defines a sample volume used to perform a diagnostic test. In some implementations, the loading module includes a metering chamber and an overflow chamber to produce a metered sample volume. The loading module may further comprise a mechanism for detecting a sufficient sample volume is present in the device. A window may be included to allow a user or an instrument to detect the mechanism indicative of a sufficient sample volume. In another implementation, the loaded sample is drawn into the cartridge using a converging channel.

[0338] In some embodiments the loading module comprises a sample port assembly ***1100*** disposed within the cartridge. Optionally, the sample port assembly ***1100*** is configured to produce a metered sample of predetermined volume. Specifically, as discussed in greater detail below regarding FIG. 71, the sample port assembly comprises an entry port ***1140***, a fill chamber ***1101***, a metering chamber ***1110***, metering channel ***1113***, an overflow chamber ***1120***, overflow channel ***1122***, vent ***1165***, and gas conduit ***1150***. The entry port ***1140*** of the assembly defines an opening of the fill chamber to receive a sample, wherein the fill chamber ***1101*** is in fluidic communication with metering chamber ***1110***. A sample loader, such as a bulb, syringe or pipette ***1060***, can be useful for loading a sample into the cartridge.

[0339] The fill chamber has dimensions including a volume, said volume being between 100 µl and 15 ml, between 200 µl and 7.5 ml, between 0.5 ml and 5 ml, between 0.5 ml and 3 ml, between 5 ml and 10 ml, between 1 ml and 3 ml, between 0.5 and 1.5 ml. While the fill chamber illustrated in the FIGs. 69-71 is configured to hold up to 2.4 ml of fluid, cartridges of the invention may accommodate larger sample volume by increasing the depth of the fill chamber. Increasing the depth of the fill chamber results in an overall thickness increase of the cartridge as a function of the depth of the fill chamber. Advantageously, increasing the thickness of the cartridge can

allow for increased volume of chambers holding liquid reagents and the waste chamber as discussed in greater detail below. Thicker cartridges can be accommodated by the instrument simply by changing the clamping position of the moving bracket assembly ***2040*** as described herein.

[0340] When implemented, the metering chamber ***1110*** is in fluidic communication with the fill chamber ***1101*** via a metering channel ***1113***. In some embodiments, the metering chamber includes a mechanism for detecting a sample volume present within the metering chamber, e.g., a buoyant ball ***1114***. The ball may be detected through a sample window ***1050*** by a user or instrument ***2000*** for indicating an adequate sample volume is in the metering chamber ***1110*** prior to executing a diagnostic test. Alternatively, the meniscus of the fluid sample can be detected through the sample window by a user or instrument. Referring to FIG. 68, the label imaging system ***2770***, that captures an image of the patient label area, can also capture an image of the meniscus or buoyant ball ***1114*** through the sample window ***1050***. The metering chamber has dimensions including a volume, which can range from 0.1 to 10 ml, from 0.5 to 5 ml, from or 1 to 3 ml.

[0341] When implementing a metering chamber, the cartridge typically further comprises an overflow chamber ***1120*** configured to capture excess sample that was loaded into the fill chamber ***1101*** and cannot be accommodated by fully filled metering chamber. The overflow chamber is in fluidic communication with the metering chamber ***1110*** through an overflow channel ***1122***, such that excess sample flows though the overflow channel and is retained in the overflow chamber. Taking advantage of the vertical orientation of the cartridge with the instrument, the sample flows from the fill chamber ***1101*** through the metering channel ***1113*** into the top of the metering chamber ***1110***. Once the metering chamber ***1110*** is filled, any excess fluid remaining in the fill chamber passes from the metering channel ***1113*** to the overflow channel ***1122*** and then to the overflow chamber ***1120*** without substantially entering the metering chamber. This geometry can be advantageous for instances in which the metering chamber comprises a chemical or enzymatic agent to pretreat the sample prior to passing the sample on to the lysis module. After metering, the meter fluid within the metering channel can be withdrawn from the metering chamber though a channel at the bottom of the metering chamber. In a preferred embodiment, the lower bound of the metering chamber is angled toward the exit such that gravity will assist in emptying the chamber.

[0342] The sample port assembly ***1100*** further comprises a structure for separating the sample from the outside environment, e.g. a cap ***1181*** configured to be opened to permit addition of a sample and then resealed prior to the sample being loaded to the device. Given the pressurization inherent to the devices described herein, the closure, i.e. the cap, preferably is air tight. The configuration, as described herein, creates an airlock within the loading module to prevent sample within fill chamber ***1001*** from passing to the metering chamber ***1110*** until actuated by pressurization. Such an airlock further prevents liquid from entering the metering chamber when the device is tilted vertically.

[0343] In some embodiments, the loading module is configured to be emptied using pneumatic force. Specifically, a sample is transferred from the fill chamber ***1001*** to the metering chamber ***1114*** when pneumatic line ***1171*** to the fill chamber is pressurized. In one implementation, the port is pressurized using constant pressure. In another implementation the port is pressurized using a series of applied pressure pulses each followed by a period of zero applied pressure. In the instance where an excess sample volume is present, excess sample enters overflow channel ***1121*** and is retained in the overflow chamber ***1120***.

[0344] In an additional specific aspect implemented to mitigate or eliminate bubble formation during sample processing, embodiments of the integrated diagnostic cartridge may incorporate an antifoaming agent that is mixed with the sample during sample processing. The use of an antifoaming agent may be advantageous for reducing the bubbling of protein-rich and/or surfactant-rich mixtures, e.g. a sample combined with lysis reagents, in implementations where pneumatic pressure drives fluid movement. Surfactant- and protein-rich mixtures can easily generate bubbles and/or become foamy. The resulting bubbles increase the difficultly of directing fluids by means of gravity in a vertically oriented cartridge and may interfere with downstream optical visualization of reactions within assay chambers. In one embodiment, an antifoaming agent is including within the loading module. The antifoaming agent may be in liquid or dried form. Depending on the properties of a selected antifoam agent, the antifoam agent may be used in full strength or in any suitable concentration based on properties of the antifoam agent, sample type, specific cartridge designs, cartridge volume and other factors. In one embodiment, the antifoaming agent may be diluted by combination with another a fluid such as an engineered fluid or a solvent. In one exemplary embodiment, the antifoam agent is the silicone fluid antifoam compound available commercially from The Dow chemical company under the trade name XIAMETER™ ACP-0001. In one exemplary embodiment, the antifoam agent is diluted with an engineered fluid solvent available under the trade name Novec™ 7000 commercially available from Sigma Aldrich, Inc. In one specific implementation, 2 microliters of the antifoam agent is combined with 100 microliters of the engineered fluid solvent and dried to produce a dried antifoam agent. During cartridge manufacturing, the dried antifoam agent is included in the sample fill chamber. As a result, for integrated cartridge embodiments that include a dried antifoam agent, when a user introduces a sample into the fill chamber as part of the sample loading process (see FIGs. 2A-2C), the sample is combined with the dried antifoam agent. Thereafter, the cartridge is accepted as explained with regard to FIGs. 3, 4A and 4B and will proceed to process the sample combined with the antifoam agent.

[0345] In an alternative embodiment shown in FIG. 72, the loading module may comprise an entry port in fluidic communication with a reservoir containing a converging channel and one or more diverging channels. Such a configuration enables a loaded sample to be drawn to the distal end of the converging channel, wherein the sample exits the converging channel and fills the one or more diverging channels. Further description of a sample port configured to wick a sample can

be found in U.S. patent application titled "Vented Converging Capillary Sample Port and Reservoir," filed 13 September 2018, and assigned application serial no. 16/130,927, which is incorporated by reference herein.

[0346] Lysis Module

[0347] The cartridge further comprises a lysis module configured to disrupt cell walls and/or cells membrane to release inter-cellular materials such as nucleic acids (DNA, RNA), protein or organelles from a cell. In a one implementation, the lysis module comprises a lysis chamber ***1371*** and a stir bar ***1390***. In one aspect, the lysis module may further include a filter assembly to rid the sample of cell debris after lysis to minimize the potential for clogging downstream features in the cartridge.

[0348] In a preferred implementation, the lysis module comprises a mixing assembly for combining the sample with one or more lysis agents. In one embodiment, illustrated in FIG. 70A, the cartridge includes a mixing assembly comprising a lysis chamber *1371* and a stir bar ***1390***. The lysis chamber ***1371*** is configured to receive the sample from a sample transfer channel ***1386*** through inlet ***1373*** that is preferably located at or near the top of the lysis chamber ***1371***. In some implementations, the cartridge comprises a chemical lysis reagent prior to loading the sample. When the chemical lysis reagent is a liquid, the reagent preferably is sealed into the lysis chamber prior to use of the cartridge. In one such embodiment, the channel leading to the lysis chamber ***1386*** and the channel for draining the lysis chamber ***1388*** are both closed with a frangible seal prior to use. When the cartridge is inserted into the instrument and readied for use, the frangible seals are broken, thus permitting pressurized air to transfer the sample into the lysis chamber for lysis. The magnetic mixing assembly, as described herein with regard to instrument ***2000***, is activated to rotate the stir bar ***1390*** and thereby mix the sample with the one or more lysis reagents.

[0349] When used with the balanced magnet mixing assembly ***2300*** described above, the stir bar ***1390*** need not be a permanent magnet. In a preferred implementation, the stir bar is a composed of a ferromagnetic material, which is not magnetized in the absence of an external magnetic field. In some embodiments, the ferromagnetic material of the stir bar is ferritic stainless steel or duplex stainless steel. In additional embodiments, a relative magnetic permeability of the stir bar can be between 500-1,000,000. The stir bar can comprise any shape and/or volume. For example, the shape of the stir bar can be selected from a group consisting of cylindrical, spherical, and triangular-prism-shaped. Further description of stir bar, lysis chamber and balanced magnetic mixing assembly can be found in US patent publication 2019/0160443 A1, titled "Magnetic Mixing Apparatus," which is incorporated by reference herein.

[0350] In embodiments where one of the one or more lysis reagents is a chemical agent, the ferromagnetic material is preferably coated with an inert material to protect the stir bar from corrosion and to deter release of iron, a suspected inhibitor of amplification, from being released into the lysate. One of ordinary skill in the art would be able to select an appropriate impermeable

material that would not interfere with magnetic flux through the stir bar. Example materials include, but are not limited to PTFE, parylene C, parylene D, functionalized perfluoropolyethers (PFPEs), FEP, Xylan Fluoropolymer, epoxy, and urethane. Similarly, the impermeable material can be applied to the stir bar by any method known in the art, such as by tumble coating. In one implementation, the ferromagnetic material of the stir bar is passivated prior to coating. In a preferred implementation, the stir bar is tumble-coated with a layer of parylene C between 20 µm and 200 µm thick.

[0351] By placing a ferromagnetic stir bar within the lysis chamber ***1371*** located in a gap between a driving magnet system ***2310*** and a driven magnet system ***2350*** of instrument ***2000***, a magnetic dipole can be induced across and within the stir bar. This dipole of the stir bar effectuates a magnetic coupling between the stir bar ***1390***, the one or more driving magnets of the driving magnet system ***2310***, and the one or more driven magnets of the driven magnet system ***2350***. Specifically, the introduction of the stir bar ***1390*** into the magnetic field causes the stir bar to be attracted to the one or more driving magnets and the one or more driven magnets. In preferred embodiments in which a magnetic strength of the corresponding driving magnet equals a magnetic strength of the driven magnet, and the driving magnet magnetic axis is substantially collinear with the driven magnet magnetic axis, attraction of the stir bar to the driving magnet and driven magnet causes the stir bar to be located roughly equidistant from driving magnet and driven magnet. In an even further preferred embodiment in which a center of the lysis chamber ***1371*** is located an equal distance from the driving magnet system and the driven magnet system, as a result of the attractive forces between the stir bar and the one or more driving magnets and the stir bar and the one or more driven magnets, the stir bar can be centered within the lysis chamber thereby minimizing the amount of contact between the stir bar and the bounding surface.

[0352] In some embodiments, the lysis chamber ***1371*** further comprises beads. In such embodiments, mixing the fluid sample with the beads promotes lysis of the one or more cells. Preferably, the sample and beads, plus optionally one or more additional lysis reagents, are stirred at least 500 rpm, at least 1000 rpm, at least 2000 rpm or at least 3000 rpm for at least 15 seconds 30 seconds, 60 seconds or 2 minutes to generate a lysed sample, or lysate. Following mixing of the fluid sample with the beads, the lysate is removed from the lysis chamber. In a preferred embodiment, the beads are separated from the fluid sample in conjunction with the sample being removed from the lysis chamber. To separate the beads from the fluid sample, in some embodiments, bead filter channels ***1387*** are appended to the lysis chamber. The bead filter channels are located along an edge of the lysis chamber and are configured to retain the beads in the lysis chamber while allowing the fluid sample to exit. Preferably the bead filter channels are located at the bottom of the lysis chamber to take advantage of gravitation forces to move the lysate from the lysis chamber without generating bubbles or foam in the lysate. In a preferred implementation, a cross sectional area of each bead filter channel comprises a first dimension such that the beads are too large to enter the bead filter channels, and a second dimension such

that the beads are unable to block fluid flow. In this way use of the bead filter channels enables fluid to be drawn from the lysis chamber without beads.

[0353] In some implementations, the lysis module further comprises a process control. A process control establishes a factor of confidence in a test result when executing a diagnostic test. Controls are treated and tested in parallel with target pathogen and are used to generate a predetermined expected result. When the expected result is reported, one or more aspects of the diagnostic test are confirmed to be working as intended, enabling the user of to verify the diagnostic test as valid. However, when the predetermined result is not obtained, one or more aspects of the test does not meet the expected performance and would invalidate the test results obtained from a cartridge. In one embodiment, a cartridge can include a process control chamber ***1130*** comprising an inlet ***1131***, an outlet ***1132***, and a control plug ***1133*** for doping a sample with a process control. In one aspect, sample within the metering chamber is flowed through the process control chamber to dope a sample with a process control. In a further embodiment, the process control is a positive control. Prior to mixing the sample with at least one lysis agent, a process control can be added to the sample. In such an implementation, one of the assay chambers will comprise a primer set specific to a nucleic acid sequence found in the process control. The process control chamber is exemplified in FIGs. 69-71.

[0354] Preferably, the process control can function as a positive control for lysis, purification and amplification within the cartridges described herein. One exemplified process control is a bacterial spore, such as a spore of a *Bacillus* species. Bacterial spores typically are more difficult to lyse than any other target cell and can therefore serve as a universal control for cell lysis. Suitable spores can be comprised of any species of *Bacillus*, including, e.g., *Bacillus globgii*, *Bacillus atrophaeus*, *Bacillus subtilis*, and *Bacillus stearothermophilus*. Alternatively, a process control can be added to the lysed sample prior to passing the lysed sample through the porous solid support. Such a process control would act as a positive control for purification and amplification, but not lysis.

[0355] In some implementations, the cartridge further comprises one or more filter assemblies ***1330*** to remove undesired cellular material and debris from a sample by passing the sample through filter assembly ***1330***. A filter assembly comprises at least a filter, an inlet, and an outlet. In one implementation, the lysis module comprises a filter assembly located before the lysis chamber to filter a sample before lysing. In another embodiment, the lysis module comprises a filter assembly placed after the lysis chamber to filter a lysed sample. Specifically, the lysis module can comprise one or more filter assemblies located downstream of the lysis chamber.

[0356] FIGs. 73-75B illustrate a filter assembly according to one embodiment described herein. FIGs. 74 and 75A provide section views through the cartridge depicting a filter assembly ***1330***. FIG. 75B illustrates an enlarged in view of the exemplary filter assembly during operation when pressurized. In one embodiment, the filter assembly ***1330*** comprises a filter ***1331***, an inlet via ***1332***, an outlet via ***1333***, flow directors ***1334***, a filter plug ***1336***, and a pneumatic interface cover

adaptor ***1172***. Filter ***1331*** can be configured to capture one substance, e.g., larger cells, more effectively, e.g., substantially more effectively, than another substance, e.g., a liquid, such as a sample suspected on contained a target pathogen, when the substances are exposed to the filter and at least one of them is moved substantially therethrough. For example, a filter ***1331*** can enable the solid components, such as, e.g., cells, debris or contaminant, to be separated from the liquid components of the solution. Alternatively, a filter can enable larger solid components, such as, e.g., proteinaceous aggregates, aggregated cell debris, or larger cell, to be separated from smaller components, e.g. virus, bacterial cells or nucleic acid, from a solution. In aspects of this embodiment, a filter useful for separating components contained in a solution can be, e.g., a size-exclusion filter, a plasma filter, an ion-exclusion filter, a magnetic filter, or an affinity filter. In other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., 0.1 µm, 0.2 µm, 0.5 µm, 1.0 µm, 2.0 µm, 5.0 µm, 10.0 µm, 20.0 µm, 30 µm, 40 µm, 50 µm, 60 µm, 70 µm, 80 µm, 90 µm, 100 µm, or more. In yet other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., at least 0.2 µm, at least 0.5 µm, at least 1.0 µm, at least 2.0 µm, at least 5.0 µm, at least 10.0 µm, at least 20.0 µm, at least 30.0 µm, at least 40.0 µm, at least 50.0 µm, at least 60.0 µm, at least 70.0 µm, at least 80.0 µm, at least 90.0 µm, or at least 100.0 µm. In still other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., at most 0.1 µm, at most 0.2 µm, at most 0.5 µm, at most 1.0 µm, at most 2.0 µm, at most 5.0 µm, at most 10.0 µm, at most 20.0 µm, at most 30.0 µm, at most 40.0 µm, at most 50.0 µm, at most 60.0 µm, at most 70.0 µm, at most 80.0 µm, at most 90.0 µm, or at most 100.0 µm. In other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size between, e.g., about 0.2 µm to about 0.5 µm, about 0.2 µm to about 1.0 µm, about 0.2 µm to about 2.0 µm, about 0.2 µm to about 5.0 µm, about 0.2 µm to about 10.0 µm, about 0.2 µm to about 20.0 µm, about 0.2 µm to about 30.0 µm, about 0.2 µm to about 40.0 µm, about 0.2 µm to about 50.0 µm, about 0.5 µm to about 1.0 µm, about 0.5 µm to about 2.0 µm, about 0.5 µm to about 5.0 µm, about 0.5 µm to about 10.0 µm, about 0.5 µm to about 20.0 µm, about 0.5 µm to about 30.0 µm, about 0.5 µm to about 40.0 µm, about 0.5 µm to about 50.0 µm, about 1.0 µm to about 2.0 µm, about 1.0 µm to about 5.0 µm, about 1.0 µm to about 10.0 µm, about 1.0 µm to about 20.0 µm, about 1.0 µm to about 30.0 µm, about 1.0 µm to about 40.0 µm, about 1.0 µm to about 50.0 µm, about 2.0 µm to about 5.0 µm, about 2.0 µm to about 10.0 µm, about 2.0 µm to about 20.0 µm, about 2.0 µm to about 30.0 µm, about 2.0 µm to about 40.0 µm, about 2.0 µm to about 50.0 µm, about 5.0 µm to about 10.0 µm, about 5.0 µm to about 20.0 µm, about 5.0 µm to about 30.0 µm, about 5.0 µm to about 40.0 µm, about 5.0 µm to about 50.0 µm, about 10.0 µm to about 20.0 µm, about 10.0 µm to about 30.0 µm, about 10.0 µm to about 40.0 µm, about 10.0 µm to about 50.0 µm, about 10.0 µm to about 60.0 µm, about 10.0 µm to about 70.0 µm, about 20.0 µm to about 30.0 µm, about 20.0 µm to about 40.0 µm, about 20.0 µm to about 50.0 µm,

about 20.0 µm to about 60.0 µm, about 20.0 µm to about 70.0 µm, about 20.0 µm to about 80.0 µm, about 20.0 µm to about 90.0 µm, about 20.0 µm to about 100.0 µm, about 30.0 µm to about 40.0 µm, about 30.0 µm to about 50.0 µm, about 30.0 µm to about 60.0 µm, about 30.0 µm to about 70.0 µm, about 30.0 µm to about 80.0 µm, about 30.0 µm to about 90.0 µm, about 30.0 µm to about 100.0 µm, about 40.0 µm to about 50.0 µm, about 40.0 µm to about 60.0 µm, about 40.0 µm to about 70.0 µm, about 40.0 µm to about 80.0 µm, about 40.0 µm to about 90.0 µm, about 40.0 µm to about 100.0 µm, about 50.0 µm to about 60.0 µm, about 50.0 µm to about 70.0 µm, about 50.0 µm to about 80.0 µm, about 50.0 µm to about 90.0 µm, or about 50.0 µm to about 100.0 µm. An artisan of ordinary skill may select an appropriate filter based on considerations such as sample type and the target pathogen of interest.

[0357] In certain implementations, the filter can be a depth filter. Depth filters consist of a matrix of randomly oriented, bonded fibers that capture particulates within the depth of the filter, as opposed to on the surface. The fibers in the depth filter can be comprised of glass, cotton or any of a variety of polymers. Exemplified depth filter materials may include, type GF/F, GF/C and GMF150 (glass fiber, Whatman), Metrigard® (glass fiber, Pall-Gelman), APIS (glass fiber, Millipore), as well as a variety of cellulose, polyester, polypropylene or other fiber or particulate filters, so long as the filter media can retain a sufficient contaminant to allow further processing of the sample.

[0358] In alternate implementations, the size-exclusion filter can be a membrane filter, or mesh filter. Membrane filters typically performs separations by retaining particles larger than its pore size on the upstream surface of the filter. Particles with a diameter below the rated pore size may either pass through the membrane or be captured by other mechanisms within the membrane structure. Membrane filters can support smaller pore sizes, including small enough to exclude bacterial cells. Membrane filters can be used to concentrate solutions, e.g. bacterial cell suspensions, by filtering a first larger volume through the membrane filter, thereby holding the bacterial cells to the upstream surface of the membrane filter (or suspended in residual fluid retained on the upstream side of the filter). The bacterial cells can then be resuspended in a second small volume of fluid by either passing the suspension fluid in the reverse direction to float the bacterial cells off the membrane surface or by washing the suspension fluid across the upstream surface of the filter to wash the bacterial cells off the filter. Exemplified membranes may include, polyethersulfone (PES) membranes (e.g., Supor® 200, Supor® 450, Supor® MachV (Pall-Gelman, Port Washington, N.Y.), Millipore Express PLUS® (Millipore)). Other possible filter materials may include, HT Tuffryn® (polysulfone), GN Metricel® (mixed cellulose ester), Nylaflo® (Nylon), FP Verticel (PVDF), all from Pall-Gelman (Port Washington, N.Y.), and Nuclepore (polycarbonate) from Whatman (Kent, UK).

[0359] In various embodiments, the filter can be enclosed by a frame, a chamber, or any other housing for containing the filter material. In some embodiments, the frame holding the filter can be integrated into another cartridge structure adjacent to the filter assembly. In one

implementation, the filter assembly comprises a filter fixedly attached, e.g., laser welded, to a feature side ***1007*** of a fluidics card ***1001***, as shown in FIGs. 74, 75A, and 75B. Further, the illustrated embodiment depicts that the frame enclosing filter ***1331*** is provided by pneumatic interface cover adaptor ***1172***. Such arrangement produces deformation space ***1335*** which is formed between the filter ***1331*** and the pneumatic interface cover adaptor ***1172***. The deformation space is most readily apparent in FIG. 75A, wherein the filter ***1331*** flush against fluidic card ***1001*** prior to pressurizing the cartridge during operation. In another implementation, the filter frame, i.e. the pneumatic interface cover adaptor ***1172***, is configured with a plurality of flow directors ***1334*** integrally formed within the body of the pneumatic interface cover adaptor for directing a filtered liquid to an outlet via.

[0360] In many implementations, a filter assembly is configured to filter a liquid, e.g. a sample or lysate, when pressurized by the instrument pneumatic subsystem. FIG. 73 shows a top view of the exemplary filter assembly ***1330*** described herein. An inlet via ***1332*** provides the opening for a fluid to enter the filter assembly. As best shown in the cross section view of FIG. 74, inlet ***1332*** permits a liquid to advance from fluidic side ***1006*** to the feature side ***1007.*** In the exemplary embodiment where the filter is fixedly attached to the fluidic card, fluid pressure is generated as a result of the liquid entering the filter assembly ***1330*** and causes filter ***1331*** to expand, i.e. deflect away from feature side of fluidic card ***1101***. FIG. 75B is an enlarged section view of the filter assembly shown in FIG. 75A when pressurized during cartridge operation. Expansion of the filter is accommodated by deformation space ***1335*** (FIG. 75A), such that the filter ***1331*** is permitted to expand until contacted by flow directors ***1334***. The contacting of filter ***1331*** against flow directors ***1334*** transforms deformation space ***1335*** into a plurality of defined channels, wherein three surfaces are formed by the pneumatic interface cover adaptor ***1172*** (specifically, two surfaces are formed by flow directors ***1334***), and one surface of each channel is formed by the porous filter ***1331***. Accordingly, the active pressurization advances the liquid through the filter. Substances, e.g., a sample, such as a lysed sample, is transmitted through the filter while other substances, e.g., particles, such as larger cells or cell debris, is prevented from passing therethrough to produce a filtered sample. The resulting filtered liquid is collected in the plurality of channels formed by the flow directors ***1134*** and is directed toward outlet ***1333*** shown at the bottom of the filter assembly in FIG. 73. Further, filter ***1331*** includes a cutout around outlet via ***1333*** to permit the filtered sample to enter the outlet via and travel from the feature side ***1007*** to the fluidic side ***1006*** of fluidic card ***1001***.

[0361] In other aspects, the pneumatic interface cover adaptor can be a structure which receives pressurized air from the instrument pneumatic interface ***2100***. In various embodiments, the pneumatic interface cover adaptor is configured to hold a filter plug ***1336*** for filtering the pressurized air input. As illustrated in FIG. 75A, pressurized air enters input via ***1195*** of the cartridge pneumatic interface ***1170*** and is filtered by filter plug ***1336*** before exiting the main pneumatic via ***1193*** and pneumatic via ***1194***, such that main pneumatic via ***1193*** is fluidically

coupled to the main pneumatic line ***1171*** and pneumatic via ***1194*** is fluidically coupled to pneumatic line ***1178***.

2. Purification Module

[0362] The cartridges of the invention further comprise a purification module for capturing nucleic acids from a lysed sample. In one aspect the purification module is configured to purify a lysed sample using a rotary valve, wherein the rotary valve comprises a porous solid support. The porous solid support captures nucleic acid while allowing the remainder of the sample and liquid waste to be directed to a waste collection element. In such an embodiment, the device additionally includes reagent reservoirs to store on-board reagents necessary for sample purification.

[0363] In one aspect, the purification module comprises one or more rotary valves comprising an integrated flow channel containing a porous solid support for filtering, binding and/or purifying analytes within a fluid stream. In one implementation, the rotary valve comprises a stator ***1450*** comprising a stator face and a plurality of passages ***1454***, each passage comprising a port ***1453*** at the stator face; a rotor ***1410*** operably connected to the stator and comprising a rotational axis, a rotor valving face, and a flow channel having an inlet ***1441*** and an outlet ***1442*** at the rotor valving face, wherein the flow channel comprises a porous solid support ***1445***; and a retention element ***1490*** biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal.

[0364] The rotor usable in the devices and methods described herein typically include a first face, e.g., a valving face ***1412***, and a second face, e.g., outer face ***1413*** (not shown), opposite the first face. The valving face and/or outer face can each be planar or have a planar portion. In such circumstances, the rotational axis of the rotor is perpendicular or substantially perpendicular to the valving face and/or the outer face. Also, in a cylindrical rotor, a rotational axis can be defined by and/or be a portion of the rotor located equidistant or substantially equidistant from all points on an outermost radial edge of the rotor or on an outermost radial edge of the rotor and/or outer face. The rotor valve face ***1412*** optionally comprises a gasket ***80***. The valving face typically also will comprise one or more fluid handling features, such as an inlet and/or outlet to a flow channel, a fluidic connector or a fluidic selector. In some embodiments, it may be advantageous to use fluidic handling features, e.g. a connector or selector further described herein, integrally formed within the rotor valving face or a gasket to deliver exact volumes of fluid to selected portions of the cartridge. In one exemplary embodiment, in operation, a rotor may be indexed to a position allowing fluidic communication between one stator port and a fluidic connector comprising a known connector volume. A fluid may be introduced into the fluidic connector within the rotor valving face, via the stator port, thus filling the fluidic connector volume with fluid against a hard stop. Subsequently, the rotor may be indexed to a second position to transfer the volume of fluid within the connector to a desired cartridge location when fluidic communication is established between an inlet and outlet stator port. Such embodiment is advantageous in methods where delivery of exact fluid volumes is desired, e.g. aliquoting and performing dilutions.

[0365] In some aspects, a rotary valve includes a gasket between the stator face and the rotor valving face. A gasket is a mechanical seal that fills a space between two or more mating surfaces of objects, generally to prevent leakage from or into the joined objects while the gasket is under compression. In various aspects, the gasket is composed, e.g., entirely composed, of an elastic and/or compressible material. In some versions, the rotor comprises the gasket and in other versions, the stator comprises the gasket. In embodiments wherein the rotor comprises a gasket, is fixedly, e.g., adhesively, attached to a rotor and forms a sliding interface along the stator. Also, in those embodiment where the stator comprises the gasket, the gasket is fixedly, e.g., adhesively, attached to a stator and forms a sliding interface along the rotor.

[0366] One embodiment of a rotary valve gasket is shown in FIGS. 76B and 76C. Specifically, FIGS. 76B and 76C illustrate a gasket ***1480*** configured to slidably engage a stator. The gasket ***1480*** also includes a first inlet ***1484*** aligned with the inlet ***1441*** of the rotor's flow channel, and a outlet ***1485*** aligned with the outlet ***1442***.

[0367] As used herein, a fluid handling feature is a physical structure in the rotor or gasket that, when aligned with two stator ports, fluidically connect the two ports and associated passages to form a continuous fluidic path. In some embodiments, the fluid handling feature is a fluidic connector ***1486***. A fluidic connector is configured to fluidically connect a first stator port to a second stator port. In implementations, such as illustrated in FIGS. 76B and 76C, the fluidic connector is an elongated groove in the rotor or gasket with the longest dimension along a line radiating from the center of the rotor. Such a radially aligned fluidic connector is capable of sequentially connecting a plurality of pairs of stator ports, wherein each of the plurality of pairs has one proximal port and one distal port, wherein all proximal ports are one distance from the rotational axis and all distal ports are a second, larger, distance from the axis. Alternatively, a fluidic connector may be configured to fluidically connect a first stator port to a second stator port along the same arc, i.e. stator ports along different points around the rotational axis with an equal radial distance. In some embodiments, the fluid handling feature is a flow channel, wherein when the flow channel inlet is aligned with one stator port and the flow channel outlet is aligned with a second stator port, the full volume of the flow channel fluidically connects the two stator ports. Accordingly, the flow channel can act as a fluidic connector. In some embodiments, the fluid handling feature is a fluidic selector **77** having a first portion that is an arc with all points along the first portion being equidistant from the rotational axis, and a second portion extending radially toward or away from the center of the rotor.

[0368] One aspect of the invention provides a rotary valve having a rotor wherein the rotor valving face comprises a first fluidic connector, wherein in a first rotor position a first port of the stator is fluidically connected to a second port of the stator via the first connector. In a second rotor position, a third port is fluidically connected to a fourth port via the first fluidic connector. Optionally, in a third rotor position, a fifth port is fluidically connected to a sixth port via the first

fluidic connector. In one implementation, the fluidic connector is an elongate groove. In another implementation, the fluidic connector is a flow channel in the rotor.

[0369] In various aspects, a gasket is substantially cylindrical and/or disk-shaped wherein the distance between the axis of rotation and the outer circumference of the gasket is greater than the distance between the axis of rotation to the most distant port on the stator. In some embodiments, such as illustrated in FIGS. 76B and 76C, the gasket is annular having an outer circumference beyond the most distant stator port as described above and wherein the distance between the axis of rotation and inner circumference of the annulus is less than the distance between the axis and the most proximal stator port. A gasket can have an outer cross-sectional diameter such as any of the rotor diameters provided herein. A gasket can have an out cross-sectional diameter, for example, of 100 mm or less, such as 45 mm or less, such as 50 mm or less, such as 40 mm or less, such as 20 mm or less, such as 10 mm or less. The inner and outer gasket diameters can range, for example, from to 1 mm to 100 mm, 3 mm to 50 mm, 3 mm to 25 mm or 5 mm to 35 mm. A gasket can also have a thickness such as any of the thicknesses of device components provided herein, such as 10 mm or less, such as 5 mm or less, such as 1 mm or less or 1 mm or more, 5 mm or more, or 10 mm or more.

[0370] In various aspects, a gasket is composed, e.g., entirely composed, of one or more polymeric materials (e.g., materials having one or more polymers including, for example, plastic and/or rubber and/or foam). A gasket can also be composed of a silicone material. A gasket can be composed of any of the elastic materials provided herein. Gasket materials of interest include, but are not limited to: polymeric materials, e.g., plastics and/or rubbers, such as polytetrafluoroethene or polytetrafluoroethylene (PTFE), including expanded polytetrafluoroethylene (e-PTFE), polyester (Dacron™), nylon, polypropylene, polyethylene, , polyurethane, etc., or any combinations thereof. In some embodiments, the gasket comprises Neoprene (polychloroprene), a polysiloxane, a polydimethylsiloxane, a fluoropolymer elastomer (e.g. VITON™), a polyurethane, a thermoplastic vulcanizate (TPV, such as Santoprene™), butyl, or a styrenic block copolymer (TES/SEBS).

[0371] According to some embodiments, a gasket includes one or more apertures fully penetrating the thickness of the gasket. In those implementations, wherein the gasket is affixed to the stator, the gasket comprises an aperture corresponding to and aligned with each stator port, to permit fluid flow therethrough. In implementations wherein the gasket is affixed to the rotor, the gasket comprises an aperture corresponding to and aligned with each of the flow channel inlet and outlet, if present, to permit flow across the rotor-stator interface. In alternative embodiments, the gasket may include one or more apertures, structures, or geometries partially formed therethrough. Such embodiment may be useful for delivering small precise volumes of fluid to various locations within the cartridge. FIGs. 12, 17A, 19A, 19C, and 29 illustrate an exemplary embodiment of a rotary valve ***1400*** in an operational position within a vertically oriented diagnostic cartridge. For the purposes of understanding, the cross section view of FIG. 76A

illustrates an orientation of the rotor and the stator not in an operational position. However, associated cartridge axis are depicted alongside the rotor cross section to demonstrate the operational orientation and is further described below. The rotor may be indexed rotationally, with respect to to the stator, e.g. a fluidic card, such that a fluid pathway is established through a plurality of features formed within a stator, a rotor and optionally a gasket. As a result, a flow channel ***1440*** within the rotor body ***1411*** provides fluid communication with the porous solid support ***1445*** within the solid support chamber ***1446*** to purify a lysed sample and produce an enriched nucleic acid when introduced into the solid support chamber. In many embodiments, fluid communication to the flow channel ***1440*** is accessed when stator ports within the stator face align with fluid conduits integrally formed within the rotor. In a further embodiment, a gasket, may interpose the rotor and stator at a rotor-stator interface to facilitate a fluid-tight seal.

[0372] Specifically, the exemplary fluid pathway in FIG. 76A begins at the stator ***1450***. A lysed sample first enters at a first stator fluid passage ***1454a*** and stator port ***1453a*** and advances through the gasket ***1480*** via the gasket inlet ***1484***. The fluid enters the rotor body ***1411*** via inlet ***1441*** and traverses through the first fluid conduit ***1443***. The outlet of the first conduit ***1443*** leads to a fluid pathway defined by a spacing between the rotor upper surface and a bottom surface of the cap cover ***1430***. The upper surface of the rotor body in this region is shaped to include a short channel to provide a portion of desired flow path between the first fluid conduit **1443** and the solid support chamber ***1446***. The partial flow path is completed when the cap cover ***1430*** is secured to the rotor top surface, thus allowing a filtered sample exiting the first fluid conduit to travel in a direction along the cartridge width axis **1025** (additionally see FIGs. 68-72) when oriented in an operational position as shown in FIG. 29. Next, the fluid enters the solid support chamber ***1446*** containing the porous solid support ***1445***. Fluid passes through the porous solid support ***1445*** to generate a purified or enriched nucleic acid and is directed to the bottom of solid support chamber to the second conduit ***1444*** and exits via a rotor outlet ***1442***. Fluid exits the rotor, passes through the gasket ***1480*** via outlet ***1485*** and enters the stator, i.e. fluidic card ***1001***, through stator opening ***1453b*** and fluid passage ***1454b***.

[0373] The rotary valve for use in the cartridges of the invention are described in greater detail in U.S. patent application titled “Rotary Valve,” filed 15 February 2018, and assigned application serial no. 15/898,064, and in international patent application, also titled “Rotary Valve,” filed 15 February 2019 and assigned application no. PCT/US2019/018351, each of which is incorporated by reference herein.

[0374] As an integral part of the rotor, a flow channel is configured for rotational motion, rotating with the other portions of the rotor with respect to other valve aspects, such as a stator. In a preferred implementation, the flow channel is not concentric with the rotational axis of the rotor. As illustrated in FIG. 76A, a flow channel can include one or more inlets ***1441*** and one or more outlets ***1442*** and provide fluidic communication between the inlet and the outlet. In a preferred implementation, each flow channel will comprise a single inlet and a single outlet. The inlet and

outlet typically, but not necessarily, will adopt the same form as a cross-section of the flow channel immediately adjacent to that inlet or outlet. The inlet and/or outlet can be circular, rectangular or any other appropriate shape consistent with forming fluid-tight fluidic connections within the valve interface.

[0375] The rotor can be configured to hold one or more porous solid supports. As shown in FIG. 77, each support chamber *1446a-1446d* may vary in shape, size, dimension, volume or by the content of the solid support contained in a specific support chamber ***1446***.

[0376] Optionally, the flow channel also includes a flow channel spacer ***1449*** for spacing a porous solid support from a surface, e.g., a bottom surface, of a porous solid support chamber ***1446***. In various embodiments, a flow channel spacer can be crescent shaped and extend in an arcuate manner along its length. The flow channel spacer can facilitate fluid flow through the outlet by preventing the porous solid support, e.g., beads or fibers, from physically blocking the exit from the solid support chamber. Illustrious flow channel spacer variations include: (a) a flow channel spacer may be segmented rather than a continuous structure; (b) a flow channel spacer may include more than one structure along a surface of the solid support chamber such as a sidewall or bottom; (c) a flow channel spacer may be spaced apart from the chamber exit or terminate at the edge of the exit; and (d) a flow channel spacer may be raised above a chamber interior surface such as a bottom or a sidewall, recessed into a chamber interior surface such as a bottom or a sidewall.

[0377] Porous solid supports can be configured to capture and thereby concentrate analyte, e.g., concentrate analyte from a first concentration to a second concentration, from a sample flowed therethrough by an amount of analyte concentration, such as 1000 X or more in any of the time amounts described herein, such as in 30 min or less, such as 1 hour or less. In various embodiments, a porous solid support is bounded, such as bounded at an upstream face and/or a downstream face by a frit.

[0378] In some aspects, a porous solid support can be a selective membrane or a selective matrix. As used herein, the terms “selective membrane” or “selective matrix” as referred to herein is a membrane or matrix which retains one substance, e.g., an analyte, more effectively, e.g., substantially more effectively, than another substance, e.g., a liquid, such as portions of a sample other than the analyte and/or water and/or buffer, when the substances are exposed to the porous solid support and at least one of them is moved at least partially therethrough. For example, a porous solid support, such as a selective matrix, having a biological sample flowed therethrough can retain an analyte, e.g., nucleic acids, while the remainder of the sample passes through the porous solid support.

[0379] Examples of porous solid supports include, but are not limited to: alumina, silica, celite, ceramics, metal oxides, porous glass, controlled pore glass, carbohydrate polymers, polysaccharides, agarose, Sepharose™, Sephadex™, dextran, cellulose, starch, chitin, zeolites, synthetic polymers, polyvinyl ether, polyethylene, polypropylene, polystyrene, nylons,

polyacrylates, polymethacrylates, polyacrylamides, polymaleic anhydride, membranes, hollow fibers and fibers, or any combinations thereof. The choice of matrix material is based on such considerations as the chemical nature of the affinity ligand pair, how readily the matrix can be adapted for the desired specific binding.

[0380] In some embodiments, a porous solid support is a polymeric solid support and includes a polymer selected from polyvinylether, polyvinylalcohol, polymethacrylate, polyacrylate, polystyrene, polyacrylamide, polymethacrylamide, polycarbonate, or any combinations thereof. In one embodiment, the solid support is a glass-fiber based solid support and includes glass fibers that optionally can be funcationalized. In some embodiments, the solid support is a gel and/or matrix. In some embodiments, the solid support is in bead, particle or nanoparticle form.

[0381] A myriad of functional groups can be employed with the subject embodiments to facilitate attachment of a sample analyte or ligand to a porous solid support. Non-limiting examples of such functional groups which can be on the porous solid support include: amine, thiol, furan, maleimide, epoxy, aldehyde, alkene, alkyne, azide, azlactone, carboxyl, activated esters, triazine, and sulfonyl chloride. In one embodiment, an amine group is used as a functional group. A porous solid support can also be modified and/or activated to include one or more of the functional groups provided that facilitate immobilization of a suitable ligand or ligands to the support.

[0382] In some embodiments, a porous solid support has a surface which includes a reactive chemical group that is capable of reacting with a surface modifying agent which attaches a surface moiety, such as a surface moiety of an analyte or ligand of a sample, to the solid support. A surface modifying agent can be applied to attach the surface moiety to the solid support. Any surface modifying agent that can attach the desired surface moiety to the solid support may be used in the practice of the present invention. A discussion of the reaction a surface modifying agent with a solid support is provided in: “An Introduction to Modern Liquid Chromatography,” L. R. Snyder and Kirkland, J. J., Chapter 7, John Wiley and Sons, New York, N.Y. (1979), the entire disclosure of which is incorporated herein by reference for all purposes. The reaction of a surface modifying agent with a porous solid support is described in “Porous Silica,” K. K. Unger, page 108, Elsevier Scientific Publishing Co., New York, N.Y. (1979), the entire disclosure of which is incorporated herein by reference for all purposes. A description of the reaction of a surface modifying agent with a variety of solid support materials is provided in “Chemistry and Technology of Silicones,” W. Noll, Academic Press, New York, N.Y. (1968), the entire disclosure of which is incorporated herein by reference for all purposes.

[0383] As described above, in some versions of the rotary valve, the valve includes a gasket between the stator face and the rotor valving face, and a structure for maintaining the valve in a storage configuration wherein the rotor and stator are spaced apart such that the gasket is not compressed at the rotor-stator interface. Gaskets, typically formed of compressible, elastomeric materials, are susceptible to compression-set and adhesion to adjacent surfaces if stored under

compression for extended periods of time. Accordingly, described herein is a preferred implementation of a rotary valve that includes a threaded rotor and a threaded retention ring for maintaining a gap between the gasket and at least one of the rotor and stator, thereby preventing the gasket from sealing against at least one of the rotor and stator, wherein when threaded rotor is rotated, the gasket seals the rotor and stator together in a fluid tight manner. In a preferred embodiment, the mechanism for sealing the rotor and stator together in a fluid tight manner is irreversible.

[0384] As best seen in FIGs. 78 and 79, a retention ring ***1491*** includes a threaded portion ***1492***. In the illustrated embodiment, the threaded portion ***1492*** includes threads. A rotor includes an outer wall having a threaded portion ***1411***. In the illustrated embodiment, the threaded portion includes grooves ***1411*** that correspond to the threads ***1492*** of the retention ring. In the shipping configuration shown in FIGs. 78 and 79, a biasing element ***1496*** maintains engagement between threads ***1492*** and grooves ***1411*** aiding in maintaining the desired gap between the rotor sealing surface (gasket ***1480***) and the stator valving face ***1452***. As best seen in FIG. 79, the top of rotor cap ***1430*** is substantially flush with an upper surface of retention ring ***1491*** maintaining a low-profile rotary valve design factor. Rotation of the rotor relative to the retention ring ***1491*** moves the rotor towards the stator and into the operational configuration shown in FIGs. 80 and 81. The transition out of the storage configuration is clear in this view, as the rotor cap is recessed below the top surface of the retention ring ***1491*** and the gasket ***1480*** provides a fluidic seal between the rotor and the stator. Also visible in FIG. 81 it is that the rotor is detached from the threaded portion ***1492*** of the retention ring ***1491***. Movement of the threaded rotor into this position ensures that the rotor is free to be indexed relative to the stator as described herein.

[0385] In consideration of FIGs. 79-81, there is provided a rotary valve comprising a rotor ***1410*** having a rotational axis, a rotor valving face, an outer face opposite the rotor valving face. Additionally, there is a stator ***1450*** having a stator valving face positioned opposite the rotor valving face. The rotary valve also includes a retention element ***1490*** biasing the rotor and stator towards one another comprising a retention ring ***1491*** and a biasing element ***1496***. The rotary valve is maintained in a shipping configuration while a threaded portion of the retention ring is engaged with a threaded portion of the rotor. In one configuration, a relative motion between the rotor and the stator produces a fluid tight arrangement between the rotor valving surface and the stator valving surface or the relative motion between the rotor and the stator is rotation of the rotor so as to move the rotor along the threaded portion of the retention ring until released to seal against the stator. As such, a rotary valve having a threaded rotor used for engagement in a shipping configuration may be configured to transition to provide a fluid tight seal within the rotary valve with a rotation of less than one revolution, half a revolution, a quarter of a revolution or one-eighth of a revolution of the threaded rotor. Still further, it is to be appreciated that while the threaded components of a threaded rotor rotary valve are engaged a gasket disposed between

the rotor valving face and the stator valving face does not form a fluid tight seal with the stator valving surface.

[0386] In an alternative embodiment, one or more displaceable spacers may configured for preventing the gasket from sealing against at least one of the rotor and the stator. When the spacers are displaced, e.g., displaced from a pre-activated configuration to an activated configuration, the gasket seals the rotor and stator together in a fluid-tight manner. According to the subject embodiments, displaceable spacers can be part of and/or integral with a stator or rotor.

[0387] In one implementation, in the storage configuration, a displaceable spacer comprises a plurality of tabs that contact a lip on the rotor to hold the rotor away from the stator. Each of the plurality of tabs is displaceable to disengage from the lip in the operational configuration. In one embodiment, the displaceable spacer comprises a plurality of tabs displaceable from a first tab configuration, i.e. storage configuration, to a second tab configuration, i.e. operational configuration. In a further embodiment, the stator comprises the plurality of tabs. Displaceable spacers, such as tabs, can be shaped substantially as a three-dimensional box or rectangular shape so as to contact a lip of a rotor during a storage configuration. To facilitate displacement of the spacer when transitioning from a storage configuration to an operational configuration, the rotor can comprise one or more ramping portions adjacent to the lip on the rotor to interact with each of the spacers. Specifically, the ramping portions exert a force in an outward or substantially outward direction, such as a direction away from a rotational axis of a rotor, on the displaceable spacers, thus allowing the retention element to bias the rotor and stator to form a fluid-tight seal in the operational configuration.

[0388] In one aspect of the invention, the purification module stores on-board liquid reagents in reagent reservoirs for easy delivery of reagents used herein to prepare a sample suspected of containing a target pathogen. Such reagent reservoirs can be of any structure formed in the fluidics card ***1001*** configured to contain liquid therein, such that the fluidics card forms a first bounding surface. In one embodiment reagent reservoirs may comprise a second bounding surface provided by one or more sealing films fixedly attached, e.g. welded, to the fluidics side ***1006*** of the fluidics card (see, e.g. FIG. 89, discussed in greater detail below). In one implementation, reagent reservoirs are sealed by frangible seals to define a receptacle for long-term storage of the liquid reagents contained therein. Reagent reservoirs are rendered fluidically active when actuated to break the frangible seal allowing fluid to be emptied from the reagent reservoir and redirected throughout the cartridge. In one implementation, reagent reservoirs are in direct fluidic communication with main pneumatic line ***1171*** to deliver pressurized air to a reagent reservoir inlet to empty the contents of the reagent reservoir. The reagent reservoir additionally includes a reagent reservoir outlet to transfer the contents of the reagent reservoir from its holding receptacle to appropriate sample processing locations on the cartridge.

[0389] In one implementation, the device comprises one or more frangible seals configured to seal the device and allow on board liquids to be stored therein. In some implementations, breaking the one or more frangible seals renders the device fluidically active to allow liquid substances contained therein, e.g., lysis buffer or wash buffer, to be directed through the fluidic network of channels. A variety of configurations of frangible seals for closing microfluidic channels are well known in the art, any of which can be used in conjunction with the cartridges disclosed herein. For example, a description of frangible seals can be found in U.S. Patent No. 10,183,293, U.S. Patent No. 10,173,215, U.S. Patent 9,309,879, U.S. Patent 9,108,192, U.S. Patent Application publication 2017/0157611, and published European Patent Application 3406340 A1, all of which are incorporated by reference herein.

[0390] In one implementation, a reagent reservoir is configured to store a wash buffer to form a wash buffer reservoir ***1475*** (see, e.g. FIG. 70A). Wash buffer removes unbound or loosely bound contaminants from a porous solid support while target analyte, e.g. nucleic acids, remain bound to the porous solid support. In one embodiment, the wash buffer reservoir is in direct fluidic communication with the main pneumatic line ***1171*** such that pressurized air can be sent to the wash buffer reservoir through a wash reservoir inlet ***1476*** to empty the wash buffer reservoir. The wash buffer exits the wash buffer reservoir through wash outlet ***1477*** and is transferred to the porous solid support chamber within the rotary valve, thus washing the porous solid support of contaminants. Wash buffer exits the porous solid support chamber, and the wash buffer containing cell debris is then conveyed to the waste collection element ***1470***.

[0391] In another one implementation, a reagent reservoir is configured to store an elution buffer to form an elution buffer reservoir ***1500*** (see, e.g. FIG. 70A). Elution buffer enables the release of target nucleic acids bound to a porous solid support in a sample to form a purified sample. The elution buffer reservoir further includes an elution reservoir inlet ***1501*** from which pressurized air can enter the elution buffer reservoir to empty the contents. The inlet of the elution buffer reservoir can be in direct fluidic communication with main pneumatic line ***1171*** to deliver the pressurized air. The elution buffer is emptied from the elution buffer reservoir through an elution reservoir outlet ***1502*** and flowed over the porous solid support in the rotary valve to release target nucleic acid to produce an eluate, or eluted nucleic acid. In another embodiment, the eluate may subsequently be directed to a rehydration chamber, described in further detail below.

a) Waste Collection Element

[0392] The waste collection element ***1470*** is configured for receiving and storing liquid waste in a secure manner. In some embodiments, the waste collection element ***1470*** comprises a waste inlet ***1471***, a vent channel ***1472***, vent ***1473***, at least one waste outlet ***1474***, and outlet filter plug ***1478***. Accordingly, liquid waste is directed to the waste collection element from channel ***1362*** through a waste inlet ***1472***. The waste collection element will include at least one, but preferably more than one, waste outlets in fluidic communication with a vent channel ***1472***. Multiple waste outlets coupled to the vent channel allow continuous venting in the instance that one or more

waste outlets become clogged or obstructed by fluid. In one embodiment, the vent channel terminates at a vent ***1473***. The vent ***1473***, optionally, can include an outlet filter plug ***1478*** configured to capture aerosolized liquid particles that may travel through the waste collection element given the pressurization applied to the device.

[0393] In one aspect of the invention, the cartridge uses the force of gravity for retaining fluids within the waste collection element during a diagnostic test run. Active pressurization applied to the cartridge motivates fluids, e.g., the sample, reagents, and air, through the fluidic network of the cartridge. Specifically, liquid waste is directed through channels and enters the to the waste collection element through channel ***1362***. The vertical orientation of the cartridge within the instrument ***2000*** allows the waste collection element to be configured as a liquid trap until all incoming and outgoing channels in fluidic communication with the waste collection element are either temporarily or permanently isolated from other portions of the cartridge. In one implementation of permanent isolation all incoming and outgoing channels in fluidic communication with the waste collection are sealed. In one specific implementation, the channels are heat staked separately or simultaneously as part of isolating one or more assay chambers. Sealing the channels to and from the waste collection element forms a closed system to prevent liquid waste contained therein to escape the waste collection element regardless of the cartridge orientation.

[0394] As mentioned above, sealing the channels leading to and exiting from the waste collection element to retain fluids therein, regardless of cartridge orientation, can be achieved by selectively heat staking a portion of the device. In one embodiment, the cartridge prevents liquid waste from exiting the waste collection element to mitigate contamination control by heat staking channel ***1362*** leading to the waste collection element and vent channel ***1472*** in a process described herein. Heat staking the channel ***1362*** and channel ***1472*** seals all access channels leading to the waste collection element. In some embodiments, a portion of the cartridge may be configured to include a raised platform ***1605*** to facilitate heat staking. Further description of the raised platform in context to sealing the cartridge by heat staking is discussed in the sections to follow. In another implementation, the waste collection element can contain a bibulous pad for absorbing liquid waste captured by the waste collection element.

3. Amplification Module

[0395] In an additional embodiment, a device comprises an amplification module configured to supply amplification reagents required to perform an assay, amplify nucleic acid from a purified sample and detect a signal indicative of the presence of a target pathogen. The amplification module has a reaction area comprising a plurality of assay chambers of defined volume, each configured to receive nucleic acids, where the said nucleic acids are amplified to yield a greater copy number of the nucleic acid sequence for detection. One or more nucleic acid targets can be read on a chamber-by-chamber basis to permit multiplex amplification and detection. The large number of amplicons generated in nucleic acid amplification poses a threat for contamination to

laboratory work surfaces. In some implementations, the amplification module includes a mechanism for amplicon containment.

[0396] In various aspects, the amplification module includes one or more rehydration chambers for rehydrating dried reagents with a substance, e.g., a liquid, such as a purified sample. As illustrated in FIG 70A, the cartridge can comprise a rehydration chamber ***1520*** that accepts nucleic acid solution eluted from the porous solid support of the rotary valve. Referring to FIG. 82, one exemplified rehydration chamber comprises a double tapered chamber which in turn comprises tapered inlet ***1521***, a tapered outlet ***1522***, two curved boundaries ***1525***, and a reagent plug ***1523***. In certain implementations, a first bounding surface is formed by the fluidics card ***1001***, and a second bounding surface is formed by a plug. The plug comprises a body and a cap. The body of the plug protrudes into the fluidics card ***1001*** of the rehydration chamber ***1520*** to form the second bounding surface of the rehydration chamber. In further embodiments, one or more films form a third bounding surface of the rehydration chamber such that the first bounding surface, the second bounding surface, and the third bounding surface together enclose the rehydration chamber volume. In some embodiments, the plug cap comprises an internal cavity ***1774***configured to hold one or more dried amplification reagents for use in an assay to take place in the assay chambers, described in greater detail in the following section. Additionally, a magnetic mixing element may be located in the rehydration chamber to facilitate actuation of an assay in the assay chamber. In one implementation, the magnetic mixing element is a magnetic ball ***1524***.

[0397] In various embodiments, the amplification module of the cartridge comprises one or more assay chambers ***1621*** configured to detect a signal indicative of target amplicon generated from the nucleic acid. Referring to FIG. 70A, the assay chambers are located within the reaction area ***1600*** and are visible to the reaction camera ***2701*** of the reaction imaging assembly ***2700***.

[0398] In one implementation, the assay chambers ***1621***comprise a double tapered chamber which in turn comprises tapered inlet ***1641***, a tapered outlet ***1642***, two curved boundaries, and a reagent plug ***1770***. In certain embodiments, the assay chamber comprises a first bounding surface formed in a monolithic substrate (i.e., fluidics card ***1001***), and a second bounding surface formed by a plug. The plug comprises a body and a reagent surface. The body of the plug protrudes into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber. In particular, the reagent surface of the plug forms the second bounding surface of the assay chamber. In further embodiments, a film may form a third bounding surface of the assay chamber such that the first bounding surface, the second bounding surface, and the third bounding surface together enclose the assay chamber volume. In some embodiments, the plug reagent surface comprises an internal cavity ***1774*** configured to hold one or more dried reagents for use in an assay for a diagnostic test to take place in the assay chamber.

[0399] In one implementation of an assay chamber of an integrated diagnostic cartridge can include a plug 1770 having one or more or a combination of the following features. The bottom surface of the plug body can include a cavity in the bottom surface with the dried reagent within the cavity. The plug can have a plug thickness between a central opening bottom and the plug body bottom, and further wherein a depth of the cavity is less than 90% of the plug thickness, is less than 70% of the plug thickness or is less than 50% of the plug thickness. The plug can have a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths. The plug may have a dried reagent that can be selected from the group consisting of nucleic acid synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, monomers, detection reagents, catalysts or combinations thereof. The dried reagent can be a continuous film adhered to the plug bottom surface. The dried reagent can be a lyophilized reagent. The body of the plug can protrude into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber. In some embodiments, during the combining the enriched nucleic acid step in each of the two or more assay chambers, the enriched nucleic acid can combine with a dried reagent contained in each one of the two or more assay chambers. The dried reagent can be on a surface of a plug in each one of the two or more assay chambers. The dried reagent can be on a surface of the plug formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum used during the performing step. In one aspect, the surface of the plug having the dried reagent is also used during the performing an isothermal amplification reaction step. Images collected through the plug surface that contained the dried reagent are processed as part of the detection of an amplification product within an assay chamber.

[0400] With regard to FIG. 83A and 83B, in some embodiments, the plug further comprises a flange ***1773*** that can be welded and/or adhered to a surface of the assay chamber to stabilize the position of the plug body within the opening of the fluidic card of the assay chamber. The plug body further includes a central opening ***1777*** with a side wall ***1778*** and a bottom surface ***1776***. The plug protrudes into the monolithic substrate at a depth such that the component of the plug that is visible on the exterior of the assay chamber is the surfaces of the central opening of the plug. In embodiments in which the plug cap includes a flange, the flange is also visible on the exterior of the assay chamber as shown in FIG. 83A and 83B. FIG. 83A is a cross section view of an assay chamber taken through the tapered inlet ***1622*** and the tapered outlet ***1632*** which shows the plug flange ***1753*** supported by a raised annulus ***1797*** integrally formed within a fluidics card ***1001***. FIG. 83B is a cross section view of an assay chamber taken through the midpoint of the assay chamber showing the flange supporting the plug and the double tapered sidewalls towards the inlet.

[0401] In some embodiments, such as embodiments in which the assay chamber is used to contain an assay, the plug is transparent such that the assay within the assay chamber is optically detectable from outside of the assay chamber. FIG. 84 shows a signal indicative of the presence of target nucleic acids from a target pathogen viewed through a transparent plug as described herein. In a preferred embodiment, the signal visible through the transparent plug is a fluorescent signal. Alternatively, the signal visible through the transparent plug is a colorimetric (i.e. color change) signal.

[0402] The one or more dried reagents, used in combination with a plurality of assay chambers, generates an amplification solution and enables multiplexing to test a sample for the presence of more than one target nucleic acids. The cartridge exemplified herein can achieve multiplexing through several methods. First, the cartridge can comprise a plurality of assay chambers, with each chamber comprising primers and probes specific to a different target pathogen or process control. Additionally, a single assay chamber can comprise multiple primer/probe sets, each set specific to a different target pathogen or process control. Alternatively, a single assay chamber can comprise multiple prime sets with the same probe, where each set is specific to the same target pathogen or process control. The probe for each different target can be differentiated by the signal generated by the probe. For example a single assay chamber can contain a first primer/probe set in which the probe comprises a Texas Red fluorophore and a second primer/probe set in which the probe comprises a fluorescein (green) fluorophore. A wide variety of fluorophores are known in the art, as well as the mechanisms and filters one can use to differentiate signals from multiple fluorophores in the same assay chamber. In one implementation, the plurality of assay chambers can detect the presence of up to 3 target nucleic acids. In one implementation, the plurality of assay chambers can detect the presence of up to 5 target nucleic acids. Similarly stated, in some embodiments, the assay chambers can produce a visible signal, wherein the visible signal is associated with the presence of the target amplicon and/or target pathogen.

[0403] In an alternative embodiment, the cartridge can comprise a plurality of assay chambers with two or more chambers comprising primers and probes specific to a single target pathogen. For example, a single assay chamber can contain a first primer/probe set for detecting a specific target pathogen and a second assay chamber can contain a second primer//probe set for detecting the same target pathogen. In yet another alternative embodiment, multiple assay chambers can comprise the same primer/probe set such that identical amplification solutions are generated within at least two or more assay chambers.

[0404] In some implementations, it may be desirable to fill the assay chambers simultaneously regardless of the assay chamber fluid volume. In such implementations, one or more air chambers ***1631*** are included in a cartridge to balance a ratio of the volume of the assay chamber to the volume of the air chamber to fill simultaneously (FIG. 70A). For example, air chambers may be described in U.S. Patent Number 10,046,322, titled “Reaction Well for Assay Device” and

assigned application no. PCT/US19/23764, all of which are incorporated herein by reference. The invention contemplates cartridges having assay chambers that differ in volume, see for example, the assay chambers ***1621*** illustrated in FIG. 70A. In such embodiments, each assay chamber will be associated with its own air chamber. In order to achieve concurrent filling of each assay chambers that differ in volume, the ratio of the assay chamber volume to its associated air spring volume will be approximately the same for each assay chamber/air spring pair on the cartridge.

[0405] In an alternative embodiment, the above described features, characteristics and functionality of the various the reagent plug 1523, plug cap or plug 1770 embodiments may be provided by an plug that similarly forms part of an associated assay chamber without extending into the diagnostic cartridge as in FIGs. 82, 83A, 83B, 84, 88 and 89. In contrast, these alternative reagent plugs embodiments may be positioned in a planar or raised aspect to the assay chamber or other associated component. In one variation the plug functionality is provided by a capsule design that is raised above surface of the diagnostic cartridge. Additionally or optionally, the capsule style plug may be mounted to the surface of diagnostic cartridge with appropriately shaped raised or recessed support elements to aid in readily mounting the capsule plug in position. The capsule plug mounts may provide appropriately sized and shaped raised or recessed mounting features similar to the plug cap flange 1773. Appropriate capsule plug flanges or mounting features may be incorporated which ensure placement of the capsule relative to the assay chamber or other chamber while ensuring appropriate fluidic communication relative to inlets, outlets or other conduits associated with the chamber.

[0406] As a result, in general, in one embodiment, an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module. The reaction module includes a reagent storage component including a capsule capable of holding a liquid or solid sample. In one embodiment the capsule includes an opening, a closed end and a wall extending from the closed end to the opening. The capsule is oval-shaped and the wall is rounded, and the closed end and wall define an interior volume having a substantially smooth surface.

[0407] In still another alterative capsule style plug embodiment, there is an integrated diagnostic cartridge includes a loading module, a lysis module, a purification module, and a reaction module. The reaction module includes a capsule capable of holding a liquid or a solid sample. The capsule includes an inner surface extending from the bottom of said capsule to an oval-shaped opening at the top of the capsule, wherein said inner surface is substantially smooth and includes a concave shape extending from the bottom of the capsule, and a planar layer affixed around the oval-shaped opening of said capsule and oriented in the same plane as the oval-shaped opening of said capsule. The planar layer includes a top surface and a bottom surface. The top surface is aligned with the inner surface of said capsule at said oval-shaped opening to provide a continuous surface.

[0408] This and other capsule style plug embodiments may include one or more of the following features. The capsule can be capable of holding a volume from approximately 50 µL to approximately 200 µL. Still other embodiments provide for an oval-shaped opening contained within an area of 9 mm x 9 mm. Still further, the capsule can include a dried reagent as described elsewhere in this specification. Additional details of these and additional embodiments are provided in Published International Patent Application WO 2018/111728 entitled "Capsule Containment of Dried Reagents" having International Application Number PCT/US2017/065444 filed on December 8, 2017, incorporated herein by reference. In particular, the details of the embodiment of the capsule plug configuration illustrated and described with regard to FIG. 6 as well as the contents of paragraphs [0149-0152] are incorporated herein specifically.

[0409] The cartridge of the invention can be configured to be provide isolation between cartridge elements either temporarily or permanently. In one specific implementation of a form of permanent isolation, one or more heat stake regions are used to seal off and maintain sample within each assay chamber. In one implementation, the configuration of the main loading channel ***1671*** may consist of a u-bend ***1607*** (FIG. 85). By sealing off a connection between a main channel ***1671*** and any loading channels ***1672***, the loading channel, assay chamber ***1621*** and air chambers ***1631*** form a completely closed system from which matter cannot travel in or out, and for which, internal pressure within the assay chamber, loading channel, and air chamber remains constant, unless the environment is substantially changed, e.g. by heating the cartridge. One acceptable method of isolating the loading channels ***1672*** is heat staking with a heated element such that the loading channels are sealed off from the main channel. In one implementation of the method, the heated element is heat staker assembly ***2640*** of instrument ***2000***. Note that the supply pressure of the fluid sample is maintained during the heat staking process.

[0410] In some embodiments, as described herein, a first film is adhered to the fluidics side ***1006*** of a fluidics card ***1001***, such that the first film forms one wall of the main channel and loading channels. In one implementation the first film has a similar melting point as the substrate of the device. In further embodiments, a second film is adhered to the first film. In such embodiments, the second film has a higher melting point than the first film and the surface of the device such that when heat is applied to the device via the heat staker assembly ***2640*** to heat stake the loading channel, the first film and the surface of the device melt prior to the second film. This higher melting point of the second film prevents the pressurized sample from escaping from the loading channels, thus emptying assay chambers, as the first film and the surface of the device re melted. The result of this heat staking process is a melted first film, which forms a heat stake ***1603*** seen in FIG. 101 and 102.

[0411] In some embodiments, the fluidics card ***1001*** can further include a raised platform ***1605*** within each of the loading channels ***1672*** such that, the raised feature is positioned between an inlet to the assay chamber and the main channel. The heat staked region can be formed using a portion of the raised platform, as depicted in FIGs 85, 86, and 87. In various implementations, the

raised platform may further extend throughout a fluidics card ***1001*** to include one or more channels from different modules. For example, the raised platform may extend to include channel ***1362*** leading to the waste collection element and vent channel ***1472*** exiting the waste collection element as seen in FIG. 88. In such a configuration, the heat staker assembly ***2640*** contacts the main channel ***1671***, each of the plurality of loading channels ***1672***, u-bend ***1607***, channel ***1362***, and vent channel ***1472*** to selectively melt these areas of the cartridge to form closed systems.

4. Example Cartridge

a) 4 Module Cartridge - Sample Prep + Amp

[0412] FIG. 89 is an exploded view of the exemplary cartridge illustrated in FIGs. 69A and 70A, configured for a disposable, single use diagnostic test. The cartridge, according to the exemplified embodiment, comprises a loading module, a lysing module, a purification module, and an amplification module. Cartridge ***1000*** comprises a fluidics card ***1001***, wherein the fluidics card further comprises a fluidic side ***1006*** and a feature side ***1007***, a first film ***1002***, a second film ***1003***, and a cartridge cover ***1004***. The loading module, lysing module, purification module, and amplification module are integrally formed, e.g., molded, within the fluidics card ***1001*** to provide the structures necessary to perform each sample processing step for a diagnostic test. In some embodiments, the cartridge is between 150 and 200 mm long, 75 mm to 100 mm wide and 10 to 30 mm tall. The cartridge can be 175 to 200 mm long, 80 to 90 mm wide and 10 to 20 mm tall. In a particularly preferred embodiment, as illustrated in FIG. 70A, the cartridge is approximately 180 mm long, about 90 mm and about 12 mm tall.

[0413] The loading module is configured to accept and seal a sample. As described herein, the loading module is configured to define a metered sample and comprises an entry port ***1140***, a fill chamber ***1101***, a metering chamber ***1110*** and an overflow chamber ***1120***. Such a configuration defines the volume of the sample and can accommodate for excess sample loaded into the fill chamber by directing the excess sample to the overflow chamber.

[0414] The lysis module is configured to lyse a metered sample generated by the loading module. The lysing module produces a lysed sample upon mixing a sample in the lysis chamber ***1371*** with one or more lysis reagents and subsequently produces a filtered lysate after flowing the lysed sample through a filter assembly ***1330***. The lysis chamber ***1371*** formed within the fluidics card ***1001*** is configured to hold a stir bar ***1390*** to mix the metered sample with a substance contained therein, e.g., lysis buffer, to disrupt the cell wall and/or outer membrane of cells. Lysing a sample releases the contents of cells including various organelles, proteins, and nucleic acids. As exemplified, the lysing module includes a filter assembly ***1330*** through which the lysed sample flows. The filter assembly is fluidically downstream from the lysis chamber ***1371*** to filter a lysed sample. An inlet via ***1332*** allows the lysed sample to enter the filter assembly where filter ***1331*** is configured to filter the lysed sample of cellular debris and other contaminants. Flow directors ***1334*** direct a filtered sample to the outlet, wherein outlet via ***1333*** enables the filtered sample to exit the filter assembly and be directed to amplification module.

[0415] In cartridges described herein, the purification module is configured to purify a filtered sample to capture nucleic acids associated with a suspected target pathogen. As exemplified, the purification module includes a rotary valve ***1400*** comprising a porous solid support ***1445***. In such a configuration, the porous solid support ***1445*** allows a filtered lysate to flow through the porous solid support ***1445*** to capture nucleic acids while passing proteins, lipids and other cell debris. The purification module includes a waste collection element ***1470*** to which liquid waste from the filtering module and purification module is conveyed. In this embodiment, the waste collection element ***1470*** comprises an output filter plug ***1478*** configured to capture aerosolized liquid particles, thus avoiding contamination of the instrument or laboratory environment. Furthermore, the waste collection element ***1470*** is configured to be isolated so as not cause any other areas of the cartridge or the inside of instrument ***2000*** to be contaminated by previously used substances, e.g., liquids, such as the sample or wash buffer. Another feature of the purification module, reagent reservoirs, are formed within the fluidics card ***1001*** to for on-board storage of liquid substances, including a wash buffer and an elution buffer. Prior to operation of the cartridge, the reagent reservoirs are sealed by frangible seals to form closed systems to prevent the cartridge from being fluidically activated until actuated at the time of the diagnostic test.

[0416] In the exemplified cartridge, the amplification module provides a plurality of assay chambers ***1621*** such that the amplification module can perform an isothermal nucleic acid amplification on the sample deposited into the loading module. In this embodiment, each of the assay chambers is a double tapered chamber which comprises tapered inlet ***1641***, a tapered outlet ***1642***, two curved boundaries, and a reagent plug ***1770***. In some embodiments, the plug cap comprises an internal cavity ***1774*** configured to hold one or more dried reagents for use in an assay for a diagnostic test to take place in the assay chamber. In such embodiments, the one or more dried reagents are configured to produce a visual signal, e.g., fluorescent signal, to indicate the presence of nucleic acids from a target pathogen within the sample. The reagent plugs are configured to be transparent such that the assay within the assay chamber ***1621*** is optically detectable from outside of the assay chamber.

[0417] As exemplified, the cartridge includes a rehydration chamber ***1520*** and a portion of the cartridge is configured to be heat staked, as described above. The rehydration chamber comprises tapered inlet ***1521***, a tapered outlet ***1522***, two curved boundaries, and a reagent plug ***1770***. The reagent plug of the rehydration chamber comprises an internal cavity ***1774*** configured to hold one or more dried reagents. A portion of the cartridge includes a raised platform feature ***1605*** to heat stake a cartridge to maintain the sample level in the plurality of assay chambers therein without active pressurization. As described herein, heat staking seals the assay chambers ***1621*** and the waste collection element ***1470*** from the remainder of the features of the cartridge and from the outside environment. Specifically, a portion of the main channel ***1671***, loading channels ***1672***, channel leading to the waste collection element ***1362***, and the vent channel ***1472***

exiting the waste collection element are configured to include a raised platform feature ***1605*** to melt the two films attached to the fluidics side of a fluidics card to retain liquids therein.

b) 3 Module Cartridge - Sample Prep

[0418] An alternate configuration of the cartridge is depicted in FIG. 92 In this alternative configuration, the device comprises a loading module, a lysing module, and a purification module configured to receive a sample, lyse cells in the sample, and subsequently purify nucleic acids from the sample. This cartridge configuration is intended to be used as a sample preparation device and is not configured to perform a nucleic acid amplification test, not to report an assay result. This sample preparation-inly configuration can be processed using an assay instrument as described herein, or on an abbreviated sample-preparation instrument that lacks the chemistry heater assembly and the reaction imaging assembly.

[0419] In such sample preparation embodiments, the loading module is configured to accept and seal a sample. As described herein, the loading module is configured to define a metered sample and comprises an entry port ***1140***, a fill chamber ***1101***, a metering chamber ***1110*** and an overflow chamber ***1120***. Such a configuration defines the volume of the sample and can accommodate for excess sample loaded into the fill chamber by directing the excess sample to the overflow chamber ***1120***.

[0420] The lysis module, in some embodiments, is configured to lyse a metered sample generated by the loading module. The lysing module produces a lysed sample upon mixing a sample with one or more lysis reagents in the lysis chamber ***1371*** with a stir bar ***1390*** as described above. In the sample preparation cartridge, the lysing module may further include a filter assembly ***1330*** to produce a filtered lysate after flowing the lysed sample through a filter assembly.

[0421] The purification module of the sample preparation cartridge, similar to a standard assay cartridge, is configured to purify a filtered lysate to enrich nucleic acids. For example, the purification module includes a rotary valve ***1400*** comprising a porous solid support ***1445***. The porous solid support ***1445*** allows a filtered lysate to flow through the porous solid support ***1445*** to capture nucleic acids while passing proteins, lipids and other cell debris therethrough. The purification module includes a waste collection element ***1470*** to which liquid waste from the purification module is conveyed. Another aspect of the purification module, reagent reservoirs, are formed within the fluidics card to for on-board storage of liquid substances, such as a wash buffer and an elution buffer. The sample preparation cartridge will include one or more frangible seals to seal reagent reservoirs, allowing the cartridge to be fluidically inactive, until actuated by a system, such as instrument ***2000*** described herein.

[0422] This embodiment of the device, as described herein, further comprises a retrieval port for retrieving a purified sample from the device. In some implementations, the retrieval port comprises a cap, similar to the cap ***1181*** configured to cover entry port ***1140*** of the loading module. Preferably the cap of the retrieval port is configured to be opened to permit retrieval of a purified sample and then resealed prior to disposal of the device. Alternatively, the sample can be

retrieved via a puncturable septa or large one-way valve. In some implementations, the retrieval port is enclosed with a film that is cut, punctured or otherwise ruptured to permit access to the purified nucleic acid. The sample preparation system can include a sample loader, such as a bulb or syringe, useful for retrieving a purified sample from the device.

[0423] Since the sample preparation cartridge does not require any structures related to the amplification module, a sample preparation cartridge having the same dimensions as a test cartridge and designed to be run on an assay instrument can process larger volumes than the corresponding test cartridge. As illustrated in FIG. 92, the waste collection element can be expanded to accept larger volumes of sample, lysis reagent, and/or wash buffer. As described above, the capacity of the sample preparation cartridge can be further augmented by increasing the thickness of the cartridge.

H. Methods of Use - Cartridge

[0424] The cartridge, and any of the cartridges described herein, can be configured for use in a decentralized testing facility. In a further embodiment, the device can be a CLIA-waived device and/or operate in accordance with methods that are CLIA-waived. FIGs. 93 to 102 depict one exemplary method that can be used to prepare a biological sample to amplify nucleic acid and detect the presence of a suspected pathogen in a diagnostic test using one embodiment of a cartridge ***1000***, as described herein. The features of the cartridge used to perform the method for a diagnostic test is depicted in FIG. 93. The relative size of the features and the routing between features is for illustration of the method and are not to scale. Each step is summarized in the Table 1 below. Various processing steps and alternative embodiments are discussed in greater detail below.

TABLE 1: Cartridge Test Method Steps

Step	FIG.	Method
0	93	Load Sample
1	94	Cartridge Preparation
2	95	Lyse and Mix Sample
3	96	Filter and Bind Lysed Sample
4	97	Wash Bound Sample
5	98	Air Dry
6	99	Elute and Meter Purified Sample
7	100	Load Reaction Chambers
8	101	Isolation (e.g., Heat Stake)
9	102	Assay

[0425] FIG. 93 illustrates the state of a cartridge after a biological sample is loaded into the sample port assembly ***1100***, prior to insertion into an instrument and/or prior to actuation of any cartridge features by the instrument. Frangible seal ***1201*** is configured to maintain the sample within the sample port assembly. Frangible seals ***1202*** *and* ***1205*** are configured to maintain a lysis agent solution within the lysis chamber ***1371***. Frangible seals ***1203*** *and* ***1204*** are configured to maintain wash buffer within the wash buffer reservoir ***1475***. Frangible seals ***1206*** and ***1207***

are configured to maintain elution buffer within the elution buffer reservoir ***1500***. Prior to insertion into and actuation by the instrument, all of the frangible seals ***1201***-***1207*** remain intact. The rotary valve ***1400*** is positioned such that the rotor and stator are not in contact (indicated by dashed outline of the rotary valve feature in FIG. 93). In FIGs. 93-102, channels that conduct only air (pneumatic pressure) are indicated by dashed lines. Channels that conduct fluids are indicated by solid lines. When the fluid channels are active, i.e. subject to a motive force, such as pneumatic pressure, the solid lines 'bolded' (indicated with thicker solid lines as compared to inactive channels). Liquid within features of the cartridge is indicated by waved patterning within the relevant feature. Dried reagents are depicted with speckled patterning.

[0426] The cartridge is inserted into instrument where cartridge verification tests are performed to ensure the cartridge is suitable for use and certain cartridge preparation steps are performed. The rotary valve ***1400*** is moved into operational configuration and the cartridge is clamped by the clamping subsystem. The frangible seals ***1201***-***1207*** are ruptured with pins in the instrument. After rupture, the fluidic channels are no longer physically blocked and fluids within the cartridge are free to flow when exposed to a motive force. Rotary valve ***1400*** is rotated 360 degrees and indexed to a zero valving position to begin the series of sample processing steps. FIG. 94 illustrates the status of the cartridge features after these cartridge preparation steps are completed. All of the fluids remain in their original positions, as no motive force has yet been applied to the cartridge features.

[0427] Next, sample is transferred from the sample port assembly to the lysis chamber ***1371*** to effect lysis of any cells, including any suspected pathogen, contained in the sample. Pneumatic pressure is applied to the main pneumatic via ***1193***, permitting air to flow through the liquid trap ***1145*** positioned in the main pneumatic line ***1171*** and to the frangible seals. Rotary valve ***1400*** remains in the zero valving position to dead-end fill the lysis chamber ***1371*** under pressure. The instrument pressurizes the cartridge to transport sample through exit port ***1180***, frangible seal ***1202***, sample transfer channel ***1386*** and into the lysis chamber ***1371***. Pressure is applied to the cartridge while magnetic mixing assembly ***2300*** mixes the sample with lysis buffer by effectuating a magnetic coupling between the driving magnet system ***2310***, driven magnet system ***2350***, and stir bar ***1390*** contained in the lysis chamber ***1371*** to produce a lysed sample. The pressure applied to the cartridge is turned off after the sample is mixed for a set period. Due to the vertical orientation of the cartridge, the liquid lysate settles to the bottom of the lysis chamber and does not back flow toward the sample loading assembly when the lysis chamber is no longer under pressure. FIG. 95 illustrates the status of the cartridge features after the lysis steps are performed.

[0428] After the lysis step, the rotary valve ***1400*** is indexed to a first valving position, thereby fluidically connecting the empty sample loading assembly ***1100***, lysis chamber ***1371***, filter assembly ***1330***, via ***1370*** in fluidic communication with the solid support chamber of the rotary valve, and the waste collection element ***1470***. This alignment of features permits filtering of the

lysed sample and binding of target analyte, e.g. nucleic acid, to a porous solid support located in the rotary valve. Pressure applied at the main pneumatic via provides a motive force. The lysed sample exits the lysis chamber ***1371*** through exit channel ***1388*** passing through frangible seal ***1205***. Lysed sample advances to filter inlet via ***1332*** and flows through filter ***1330*** to produce a filtered sample. The filtered sample exits filter outlet via ***1333*** and into channel ***1361*** before entering the solid support chamber of the rotor using via ***1370***. The filter assembly captures and removes undesired cellular material and debris that may clog the porous solid support to generate a filtered sample. As the filtered sample passes through the porous solid support contained within the solid support chamber, target analyte, e.g. nucleic acid, is bound to the porous solid support. The remainder of the filtered sample, e.g., proteins, lipids, or carbohydrates, exits via ***1372*** and flows through channel ***1362*** to waste collection element ***1470***. Optionally, pressure applied to the cartridge is turned off when the pneumatic subsystem detects pushing the filtered sample over the porous solid support is complete. FIG. 96 illustrates the status of the cartridge features after the filtration and binding steps – the lysis chamber ***1371*** is empty, and fluid has passed to the waste collection element ***1470***.

[0429] In order to remove unbound or loosely bound contaminants from a porous solid support while continuing to bind the target analyte, e.g. nucleic acids, a wash buffer is passed through the porous solid support to remove the contaminants. In an exemplary embodiment, the porous solid support is a silica resin and the wash buffer is an aqueous alcohol solution. The rotary valve ***1400*** is indexed to a second valving position to flow wash buffer from the wash buffer reservoir over the matrix. Pneumatic pressure is applied to the main pneumatic via ***1193***. Wash buffer contained in wash buffer reservoir ***1475*** is pressurized to pass through frangible seal ***1204***, wash inlet via ***1460***, and porous solid support thereby removing undesired contaminants while target analyte remains bound. Wash buffer carrying contaminants travels through the wash outlet via ***1461*** and channel ***1362*** where it is directed to waste collection element *1470*. FIG. 97 illustrates the status of cartridge features after completion of the wash step.

[0430] In implementations using a wash buffer containing volatile components, such as alcohol, the excess wash buffer occupying the dead volume of the column advantageously is removed prior to releasing bound analyte by air drying the porous solid support. To execute such a step, the rotary valve ***1400*** is indexed to a third valving position permitting pressurized air to flow over the porous solid support through main pneumatic line ***1171***. Pneumatic pressure is applied to the main pneumatic via ***1193***, passes through the pneumatic line ***1177*** to air inlet via *1462* on the rotor thereby drying the porous solid support removing residual volatile components of the wash buffer from the porous solid support. Air exits the solid support chamber through air outlet via ***1463*** and channel ***1362*** where it is directed to waste collection element ***1470*** and ultimately to a vent ***1473***. Due to the vertical orientation of the waste collection element, having the inlet and outlet along the upper boundaries of the element, passing air through the waste collection element does not disturb fluid waste already stored in the waste collection element. Further to avoid

accidental release of fluidic contaminants from the cartridge, the vent ***1473***, optionally includes an outlet filter plug which is configured to capture aerosolized liquid particles that may travel through the waste collection element. FIG. 98 illustrates the status of cartridge features after completion of the air dry step.

[0431] The bound analyte, e.g. nucleic acid, is then released from the porous solid support. To effect release, the rotary valve ***1400*** is indexed to a fourth valving position thereby fluidically connecting the elution buffer reservoir ***1500*** to the porous solid support and then to the rehydration chamber ***1520***. Elution buffer exits the elution buffer reservoir ***1500*** through channel ***1551***, frangible seal ***1206***, channel ***1552***, elution inlet via ***1503*** and then over the porous solid support to release target analyte, thereby generating a purified analyte solution, e.g. an enriched nucleic acid solution. The purified analyte solution exits eluate outlet via ***1504*** and is directed to the rehydration chamber ***1520*** using channel ***1553***. The fourth valving position permits the filling of the rehydration chamber with the purified analyte solution. As previously described herein, in a preferred embodiment, the rehydration chamber contains one or more dried reagents for performing an assay. Rehydration of the one or more dried reagents with the purified analyte solution thus results an analyte/reagent solution. The exemplary cartridge embodiment further allows the metering the purified sample to a produce a desired volume. Pneumatic pressure advances the purified sample to fill the rehydration chamber and into channel ***1554***.The purified sample passes two vias, ***1580*** and ***1581***, before flowing through metering via ***1582*** to fill metering channel ***1557*** up against a metering vent ***1560***. Metering, while optional, is advantageous to avoid generating an overly dilute solution when the dried reagents are rehydrated. This implementation is particularly advantageous in assays that require exact volumes for amplification and detection conducted in the assay chambers, e.g. when detecting a target pathogen at low concentrations. In use, while pressure remains on and the purified sample is pressurized against metering vent ***1560***, a magnetic element, e.g. a rehydration motor, within the instrument rotates to cause a magnetic ball within the rehydration chamber to gyrate, thereby assisting dissolution and homogenization of dried down reagents with the purified analyte solution. Completion of this step generates an analyte/reagent solution. The pressure applied to the cartridge can be after the metering step is competed. FIG. 99 illustrates the status of cartridge features after the elution and metering step.

[0432] The analyte/reagent solution is now ready to be passed to the assay chambers. The rotary valve ***1400*** is indexed to a fifth valving position to load the assay chambers within the cartridge reaction area ***1600***. Note that in the exemplary cartridge described herein, indexing to the fifth valving position results a dead volume of analyte/reagent solution lost. The volume of analyte/reagent solution present in the metering channel ***1577*** remains in said channel after indexing to the fifth valving position, thus resulting in an exact metered volume corresponding to the sum of the plurality of assay chamber volumes.

[0433] Unlike the aforementioned steps where the rotary valve is indexed to positions that permit pressurization through main pneumatic line ***1171***, the fifth valving position blocks via ***1193*** (shown in FIG. 75A) to prevent pressurization of the main pneumatic line. Instead, the fifth valving position allows pressure to be applied to pneumatic via ***1194*** (shown in FIG. 75A), thereby pressurizing pneumatic line ***1178***. Air is directed first through via ***1192*** and ***1580***. The pressurized air subsequently travels through channel ***1554*** to push the analyte/reagent solution out of the rehydration chamber ***1520***, through channel ***1553*** to main channel ***1671*** and then loading channels ***1672*** (not shown). The analyte/reagent solution is split and distributed to the plurality of assay chambers in the reaction area ***1600***. As previously described herein, the plurality of assay chambers may be configured to provide one or more dried reagents, e.g. primers and probes, directed to a specific target pathogen or process control. In the exemplary implementation, the plurality of assay chambers contains one or more dried reagents therein, such that upon distribution of the analyte/reagent solution into said plurality of assay chambers, an amplification solution is generated within each assay chamber. Different cartridge configurations may be designed to provide different combinations and a variety of dried reagents to the plurality of assay chambers. For example, an amplification solution may be generated such that the instrument performs a plurality of identical assays in multiple assay chambers, i.e. detecting the same pathogen using multiple chambers. Alternatively, the amplification solution generated may comprise different dried reagents, such that the instrument performs two or more distinct assays in multiple assay chambers, i.e. detecting two or more target pathogens using multiple chambers. In any case, the cartridge remains pressurized after all assay chambers are successful loaded. FIG. 100 illustrates the status of cartridge features after loading the reaction chambers.

[0434] While it is possible to perform an assay while maintaining the amplification solution in the assay chambers with a temporary isolation technique such as pneumatic pressure, it is preferable to use a form of permanent isolation to physically isolate each of the assay chambers from the others to avoid cross-contamination as well as to isolate the reaction from the outside environment. As such permanent isolation is particularly advantageous when performing nucleic acid amplification reactions, as amplicon contamination is a well understood risk of such methods. To isolate the assay chambers after distributing enriched nucleic acid to two or more assay chambers, rotary valve ***1400*** remains in the fifth valving position during the permanent isolation process and pneumatic pressure continues to be applied to the pneumatic via ***1194***. When using a heat stake for isolation, the pneumatic pressure continues while the instrument heat stakes the cartridge under pressure by melting a selected area of the cartridge across the assay chambers, loading channels, as well as, channel ***1362*** leading to the waste collection element ***1470***, and venting channel ***1472*** exiting from the waste collection element to produce heat stake ***1603***. The heat stake is illustrated in FIG. 101 with a very heavy straight line across the channels. Heat stake ***1603*** seals each of the effected channels and functions to contain amplified nucleic acids

and minimize the threat of contamination when performing a diagnostic test. FIG. 101 illustrates the status of cartridge features after heat staking.

[0435] Finally, the cartridge is ready to perform a diagnostic test. Since the amplification solution is safely contained within the isolated assay chambers, pneumatic pressure applied to the cartridge is no longer required. Pneumatic pressure is released from pneumatic via ***1194***. The rotary valve remains indexed to the fifth valving position. FIG. 102 illustrates the status of cartridge features after release of pressure and during the assay step. The cartridge ***1000*** can be configured to produce the visibly detectable signal within about 30 minutes, more preferably within about 25 minutes, and most preferably within 20 minutes or less, from when the sample is received by the loading module. Since the reacted analytes and waste are contained by the heat stake or other permanent solution technique, the cartridge can be disposed without any further processing by the instrument or user.

I. Methods of Use - Instrument

[0436] FIGs. 106A-106E illustrate a detailed process flow chart of a method ***100*** running a diagnostic test executed the instruments, as described herein. The method begins at ***110*** after insertion of a cartridge into the instrument to conduct a diagnostic test. Latch and pin assembly ***2210*** drops latch ***2212*** into notch ***1021*** at the top of the cartridge to prevent the cartridge from being ejected by the loading assembly ***2230***. The instrument verifies that a cartridge is inserted at ***110*** using an load position sensor ***2236*** located within the loading assembly ***2230***. The cartridge is verified to be in a loaded position within the instrument when flag ***2237*** is detected by the load position sensor. At step ***112*** instrument ***2000*** scans a cartridge ID code indicating the type of test to be run on the cartridge. The label imaging system ***2770*** illuminates and captures an image of the patient label area during this step. At ***114*** the instrument displays the image of the patient label and the type of diagnostic test about to be run on a graphical user interface (GUI). At step *120*, the user is given the option to abort the test run, e.g. if the wrong cartridge was loaded. The instrument aborts a diagnostic test when the user elects to abort the test at *122*. In one implementation, the GUI requires user input to proceed in running the diagnostic test diagnostic test. In an alternative implementation, the method proceeds in the absence of user input within a set period of time, e.g. 10 seconds.

[0437] When the method proceeds, the instrument begins the clamping sequence to perform a series of verification checks to confirm the inserted cartridge is unused and suitable to run a diagnostic test. Instrument ***2000*** first establishes a zero clamping position to set the reference point from which all other clamping positions are measured from. The moving bracket assembly ***2040*** moves in a negative direction until tab ***2047*** triggers sensor ***2017*** fixed to the bottom of the fixed support bracket ***2011***. When sensor ***2017*** is triggered, the moving bracket assembly subsequently moves a calibrated distance in the positive or negative direction to define the zero clamping position at *124*. The instrument turns the linear actuator ***2014*** on to rotate the lead screw ***2016*** in a first rotational direction, such that rotating the lead screw pulls the moving bracket

assembly ***2040*** toward the fixed bracket assembly ***2010*** in a positive linear direction to a first clamping position, shown by *126*. In the first clamping position, the valve drive assembly ***2400*** contacts the rotary valve on a cartridge and light frame ***2686*** of the thermal clamp assembly ***2680*** contacts the reaction area ***1600***. A first rotary valve verification test on the rotary valve ***1400*** is performed in the first clamping position. At step *130*, the first verification test checks that the rotary valve is in the shipping configuration. As described herein, rotary valves with prematurely dropped rotors pose the risk of leaking due to gasket deformation from compressing a gasket for long periods of time prior to immediate use. In this embodiment, the rotary valve ***1400*** is configured for a shipping configuration to prevent the gasket from sealing against the stator until the time of operation. The valve drive assembly ***2400*** checks the shipping configuration of the rotary valve ***1400*** by using an interference sensor ***2404*** and the end of the valve drive shaft ***2405***. The valve drive shaft will not trigger the interference sensor, indicating the rotary valve is in shipping configuration, when valve drive pins ***2402*** mate correctly with engagement openings ***1417*** on the rotary valve. In this instance where the valve is confirmed to be in shipping configuration, the instrument proceeds with the next step of the method *134* to drop the rotor. An error is detected when the end of the valve drive shaft ***2405*** triggers the interference sensor at *132*. Triggering the interference sensor is indicative of the rotary valve not in a shipping configuration. Conditions that trigger an error include valve drive pins not fully inserted into engagement openings or failure to be inserted at all. This condition renders the cartridge unusable. The instrument aborts the test at step *132*, displaying a shipping configuration error to the GUI and unclamping the cartridge for ejection.

[0438] After the rotary valve is confirmed to be in the shipping configuration the valve drive assembly rotates to drop the rotor at *134* to transition the valve from a shipping configuration to an operational configuration. Rotating the rotary valve drops the rotor, as described herein, off the threaded retention ring to seal the gasket between the rotor and stator. At step *140* the instrument executes a second rotary valve verification test to determine the valve drop state. The valve drive assembly ***2400*** checks the state of the rotary valve using the interference sensor to confirm a successful rotor drop. The valve drive shaft will not trigger the interference sensor, indicating a successful rotary valve drop and proceeds onto step *144* to move the moving bracket assembly ***2040*** to a second clamping position. When the interference sensor is triggered, indicating an unsuccessful valve drop, the instrument aborts the test at step *142*, displaying a failed valve drop error at the GUI and unclamping the cartridge for ejection.

[0439] The moving bracket assembly ***2040*** moves in the positive direction to the second clamping position *142* at which hard stops ***2211*** contact the first surface of the fixed bracket assembly ***2010***. At step *150*, the instrument confirms sensor ***2019*** is triggered by the hard stops ***2211***. The second clamping position is the largest displacement in the positive direction the clamp block ***2041*** moves in this exemplified method. In the second clamping position, the door support assembly ***2280*** presses against cap ***1181***, pneumatic interface ***2100*** forms a pneumatic seal with the pneumatic

interface cover adaptor ***1172***, thermal clamp assembly ***2680*** is engaged and light frame ***2686*** forms a seal around the reaction area ***1600***, and the valve drive ***2401*** remains engaged with rotary valve ***1400***. An error is detected when sensor ***2019*** is not triggered by hard stops ***2211*** contacting the first surface of the fixed support bracket at *152*. Failing to trigger the hard stop sensor indicates the moving bracket assembly ***2040*** unsuccessfully clamped the cartridge. The instrument aborts the test at step *152*, displaying a failed hard stop error at the GUI and unclamping the cartridge for ejection. Completion of all rotary valve verification tests and successful clamping of the cartridge, as indicated by sensor ***2019***, signals instrument may begin the fluidic sequence portion of the method.

[0440] At steps *154* and *156*, the valve drive assembly ***2400*** of the instrument rotates the valve 360 degrees (step *154*) and then indexes the valve to a zero valving position (step *156*) using homing sensor ***2409***. The rotary valve is configured to seal off all inlet vias and outlet vias in the fluidics card in zero valving position, such that no fluidic communication is permitted. This configuration allows the instrument to perform a pneumatic leak test, step *160*. The instrument pressurizes the cartridge and ensures no pneumatic flow is detected. An error is detected if the pneumatic subsystem detects any pneumatic flow, thus indicating a pneumatic leak is present within the cartridge. Such a pneumatic leak renders the cartridge unreliable and/or unusable. The instrument aborts the test at step *162*, displaying a pneumatic leak error at the GUI and unclamping the cartridge for ejection. If no pneumatic leaks are detected, the instrument has completed the cartridge verification tests, indicating that the cartridge is competent to perform the diagnostic assay.

[0441] At step *164* of the method (see FIG. 106B), the frangible seal block ***2260*** moves in a positive direction to a third clamping position to break all frangible seals on the cartridge with frangible seal pins. The frangible seal block is permitted to move in the positive direction until hard stop ***2263*** contacts upper rail ***2231*** of the loading assembly ***2230***. In the third clamping position, the clamp block ***2041*** and all assemblies (i.e. door support assembly ***2280***, pneumatic interface ***2100***, valve drive ***2400***, and thermal clamp ***2680***) remain stationary in the second clamping position due to hard stops ***2211*** contacting the first surface of the fixed support bracket ***2012***. As described herein, the frangible seal block ***2260*** and the clamp block ***2041*** are separate components mechanically coupled by linear slide ***2264***. This configuration separates the clamping action from the actuation of frangible seals enabling the instrument to perform rotary valve verification tests prior to rendering the cartridge fluidically active. When the frangible seal block ***2260*** is moved in the positive direction to a third clamping position at *164*, all frangible seals are punctured. In an alternative embodiment, frangible seal pins can be varying in length to allow frangible seals to be punctured in a sequence when the frangible seal block is moved to different positions in the positive direction. At step *200* the instrument confirms frangible seals are broken using sensor ***2266***. An error is detected if sensor ***2266*** on the frangible seal block is not triggered. This condition renders the cartridge unusable due to frangible sealing failing to be actuated,

indicating that one or more flow paths within the cartridge are obstructed by an unruptured seal. The instrument aborts the test at step *202*, displaying a frangible seal actuation error at the GUI and unclamping the cartridge for ejection. Upon successful completion of steps *164* and *202*, frangible seals are fluidically active and the cartridge is ready to begin sample preparation of a sample suspected of containing a target pathogen at step *204*.

[0442] To begin sample preparation, the pneumatic subsystem pressurizes the cartridge using alternating periods of applied pressure and zero pressure to draw the sample from the fill chamber ***1101*** and into the metering chamber ***1110*** at *204*. Camera ***2271*** of the label imaging assembly ***2770*** illuminates the sample window ***1050*** and confirms adequate sample volume is loaded into the cartridge at step *210*. The instrument detects the presence of a ball ***1114*** present in the metering chamber ***1110*** to determine whether adequate sample volume is present. An error is detected when the instrument fails to identify the presence of the ball at a location indicating a sufficient sample volume is present in the metering chamber. The instrument aborts the test at step *212*, displaying an insufficient sample volume error at the GUI and unclamping the cartridge for ejection.

[0443] If sufficient sample volume is present, the instrument proceeds with pressurizing the cartridge to empty the loading module *214*, forcing the sample into the lysis chamber ***1371***, which already contains a lysis buffer comprising at least one chemical lysis agent. The instrument pressurizes the cartridge for a set period to transfer the sample from the metering chamber into the lysis chamber at *214*. The lysis chamber is filled by pneumatic pressure against a dead-end provided by the zero valving position of the rotary valve, which remains stationary when performing the lysing step. This configuration enables the instrument to perform a pressure maintenance check for a constant pressure profile reading seen at step *300*. An error is detected when the cartridge fails to maintain a constant pressure profile. Inability to maintain a constant pressure profile indicates a pneumatic leak may be present within the cartridge, rending the cartridge unreliable or inoperable. The instrument aborts the test at step *302*, displaying a pneumatic leak error to the GUI and unclamping the cartridge for ejection. Confirmation of maintenance of a constant pressure profile for the set period signals the instrument may move onto the mixing step. The instrument continues to maintain pressure in the cartridge while drive motor ***2330*** of the magnetic mixing assembly ***2300*** turns on for a set period at *304*. Magnetic coupling is effectuated between the driving magnet system ***2310***, driven magnet system ***2350***, and stir bar ***1390*** of the lysis chamber, such that when drive motor ***2330*** rotates, the driven magnet system ***2350*** and stir bar ***1390*** also rotate. Rotation of the stir bar mixes the contents of the lysis chamber and lyses the sample to produce a lysed sample.

[0444] Simultaneously during the set period of mixing, a microphone ***2380*** monitors the audible feedback of the lysis chamber and drive motor at step *310*. An error can be detected when the audible signal is not within a preset range. Conditions that cause the audible feedback to not be within the preset range include decoupling of the stir bar from the magnetic mixing assembly or

stalling of the drive motor. If the audible signal falls outside the preset range, the instrument aborts the test at step *312*, displaying an audible feedback error to the GUI and unclamping the cartridge for ejection. The drive motor is subsequently turned off at *314*, given the audible feedback of the magnetic mixing assembly remains in range for the entire duration of the set period.

[0445] After completion of a successful lysis operation, the valve drive assembly *2400* indexes the rotary valve to a first valving position at *316* and the instrument pressurizes the cartridge to empty the lysis chamber at *318*. The lysed sample containing nucleic acids and other cell debris flow over a porous solid support contained within the solid support chamber of the rotary valve according to an embodiment described herein. The porous solid support captures nucleic acids while permitting cell debris and lysis buffer to be directed to a waste collection element ***1470***. The instrument performs a pressure verification check at *400* to confirm the pressure profile is achieved in the allotted amount of time. An error is detected when the pressure profile is not achieved in the allotted time, indicating a pneumatic leak may be present within the cartridge. The instrument aborts the test at step *302*, displaying a pneumatic leak error to the GUI and unclamping the cartridge for ejection.

[0446] The instrument continues to monitor the pressure profile while the lysed sample is transferred from the lysis chamber ***1371*** to the porous solid support chamber ***1446*** of the rotary valve ***1400***. In one aspect of the present invention, pneumatic subsystem ***2130*** lacks a flow sensor and instead uses a feedback control loop based on an actuation signal sent to the proportional valve to determine when the lysed sample transfer to the porous solid support chamber is complete. At step *410* (FIG. 106C), the instrument detects that lysis buffer transfer is complete using the feedback control look of the pneumatic subsystem. A transfer time out error is identified when the instrument fails to detect a successful transfer of the lysed sample within a preset time period. The instrument aborts the test at step *412*, displaying a timeout error to the GUI and unclamping the cartridge for ejection. Upon successful detection of the lysed sample transfer, the applied pressure is turned off at step *414* and the instrument is ready to perform a washing step.

[0447] To align the wash buffer reservoir with the porous solid support chamber, the valve drive assembly ***2400*** indexes the rotary valve to a second valving position at *416* and the instrument pressurizes the cartridge to empty the wash buffer reservoir ***1475*** at *418*. Wash buffer flows out of the wash buffer reservoir and passes through the porous solid support ***1445*** contained within the solid support chamber ***1446*** of the rotary valve ***1400*** to remove unbound cell debris or other contaminants remaining in the porous solid support. The wash buffer is directed to the waste collection element ***1470*** leaving predominantly nucleic acids bound to the porous solid support ***1445***. In a manner similar to step *400*, the instrument performs a pressure verification check at *420* to confirm the pressure profile is achieved in the allotted amount of time like the test performed during the lysed sample transfer. An error is detected when the expected pressure profile is not achieved in the preset allotted time, potentially indicating a pneumatic leak within the cartridge. The instrument aborts the test at step *302*, displaying a pneumatic leak error to the GUI

and unclamping the cartridge for ejection. The instrument continues to monitor the pressure profile while the wash buffer is transferred from the wash buffer reservoir ***1475*** to the porous solid support chamber ***1446*** of the rotary valve ***1400***. The feedback control loop of the pneumatic subsystem described above monitors the actuation signal sent to the proportional valve to determine when wash buffer transfer to the porous solid support chamber is complete at *422*. An error is detected when the instrument fails to detect a successful transfer of wash buffer in the allotted time. The instrument aborts the test at step *412*, displaying a timeout error to the GUI and unclamping the cartridge for ejection. Upon successful detection of the wash buffer transfer, the applied pressure is turned off at *424* and the instrument is ready to perform an air drying step.

[0448] To air dry the porous solid support, the valve drive assembly ***2400*** indexes the rotary valve to a third valving position at *426* and the instrument pressurizes the cartridge at *428* to perform an air drying step. Pressurized air is blown through the porous solid support contained within the solid support chamber ***1446*** of the rotary valve and directed to the waste collection element ***1470*** for a set period. Performing the air drying pushes residual fluids and evaporates lingering volatile compounds that may be present in the solid support chamber after the washing step. One of ordinary skill in the art would recognize the advantages of minimizing the residual presence of lysis buffer and/or wash buffer in a final assay. During the air drying step, the instrument again performs a pressure verification check at *430* to confirm the pressure profile is achieved in the allotted amount of time. An error is detected when the pressure profile is not achieved in the allotted time, indicating a pneumatic leak may be present within the cartridge. The instrument aborts the test at step *302*, displaying a pneumatic leak error to the GUI and unclamping the cartridge for ejection. Upon successful completion of the air drying process, the pressure is turned off at step *432* and the instrument is ready to perform an elution step.

[0449] To align the elution buffer reservoir with the porous solid support chamber, the valve drive assembly ***2400*** indexes the rotary valve to a fourth valving position at *434* and the instrument pressurizes the cartridge to empty the elution buffer reservoir ***1475*** as seen by step *436*. Eluent flows out of the elution buffer reservoir ***1475***, passing through the porous solid support ***1445*** contained within the solid support chamber of the rotary valve to release nucleic acids bound to the porous solid support thereby generating an enriched nucleic acid solution. The enriched nucleic acid is directed to the rehydration chamber ***1520*** to rehydrate dried reagents deposited within the chamber. The cartridge remains pressurized while the instrument performs another pressure verification check at *500* to confirm the pressure profile is maintained for the preset period. An error is detected when the cartridge fails to maintain a constant pressure profile. Inability to maintain a constant pressure profile indicates a pneumatic leak may be present within the cartridge. The instrument aborts the test at step *302*, displaying a pneumatic leak error to the GUI and unclamping the cartridge for ejection.

[0450] The instrument continues to pressurize the cartridge to fill the rehydration chamber ***1520*** with purified sample and proceeds to push the purified sample to the metering channel ***1557***

producing a metered purified sample volume. While pressure remains applied, rehydration motor ***2510*** is turned on at step *502* for a set period to gyrate the magnetic mixing element (i.e., a ball ***1524***) within the rehydration chamber to dissolve and mix dried reagents with the metered purified sample (see FIG. 106D). At step *504* of the method, rehydration motor is turned off. Pressure is turned off at *506* and the chemistry heater ***2601*** of the chemistry heater assembly ***2600*** is simultaneously turned on at *508* to preheat the chemistry heater to a loading temperature for filling the assay chambers ***1621***.

[0451] While preheating the chemistry heater ***2601***, the reaction imaging assembly ***2700*** captures an image of the dry assay chambers at step *510*. Subsequently, the valve drive assembly ***2400*** indexes the rotary valve to a fifth valving position at *512* to align the rehydration chamber ***1520*** with the dry assay chambers. The instrument pressurizes the cartridge at *514* to pass the solution from the rehydration chamber into the assay chambers, thereby loading the assay chambers. In one implementation, the pneumatic subsystem ***2130*** pressurizes the cartridge using a stepwise ramping function to load the assay chambers. In an alternative implementation of the method, the assay chambers are loaded using a constant pressure profile. Pressure remains applied while the reaction imaging assembly captures an image of the loaded assay chambers at step *516*. The instrument uses the image to verify the assay chambers successfully loaded *600*. An error is detected when the instrument identifies incomplete loading of the assay chambers. The instrument aborts the test at step *602*, displaying an incomplete loading error to the GUI and unclamping the cartridge for ejection.

[0452] After confirmation of loaded assay chambers, the heater ***2661*** of the heat staker assembly ***2640*** is activated to bring the staker bar assembly ***2641*** up to a preset staking temperature at step *604*. The instrument uses motor ***2642*** to move the staker bar assembly ***2641*** toward the cartridge until the staker blade ***2660*** contacts the cartridge. The motor releases a spring ***2643***, which applies the force required to press the staker bar assembly into the film side of the cartridge to heat stake the cartridge at *606*, as described herein. The hot staker blade ***2660*** melts selected areas of the cartridge, e.g. across u-bend ***1607***, loading channels ***1672***, channel ***1362*** leading to the waste collection element, and venting channel ***1472*** exiting from the waste collection element ***1470***. Heat staking these specific channels prevent liquids from escaping the cartridge thereby mitigating the risk for release of amplicon or potentially contaminated biological waste into the outside environment. The heat staker assembly ***2640*** heat stakes the cartridge for a set period *606* and then turns off the heater ***2661*** of the heat staker assembly. Fan ***2644*** turns on at *608* and the heat staker blade ***2660*** is actively cooled by the fan until the instrument detects the staker bar is cooled to the desired temperature. At step *610*, motor *2642* retracts the heat staker assembly ***2640*** from the cartridge and the pneumatic pressure applied to the cartridge is turned off at *612*.

[0453] At step *614*, the reaction imaging assembly captures an image of the assay chambers ***1621*** after heat staking and release of pneumatic pressure. The image verifying assay chambers remain loaded with the sample mixture at *700* is used to confirm a successful sealing by the heat

stake ***1603***. Failure of the assay chambers to remain loaded indicate a cartridge leak due to an unsuccessful heat stake. The instrument aborts the test at step *702*, displaying a failed heat stake error to the GUI and unclamping the cartridge for ejection. Confirmation of a successful heat stake allows the instrument to proceed onto an amplification step.

[0454] At this step in the method, the chemistry heater ***2601*** of the chemistry heater assembly ***2600*** has come up to the loading temperature and is ready to facilitate an amplification of nucleic acids within the assay chambers. In an alternative embodiment, the chemistry heater ***2601*** may be fluctuated between a high and low temperature one or more times prior to being set to a reaction temperature shown by step *704*. The chemistry heater is warmed until the chemistry heater reaches the high temperature. Thereafter, the chemistry heater is turned off and actively cooled by fan ***2603*** until the assay chambers ***1621*** cool to the low temperature to complete one cycle. The assay chambers optionally may be fluctuated one or more times before being set to the reaction temperature.

[0455] The chemistry heater ***2601*** is then set to the reaction temperature for the duration of the test at *706*. At a predetermined frequency, the reaction imaging assembly ***2700*** captures images of the assay chambers ***1621*** during amplification allowing the instrument to process the images of the assay chambers. In one implementation, the instrument turns on the excitation LED ***2731*** of the excitation lens cell ***2730*** and reaction camera ***2701*** captures an image of the assay chambers every 20 seconds of the amplification. In an alternative embodiment, the instrument turns on the excitation LED ***2731*** and reaction camera ***2701*** captures an image of the assay chambers every 15 seconds of the amplification. The instrument processes the sequence of images captured by the reaction imaging assembly ***2700*** to determine the presence of a signal, such as a fluorescent signal, indicative of the presence of target nucleic acids in each of the plurality of assay chambers, as shown by step *800*. In embodiments where the device is configured to perform a multiplex assay, the instrument may detect a positive or negative signal for each of the plurality of assay chambers. In certain embodiments, e.g. cartridges containing a process control expected to generate a positive signal in at least one assay chamber, the instrument may produce a timeout error, as shown by *802*, when the expected signal is not determined within the allotted time. The instrument aborts the test at step *802*, displaying an error to the GUI and unclamping the cartridge for ejection at *900*.

[0456] Upon completion of the amplification steps, either by detecting a positive signal in each well or after an allotted time for amplification elapses, the unclamping and ejection sequence begins at with linear actuator ***2014*** on the fixed bracket assembly ***2010*** rotating the lead screw ***2016*** in a second rotational direction to first push the frangible seal block ***2260*** away from the cartridge. The lead screw continues to rotate in the second rotational direction as the frangible seal block ***2260*** contacts ledge ***2046*** of the clamp block ***2041*** and pushes entire moving bracket assembly ***2040*** in a negative direction away from the cartridge to a fourth clamping position at *900*. As the moving bracket assembly moves in the negative direction, latch release arm ***2214***

contacts the end of pin ***2216*** to lift latch ***2212*** out of notch ***1021*** on top of the cartridge at *902*. The loading assembly ***2100*** ejects the cartridge at *904* using spring ***2235*** to pull the pusher carriage ***2234*** and the cartridge to a forward most loading position towards the slot ***2072*** of the instrument to eject the cartridge. Steps *900* through *904* are executed each time an error is detected that leads to aborting the method and ejecting a cartridge. When the method is successfully completed, the last step of the method displays the result of the diagnostic result to the GUI, shown by step *906*.

VIII. METHODS OF USE – BIOLOGY

[0457] One aspect of the invention provides methods of testing a sample suspected of containing a target pathogen, comprising (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen, (b) advancing the sample to a lysis chamber having at least one lysis reagent therein, (c) mixing the sample with the at least one lysis agent to generate a lysed sample; (d) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support, (e) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid, (f) distributing the enriched nucleic acid to two or more assay chambers and combining the enriched nucleic acid with one or more amplification reagents, (g) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers, and (h) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen. The method is implemented on a modular assay system comprising a loading module, lysis module, purification module and amplification module.

A. Loading module

[0458] In some cases, the cartridge comprises a sample entry port, a sample input well, or a fill chamber. Given the pressurization inherent to the devices described herein, the entry port preferably is air tight when sealed by a cap. In certain implementations, the cap is configured to be opened to permit addition of a sample and then resealed prior to the sample being loaded to the device. Alternatively, the sample can be loaded via a puncturable septa or large one-way valve. The diagnostic system can include a sample loader, such as a bulb or syringe, useful for loading a sample into the device. The cartridge can be packaged with a sample collection device, such as a syringe, bulb, swab, scraper, biopsy punch, or other tool for a user to collect a sample.

[0459] Samples can be obtained from a subject (e.g., human subject), a food sample (e.g., including an organism), or an environmental sample (e.g., including one or more organisms). (e.g., microbiological cultures). A sample may include a specimen of synthetic origin (e.g., microbiological cultures). Samples may be obtained from a patient or person and includes

blood, feces, urine, saliva or other bodily fluid. Exemplary, non-limiting samples include blood, plasma, serum, sputum, urine, fecal matter (e.g., stool sample), swab (e.g. of skin, wound, mucosal membrane, cervix, vagina, urethra, throat or nasal cavity), sweat, cerebrospinal fluid, amniotic fluid, interstitial fluid, tear fluid, bone marrow, tissue sample (e.g., a skin sample or a biopsy sample), a buccal mouthwash sample, an aerosol (e.g., produced by coughing), a water sample, a plant sample, or a food sample. The sample can include any useful target or analyte to be detected, filtered, concentrated, and/or processed.

[0460] Analysis can indicate the presence, absence, or quantity of an analyte of interest. For example, nucleic acid amplification can provide qualitative or quantitative information about a sample, such as the presence, absence, or abundance of a cell, cell type, pathogen (e.g., bacteria, virus), toxin, pollutant, infectious agent, gene, gene expression product, methylation product, genetic mutation, or biomarker (e.g., nucleic acid, protein, or small molecule).

[0461] Analysis can indicate the presence, absence, or quantity of an analyte of interest. For example, nucleic acid amplification can provide qualitative or quantitative information about a sample, such as the presence, absence, or abundance of a cell, cell type, pathogen (e.g., bacteria, virus), toxin, pollutant, infectious agent, gene, gene expression product, methylation product, genetic mutation, or biomarker (e.g., nucleic acid, protein, or small molecule). Analytical targets of interest can include indicators of diseases or illnesses such as genetic diseases, respiratory diseases, cardiovascular diseases, cancers, neurological diseases, autoimmune diseases, pulmonary diseases, reproductive diseases, fetal diseases, Alzheimer's disease, bovine spongiform encephalopathy (Mad Cow disease), chlamydia, cholera, cryptosporidiosis, dengue, giardia, gonorrhea, human immunodeficiency virus (HIV), hepatitis (e.g., A, B, or C), herpes (e.g., oral or genital), human papilloma virus (HPV), influenza, Japanese encephalitis, malaria, measles, meningitis, methicillin-resistant Staphylococcus aureus (MRSA), Middle East Respiratory Syndrome (MERS), onchocerciasis, pneumonia, rotavirus, schistosomiasis, shigella, strep throat, syphilis, tuberculosis, trichomonas, typhoid, and yellow fever. Analytical targets can include biomarkers indicative of traumatic brain injury, kidney disease, cardiovascular disease, cardiovascular events (e.g., heart attack, stroke), or susceptibility of certain infectious agents (such as bacteria or viruses) to certain therapeutic agents. Analytical targets can include genetic markers, such as polymorphisms (e.g., single nucleotide polymorphisms (SNPs), copy number variations), gene expression products, specific proteins or modifications (e.g. glycosylation or other post-translational processing) of proteins. In preferred implementations, the analyte of interest is a nucleic acid useful in the identification of microbes including viruses, bacteria, unicellular fungi and parasites.

[0462] In many implementations, it is desirable to subject the sample to one or more treatments before attempting to lyse the target pathogen. In some implementations, the treatment occurs prior to passing the sample to the lysis chamber. In other implementations, the treatment

occurs after passing the sample to the lysis chamber, but prior to mixing the sample with at least one lysis reagent.

[0463] The diagnostic system comprising a device and instrument described herein can be used to detect target pathogens from any biological sample. Solid samples or semi-solid, such as tissue samples, require chemical, enzymatic, physical and/or mechanical treatment to release the pathogen into a fluid sample suitable to flow through the test cartridge. Similarly, other biological sample types may preferably be subjected to a chemical, enzymatic, physical or mechanical pre-treatment prior to mixing with one or more lysis agents. Such pre-treatment can be performed within a cartridge or prior to loading the sample into the cartridge. Chemical pretreatments include, e.g. n-acetylcysteine to break up mucus in sputum samples or lysis of animal cells with saponin to release intracellular pathogen or debulk the sample. Dithiothreitol is also commonly used to break up mucus as well as disintegrate solid tissue samples. In another implementation, the sample can be enzymatically pre-treated, e.g. with an elastase, collagenase or proteinase K to preferentially degrade connective tissues in a solid tissue sample. In yet another implementation, the sample can be treated with a nuclease, e.g. a DNase or RNase, to remove extracellular nucleic acid from the sample prior to lysis. Such nucleases can be deactivated by subsequent addition of a nuclease inhibitor or by denaturation with a chaotropic lysis agent. Finally, certain samples can be disrupted with bead beating prior to addition of a chemical lysis agent.

[0464] In some implementations, an undesired contaminant can be physically separated from the target pathogen(s), e.g. by filtration. Filtration enables the separation of one component or fraction of a sample from another component or fraction. For example, a filter can enable the solid components, such as, e.g., cells, debris or contaminant, to be separated from the liquid components of the solution. Alternatively, a filter can enable larger solid components, such as, e.g., proteinaceous aggregates, aggregated cell debris, or larger cell, to be separated from smaller components, e.g. virus, bacterial cells or nucleic acid, from a solution. In aspects of this embodiment, a filter useful for separating components contained in a solution can be, e.g., a size-exclusion filter, a plasma filter, an ion-exclusion filter, a magnetic filter, or an affinity filter. In other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., 0.1 µm, 0.2 µm, 0.5 µm, 1.0 µm, 2.0 µm, 5.0 µm, 10.0 µm, 20.0 µm, 30 µm, 40 µm, 50 µm, 60 µm, 70 µm, 80 µm, 90 µm, 100 µm, or more. In yet other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., at least 0.2 µm, at least 0.5 µm, at least 1.0 µm, at least 2.0 µm, at least 5.0 µm, at least 10.0 µm, at least 20.0 µm, at least 30.0 µm, at least 40.0 µm, at least 50.0 µm, at least 60.0 µm, at least 70.0 µm, at least 80.0 µm, at least 90.0 µm, or at least 100.0 µm. In still other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size of, e.g., at most 0.1 µm, at most 0.2 µm, at most 0.5 µm, at most 1.0 µm, at most 2.0 µm, at most 5.0 µm, at most 10.0 µm, at most 20.0 µm, at

most 30.0 µm, at most 40.0 µm, at most 50.0 µm, at most 60.0 µm, at most 70.0 µm, at most 80.0 µm, at most 90.0 µm, or at most 100.0 µm. In other aspects of this embodiment, a filter useful for separating components contained in a solution can have a pore size between, e.g., about 0.2 µm to about 0.5 µm, about 0.2 µm to about 1.0 µm, about 0.2 µm to about 2.0 µm, about 0.2 µm to about 5.0 µm, about 0.2 µm to about 10.0 µm, about 0.2 µm to about 20.0 µm, about 0.2 µm to about 30.0 µm, about 0.2 µm to about 40.0 µm, about 0.2 µm to about 50.0 µm, about 0.5 µm to about 1.0 µm, about 0.5 µm to about 2.0 µm, about 0.5 µm to about 5.0 µm, about 0.5 µm to about 10.0 µm, about 0.5 µm to about 20.0 µm, about 0.5 µm to about 30.0 µm, about 0.5 µm to about 40.0 µm, about 0.5 µm to about 50.0 µm, about 1.0 µm to about 2.0 µm, about 1.0 µm to about 5.0 µm, about 1.0 µm to about 10.0 µm, about 1.0 µm to about 20.0 µm, about 1.0 µm to about 30.0 µm, about 1.0 µm to about 40.0 µm, about 1.0 µm to about 50.0 µm, about 2.0 µm to about 5.0 µm, about 2.0 µm to about 10.0 µm, about 2.0 µm to about 20.0 µm, about 2.0 µm to about 30.0 µm, about 2.0 µm to about 40.0 µm, about 2.0 µm to about 50.0 µm, about 5.0 µm to about 10.0 µm, about 5.0 µm to about 20.0 µm, about 5.0 µm to about 30.0 µm, about 5.0 µm to about 40.0 µm, about 5.0 µm to about 50.0 µm, about 10.0 µm to about 20.0 µm, about 10.0 µm to about 30.0 µm, about 10.0 µm to about 40.0 µm, about 10.0 µm to about 50.0 µm, about 10.0 µm to about 60.0 µm, about 10.0 µm to about 70.0 µm, about 20.0 µm to about 30.0 µm, about 20.0 µm to about 40.0 µm, about 20.0 µm to about 50.0 µm, about 20.0 µm to about 60.0 µm, about 20.0 µm to about 70.0 µm, about 20.0 µm to about 80.0 µm, about 20.0 µm to about 90.0 µm, about 20.0 µm to about 100.0 µm, about 30.0 µm to about 40.0 µm, about 30.0 µm to about 50.0 µm, about 30.0 µm to about 60.0 µm, about 30.0 µm to about 70.0 µm, about 30.0 µm to about 80.0 µm, about 30.0 µm to about 90.0 µm, about 30.0 µm to about 100.0 µm, about 40.0 µm to about 50.0 µm, about 40.0 µm to about 60.0 µm, about 40.0 µm to about 70.0 µm, about 40.0 µm to about 80.0 µm, about 40.0 µm to about 90.0 µm, about 40.0 µm to about 100.0 µm, about 50.0 µm to about 60.0 µm, about 50.0 µm to about 70.0 µm, about 50.0 µm to about 80.0 µm, about 50.0 µm to about 90.0 µm, or about 50.0 µm to about 100.0 µm.

[0465] In certain implementations, the size-exclusion filter can be a depth filter. Depth filters consist of a matrix of randomly oriented, bonded fibers that capture particulates within the depth of the filter, as opposed to on the surface. The fibers in the depth filter can be comprised of glass, cotton or any of a variety of polymers. Exemplified depth filter materials may include, type GF/F, GF/C and GMF150 (glass fiber, Whatman), Metrigard® (glass fiber, Pall-Gelman), APIS (glass fiber, Millipore), as well as a variety of cellulose, polyester, polypropylene or other fiber or particulate filters, so long as the filter media can retain a sufficient contaminant to allow further processing of the sample.

[0466] In alternate implementations, the size-exclusion filter can be a membrane filter, or mesh filter. Membrane filters typically performs separations by retaining particles larger than its pore size on the upstream surface of the filter. Particles with a diameter below the rated pore size

may either pass through the membrane or be captured by other mechanisms within the membrane structure. Membrane filters can support smaller pore sizes, including small enough to exclude bacterial cells. Membrane filters can be used to concentrate solutions, e.g. bacterial cell suspensions, by filtering a first larger volume through the membrane filter, thereby holding the bacterial cells to the upstream surface of the membrane filter (or suspended in residual fluid retained on the upstream side of the filter). The bacterial cells can then be resuspended in a second small volume of fluid by either passing the suspension fluid in the reverse direction to float the bacterial cells off the membrane surface or by washing the suspension fluid across the upstream surface of the filter to wash the bacterial cells off the filter. Exemplified membranes may include, polyethersulfone (PES) membranes (e.g., Supor® 200, Supor® 450, Supor® MachV (Pall-Gelman, Port Washington, N.Y.), Millipore Express PLUS® (Millipore)). Other possible filter materials may include, HT Tuffryn® (polysulfone), GN Metricel® (mixed cellulose ester), Nylaflo® (Nylon), FP Verticel (PVDF), all from Pall-Gelman (Port Washington, N.Y.), and Nuclepore (polycarbonate) from Whatman (Kent, UK).

[0467] In some embodiments, an undesired contaminant can be removed from a sample by exposing the sample to a capture agent, such as a capture antibody, is immobilized on a solid substrate. The solid substrate can be contacted with the sample such that contaminant in the sample can bind to the immobilized antibody. In some embodiment, a capture antibody can be used that has binding affinity for red blood cells. The antibody may be a monoclonal antibody or a polyclonal antibody. Suitable solid substrates to which a capture antibody can be bound include, without limitation, membranes such as nylon or nitrocellulose membranes, and beads or particles (e.g., agarose, cellulose, glass, polystyrene, polyacrylamide, magnetic, or magnetizable beads or particles). In an alternate implementation, the capture agent can be any protein having specific and high affinity for binding to an undesired contaminant.

B. Lysis Module

[0468] Cell lysis refers to a process in which the outer boundary or cell membrane is broken down or destroyed in order to release inter-cellular materials such as nucleic acids (DNA, RNA), protein or organelles from a cell. Lysis resulting in release of nucleic acids can be achieved by chemical, enzymatic, physical and/or mechanical interventions.

[0469] In one implementation, the lysis agent is a chemical lysis agent. Chemical lysis methods disrupt the cell membrane, e.g., by changing pH or by addition of detergents and/or chaotropic agents to solubilize membrane proteins and thereby rupture the cell membrane to release its contents. These chemical lysis solutions can include one or more chemical lysis agents such as anionic detergents, cationic detergents, non-ionic detergents or chaotropic agents. Non limiting examples of non-ionic detergents include 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), Triton X, NP-40, Tween, and 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), a zwitterionic detergent. Suitable chaotropic agents include, but are not limited to, urea, guanidine (e.g.

guanidinium isothiocyanate or guanidinium hydrochloride), ethylenediaminetetraacetic acid (EDTA) and lithium perchlorate. In a preferred implementation, the suspected pathogen is a virus or a gram-negative bacteria and the chemical lysis reagent is a chaotropic agent.

[0470] The lysis agent can be an enzyme, or enzymatic lysis agent. Enzymatic cell lysis advantageous can allow for selective lysis of certain types of cells. For example, the enzymatic lysis agent can selectively cleave peptidoglycans found only in bacterial cell walls. Exemplified enzymatic lysis agents include achromopeptidase, lysostaphin, lysozyme, mutanolysin. Alternatively, a lyticase or chitinase, specific to yeast cells, can be used as the enzymatic lysis agent. In some cases enzymes with broad specificity and no specific target cell type, such as proteinase K, can be used as the lysis agent. Any of the enzymatic enzymes can be used in combination with mechanical or chemical lysis agents to promote faster and/or more complete lysis.

[0471] For use in the cartridges described here, bead beating is a preferred mechanical lysis mechanism, in which cells are disrupted by agitating, e.g. mixing, tiny beads made of glass, steel or ceramic with the cell suspension at high speeds. Bead beating is capably of lysing a variety of cells, including yeast and gran-positive bacteria. A preferred lysis method combines both mechanical and non-mechanical (e.g. chemical) methods, for example bead beating in a solution containing guanidine and/or Triton X-100. In some implementations, the mechanical lysis agent is ceramic beads, glass beads or steel beads, and mixing comprises rotating a stir bar at at least 500 rpm, at least 1000 rpm, at least 2000 rpm or at least 3000 rpm for at least 30 seconds, at least one minute, or at least two minutes. Bead beating is a preferred method for use in disrupting cells with significantly structured cell walls. Accordingly, in some implementations utilizing a mechanical lysis agent comprised of ceramic, glass or steel beads, the suspected pathogen is a gram-negative bacterium, a fungus such as a yeast, or a plant cell.

[0472] In samples having high organic loads, such as stool samples, blood samples, sputum samples or swab samples collected from mucus membranes, significant debris may be present in the lysed sample. In such cases, it is advantageous to filter the lysed sample prior to passing it through the porous solid support. In a preferred implementation, the lysed sample is passed through a size-exclusion filter, wherein nucleic acid passes through the filter. In a more preferred implementation, the lysed sample is passed through a depth filter. Preferably such post-lysis filters have a pore size of 20 µm or less, more preferably 10 µm or less.

C. Purification Module

[0473] After lysis, the lysed sample is passed through a first porous solid support thereby capturing nucleic acid. In some implementations, the porous solid support may preferentially bind DNA more than RNA or RNA more than DNA or certain lengths of nucleic acid (e.g. fragmented genomic DNA more than complete genomic DNA). However, a porous solid support for capturing nucleic acid in the devices described herein, preferably, binds nucleic acid regardless of the sequences present in the nucleic acids. When the lysed sample is passed

through porous solid support having affinity for nucleic acids, the nucleic acids are captured by the porous solid support while proteins, lipids, polysaccharides, and other cell debris that can inhibit nucleic acid amplification pass through the column and to the waste chamber. In some implementations, after capturing the nucleic acid, a wash solution is passed through the porous solid support to further remove contaminants. Captured nucleic acid is then released from the porous solid support with an elution buffer to generate an enriched nucleic acid.

[0474] In a preferred implementation, the porous solid support is a silica resin, e.g. silica fibers. Salt is important to binding nucleic acid to silica resins. In implementations where a chemical lysis agent, such as guanidinium isothiocyanate, is used, the lysis agent can provide the necessary salt. In other implementations, it is advantageous to supplement the lysed sample with a chaotropic salt. The addition of alcohol, such as ethanol or isopropanol, can further enhance and influence the binding of nucleic acids to the silica resin. In a preferred implementation, the silica resin column is washed with a dilute salt and/or alcohol solution. If a dilute salt solution is used, preferably a send wash buffer containing alcohol but no salt is also passed over the silica resin. Prior to elution, preferably excess alcohol is removed, e.g., by drying with forced air. Finally, enriched nucleic acid is released from the silica resin with water or buffered (e.g. 10 mM Tris) water. Higher molecular weight DNA can be preferably released from the resin with 10 mM Tris at pH 8-9. RNA can be preferentially released from the silica resin with water.

[0475] In some implementations, it may be desirable to remove additional contaminants from the enriched nucleic acid by passing is through a second solid support. In such implementations, prior to distributing the enriched nucleic acid to the assay chambers, the method further comprises passing the enriched nucleic acid through a second porous solid support. The second porous solid support can be the same as the first solid support. In cases in which the first and second solid support are comprised of the same material, the enriched nucleic acid is mixed with a matrix binding agent prior to passing through the second solid support. For example, when the first and second solid support are a silica resin, the matrix binding agent can be a salt and/or alcohol solution as described above. In an alternate implementation, the second solid support is different than the first solid support. In some such implementations, the second solid support has an affinity for nucleic acid and the method further comprises releasing the captured nucleic acid from the second solid support to generate a twice enriched nucleic acid. In other implementations, the second solid support does not have an affinity for the nucleic acid, but rather captures one or more contaminants, thereby removing the contaminant from the enriched nucleic acid.

[0476] In an alternate embodiment, it may be desirable to remove contaminant from the lysed sample prior to passing it through the first porous solid support. In such cases, the method further comprises passing the lysed sample through a second solid support, wherein the second

solid support does not bind nucleic acid, but rather has affinity for one or more contaminants, thereby removing the one or more contaminants from the lysed sample.

[0477] In order to implement a method using more than one solid support on the devices described herein, the rotor can comprise a plurality of flow channels, each flow channel comprising an inlet ***1441***, an outlet ***1442***, and a porous solid support ***1445***. In certain implementation, the rotor comprises a main body and a cap ***1430*** operably connected to the main body, and wherein one wall of the flow channel is defined by the cap. The rotor comprises an outer face ***1413*** opposite the rotor valving face, wherein the outer face can comprise an opening for engaging a spline. The multiple flow channels can have the same or different dimensions. Similarly, the multiple flow channels can contain the same or different porous solid supports. Accordingly, nucleic acid may be purified from a particularly contaminated same by binding the nucleic acid to a first column, washing the bound nucleic acid and eluting a partially purified nucleic acid solution. The partially purified nucleic acid can be mixed with a binding buffer and passed through a second solid support, binding the nucleic acid to the second support, while allowing contaminants to pass. The bound nucleic acid is washed and then eluted to generate a double-purified nucleic acid solution. Alternatively the second solid support can be specific for a contaminant, allowing nucleic acid to pass, but retaining the undesired contaminant, thereby generating a more purified nucleic acid solution.

D. Amplification Module

[0478] The amplification module comprises a plurality of assay chambers of defined volume, each configured to receive a purified nucleic acid. The amplification module includes a heater such that the amplification module can perform an isothermal or thermocycling amplification reaction on the target nucleic acid. The amplification module further is configured to detect a signal indicative of target amplicon generated from the nucleic acid. In one implementation, the distributing step is performed prior to combining the enriched nucleic acid with an amplification reagent. Alternatively, the enriched nucleic acid is combined with one or more amplification reagents before the distributing step. The amplification reagent can be any reagent that is necessary or beneficial for nucleic acid synthesis, including, but not limited to, a DNA polymerase, a reverse transcriptase, a helicase, nucleotide triphosphates (NTPs), a magnesium salt, a potassium salt, an ammonium salt, a buffer, or combinations thereof. In many implementations the one or more amplification reagents comprise a primer or primer set. The primer set can be specific to a first nucleic acid sequence present in one of the one or more target pathogens. In some implementations, a first reaction well contains a first primer set specific to a first nucleic acid sequence and a second reaction well contains a second primer set specific to a second nucleic acid sequence. The first nucleic acid sequence can be present in one or more of the target pathogen or present in a process control.

[0479] In addition to the primary amplification assay, the method can comprise the step of pre-amplifying the enriched nucleic acid. Such preamplification is particularly useful when a very

limited amount of target nucleic acid is in the sample, either due to few pathogen cells and/or to low copy of the target nucleic acid within the pathogen cells. Using a cartridge with a large number of wells, i.e. highly multiplexed, also benefits from pre-amplification. For such implementation, the isothermal amplification is initiated prior to distributing the enriched nucleic acid to the two or more assay chambers. Optionally, after the distributing step, but prior to performing the isothermal amplification reaction, the method further comprises combining the enriched nucleic acid with a primer set specific to one of the one or more target pathogens.

E. Alternate Workflows

[0480] The instruments and cartridges described herein can be adapted to analyze a variety of biological samples, including cerebrospinal fluid (CSF), urine, throat or nasal swabs, blood, genital swabs (e.g. vaginal, cervical or urethral swabs), sputum, stool or solid tissue sample. In each case the method of testing a sample suspected of containing a target pathogen, comprising the following basic steps (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) advancing the sample to a lysis chamber having at least one lysis reagent therein, (c) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (d) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (e) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (f) distributing the enriched nucleic acid to two or more assay chambers *784* and combining the enriched nucleic acid with one or more amplification reagents *780*, (g) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (h) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen. For certain sample types, between steps (a) and (c), the method further comprises pretreating the sample *182*. The pretreatment can be a chemical, physical, mechanical or enzymatic pretreatment as described above. For some sample types, subsequent to step (c) but prior to step (d), the method further comprises filtering the lysed sample *382*, preferably by passing the lysed sample through a size-exclusion filter. In many cases, after step (d) and prior to step (e), the method further comprises washing the porous solid support *482*. Some samples will contain a high level of contaminants, in such case it may be advantageous to repeats steps (d) and (e) on passing the lysed sample and then enriched nucleic acid through a first and second porous solid support.

[0481] Some sample types, e.g. blood, is expected to contain the target pathogen at very low concentrations. Some cartridges will comprise a large number of assay chambers. In cases where the concentration of pathogen is very low and the number of assay chambers is high, can lead to false negative determinations due to partitioning. For example, when an enriched

nucleic acid that contains five copies of nucleic acid associated with the suspected pathogen is assayed in a cartridge having eight wells, at least three assay chambers will not receive a copy of the nucleic acid associated with the suspected pathogen. If the primer set associated with the target pathogen is in one of those three wells, the cartridge will falsely report that no pathogen is present. This result can be avoided by pre-amplifying certain nucleic acid targets prior to distributing the enriched nucleic acid to the plurality of reaction well *782*.

[0482] For simple, clear patient samples, such as cerebrospinal fluid, urine or cell suspensions extracted from throat or nasal swabs (FIG. 108), the method comprises (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) advancing the sample to a lysis chamber having at least one lysis reagent therein, (c) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (d) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (e) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (f) distributing the enriched nucleic acid to two or more assay chambers *784* and combining the enriched nucleic acid with one or more amplification reagents *780*, (g) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (h) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen. Typically, the lysis agent is a chemical agent such as a detergent, a chaotropic agent or a combination thereof. If the target pathogen is resistant to chemical lysis, e.g. a yeast or gram positive bacterium, the one or more lysis agent further comprises a mechanical lysis agent such as beading beating.

[0483] Exemplified pathogens that may be suspected in CSF include, but are not limited to, *Brucella*, *Haemophilus influenzae*, *Bacillus anthracis*, *Listeria*, *Streptococcus pneumoniae*, *Leptospira*, *Borrelia burgdorferi* (Lyme's disease), *Mycobacterium tuberculosis*, *Cryptococcus*, and *Candida*. Exemplified pathogens that may be suspected in urine include, but are not limited to, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Pseudomonas* sp. (e.g., *Pseudomonas aeruginosa*), *Enterococcus* sp. (e.g., *Enterococcus faecalis* or *Enterococcus faecium*), *Leptospira*, *Chlamydia* sp. (e.g. *Chlamydia trachomatis*), *Mycoplasma* sp. (e.g. *Mycoplasma genitalium*),and *Trichomonas vaginalis*. Exemplified pathogens that may be suspected in a throat or nasal swab include, but are not limited to, *Haemophilus influenzae*, *Bordetella pertussis*, *Corynebacterium diphtheriae*, *Streptococcus* sp. (e.g. Group A or Group B strep), *Mycoplasma* sp. (e.g. *Mycoplasma pneumoniae*), *Candida* sp. (e.g. *Candida albicans*), Influenza, and coronavirus (e.g. MERS, SARS, or SARS-CoV-2).

[0484] Sputum is the thick mucus or phlegm that is expectorated from the lower respiratory tract (bronchi and lungs) and is important for the investigation of certain respiratory diseases, e.g.

tuberculosis. Other exemplified pathogens that may be detected in sputum include, but are not limited to, *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., *Legionella* sp., *Bordetella pertussis*, *Yersinia* sp. (e.g. *Yersinia pestis*), *Pseudomonas* sp. (e.g., *Pseudomonas aeruginosa*), *Streptococcus pneumoniae*, *Mycoplasma* sp. (e.g. *Mycoplasma pneumoniae*), *Blastomyces dermatitidis*, and *Mycobacterium* sp., (e.g. *Mycobacterium tuberculosis*).

[0485] Given the high viscosity of sputa in some patients, e.g. from patients with advanced cystic fibrosis, sputa must first be liquefied mechanically by bead beating or chemically with mucolytic agents such as n-acetylcysteine (Mucomyst; Bristol) or dithiothreitol (Sputolysin). Referring to FIG. 111, the invention provides methods of identifying one or more suspected pathogens in a sputum sample, the method comprising the steps of: (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) pretreating the sputum sample with a mucolytic agent *182*, (c) advancing the sample to a lysis chamber having at least one lysis reagent therein, (d) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (e) filtering the lysed sample *382*, preferably by passing the lysed sample through a size-exclusion filter, (f) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (g) passing a wash solution through the porous solid support *482*, (h) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (i) distributing the enriched nucleic acid to two or more assay chambers *784* and combining the enriched nucleic acid with one or more amplification reagents *780*, (j) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (k) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sputum sample suspected of containing the target pathogen. Optionally, the enriched nucleic acid is pre-amplified *782* prior to distributing the nucleic acid to the two or more assay chambers.

[0486] Referring to FIG. 110, the invention provides methods of identifying one or more suspected pathogens in a sample extracted from a genital swab (e.g. vaginal, cervical or urethral swab), the method comprising the steps of: (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) advancing the sample to a lysis chamber having at least one lysis reagent therein, (c) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (d) filtering the lysed sample *382*, preferably by passing the lysed sample through a size-exclusion filter, (e) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (f) passing a wash solution through the porous solid support *482*, (g) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (h) distributing the enriched nucleic acid to two or more assay chambers *784* and combining the

enriched nucleic acid with one or more amplification reagents *780*, (i) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (j) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen. Optionally, the enriched nucleic acid is pre-amplified prior to step (h). Exemplified pathogens that may be suspected in a urogenital swab include, but are not limited to, *Chlamydia* sp. (e.g. *Chlamydia trachomatis*), *Mycoplasma* sp. (e.g. *Mycoplasma genitalium*), *Candida* sp. (e.g. *Candida albicans*), human papilloma virus (HPV), *Trichomonas vaginalis*, *Gardnerella vaginalis*, *Lactobacillus* sp., *Bacteroides* sp., *Prevotella* sp, *Mobiluncus* sp., and *Peptostreptococcus* sp., *Atopobium vaginae*, and *Sneathia* (*Leptotrichia*).

[0487] Blood samples can be particularly difficult for nucleic acid amplification testing, as heme (a component of hemoglobin in red blood cells) is a well-known inhibitor of nucleic acid amplification. According, blood samples will require additional processing prior to the amplification steps. Referring to FIG. 109, the invention provides methods of identifying one or more suspected pathogens in a blood sample, the method comprising the steps of: (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) subjecting the blood sample to one or more chemical, enzymatic or physical pretreatments *182*, (c) advancing the sample to a lysis chamber having at least one lysis reagent therein, (d) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (e) filtering the lysed sample *382*, preferably by passing the lysed sample through a size-exclusion filter, (f) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (g) passing a wash solution through the porous solid support *482*, (h) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (i) pre-amplifying the enriched nucleic acid *782*, (j) distributing the enriched nucleic acid to two or more assay chambers and combining the enriched nucleic acid with one or more amplification reagents *784*, (k) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (l) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the blood sample suspected of containing the target pathogen. Optionally steps (f), (g) and (h) are repeated with a first and second porous solid support. Exemplified pathogens that may be suspected in a blood sample include, but are not limited to, *Brucella*, *Campylobacter* sp., *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella*, *Enterobacter*, *Serratia*, *Yersinia* (e.g. *Yersinia pestis*), *Pseudomonas* (e.g., *Pseudomonas aeruginosa*), *Salmonella* sp. (e.g. *Salmonella typhimurium* or *Salmonella typhi*), *Francisella tularensis*, *Bacillus anthracis*,

Listeria, *Staphylococcus aureus* (e.g. MRSA or MSSA), *Streptococcus* sp. (e.g. Group A or Group B strep), *Treponema pallidum* (syphilis), *Leptospira*, *Borrelia burgdorferi* (Lyme's disease), *Coccidioides immitis* (Valley fever), coronavirus (e.g. MERS, SARS, or SARS-CoV-2), hepatitis, and human immunodeficiency virus (HIV).

[0488] As with blood samples, fecal samples (e.g. stool samples) contain a high concentration of contaminants, such as organic matter and high commensal bacterial load, and may require additional processing prior to the amplification steps. Referring to FIG. 112, the invention provides methods of identifying one or more suspected pathogens in a fecal sample, the method comprising the steps of: (a) accepting a cartridge having a loading chamber containing the sample suspected of containing the target pathogen *180*, (b) subjecting the fecal sample to one or more enzymatic or mechanical pretreatments *182*, (c) advancing the sample to a lysis chamber having at least one lysis reagent therein, (d) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (e) filtering the lysed sample *382*, preferably by passing the lysed sample through one or more size-exclusion filters, (f) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (g) passing a wash solution through the porous solid support *482*, (h) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (i) distributing the enriched nucleic acid to two or more assay chambers and combining the enriched nucleic acid with one or more amplification reagents *780*, (j) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (l) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the fecal sample suspected of containing the target pathogen. Typically, the mechanical pretreatment is required to homogenize and liquify the fecal sample. Such homogenization can be achieved within the cartridges described herein by stirring the fecal sample with ceramic, glass or steel beads in the lysis chamber prior to exposing the fecal sample to the lysis agent. The enzymatic pretreatment of step (b) can be incubating the fecal sample with a protease and/or nuclease. Optionally steps (f), (g) and (h) are repeated with a first and second porous solid support. Optionally, prior to step (i), the method further comprises pre-amplifying the enriched nucleic acid *782*. Exemplified pathogens that may be suspected in a fecal sample include, but are not limited to, *Campylobacter* sp. (e.g., *Campylobacter jejuni*), *Vibrio* sp. (e.g. *Vibrio cholerae*), *Salmonella* sp. (e.g. *Salmonella typhimurium* or *Salmonella typhi*), *Shigella*, and *Bacillus anthracis*.

[0489] Finally, the cartridges and instruments described herein can be used to detect suspected pathogen in solid tissue samples. Such tissue samples require additional processing to separate the cells of the tissue sample. Referring to FIG. 113, the invention provides methods of identifying one or more suspected pathogens in a tissue sample, the method comprising the

steps of: (a) accepting a cartridge having a loading chamber containing the tissue sample suspected of containing the target pathogen *180*, (b) subjecting the tissue sample to one or more enzymatic, chemical or mechanical pretreatments *182*, (c) advancing the sample to a lysis chamber having at least one lysis reagent therein, (d) mixing the sample with the at least one lysis agent to generate a lysed sample *380*; (e) filtering the lysed sample *382*, preferably by passing the lysed sample through one or more size-exclusion filters, (f) passing the lysed sample through a porous solid support to capture a nucleic acid on the porous solid support *480*, (g) passing a wash solution through the porous solid support *482*, (h) releasing the captured nucleic acid from the porous solid support to generate an enriched nucleic acid *484*, (i) distributing the enriched nucleic acid to two or more assay chambers and combining the enriched nucleic acid with one or more amplification reagents *780*, (j) isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers and (l) performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product *786*, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the tissue sample suspected of containing the target pathogen. Typically, the mechanical pretreatment is required to disintegrate and liquify the tissue sample. Such homogenization can be achieved within the cartridges described herein by stirring the tissue sample with ceramic, glass or steel beads in the lysis chamber prior to exposing the tissue sample to the lysis agent. The enzymatic pretreatment of step (b) can comprise incubating the tissue sample with an elastase, collagenase or proteinase K. The chemical pretreatment of step (b) can comprise incubating the tissue sample with dithiothreitol (DTT). Optionally steps (f), (g) and (h) are repeated with a first and second porous solid support. Optionally, prior to step (i), the method further comprises pre-amplifying the enriched nucleic acid *782*. Exemplified pathogens that may be suspected in a solid tissue sample include, but are not limited to, *Bacillus anthracis* (e.g. from skin scraping), *Corynebacterium diphtheriae*, and *Aspergillus* (lung).

IX. EXAMPLES

[0490] To demonstrate functionality of the diagnostic system of the invention for the qualitative detection of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG), the instrument described herein was paired with an integrated diagnostic cartridge populated with CT- and NG-specific RTLAMP reagents. The embodiment of the integrated diagnostic cartridge used is shown in FIGs. 69A, 70A and 89 and is described herein. The CT-specific reagents are described in detail in US Patent No. 10,450,616 B1, incorporated herein by reference. The NG-specific reagents are described in detail in US patent application serial no. 16/523,609, filed 26 July 2019, said application incorporated herein by reference.

[0491] Fresh urine samples from healthy non-infected donors were co-spiked with live CT and NG and used as samples loaded into the cartridge loading assembly. Specifically, frozen, single

use aliquots of titered bacterial stocks, either grown in-house (NG) or purchased from ATCC (CT), underwent a 30 second thaw at 37□C followed by serial dilution at room temperature in Mueller Hinton cation-adjusted growth medium. Bacteria were diluted 1:10 into pools of negative urine to achieve a final concentration of 1 IFU/mL of CT and 1 CFU/mL of NG.

[0492] Urine samples were mixed by short vortex, 1 mL of sample removed and loaded onto the integrated diagnostic cartridge via the fill chamber using a pipette. To initiate the method (time, T=0), cartridges were inserted into the instrument. Using pneumatic pressure, the instrument advanced the urine sample to the lysis chamber, which held a chemical lysis buffer including, inter alia, guanidinium isothiocyanate and isopropanol. The urine sample and lysis buffer were mixed in the lysis chamber at 1300 rpm for 30 seconds to generate a lysed sample.

[0493] The valve drive assembly of the instrument rotated the rotary valve to fluidically connect the sample exit channel of the lysis chamber, the porous solid support chamber of the rotor, contained therein a silica fiber matrix, and the waste collection element. The instrument then pressurized the lysis chamber to forcibly pass the lysed sample through the silica fiber matrix (i.e. the porous solid support), capturing nucleic acid on the matrix and passing cell debris, urine and other contaminants to the waste collection element. The matrix was washed to further rid the matrix of contaminants, and then eluted with buffered water to release nucleic acid from the matrix to generate an enriched nucleic acid. The enriched nucleic acid was used without dilution to rehydrate a dried amplification reagent solution in a rehydration chamber with agitation from a mixing ball for 20 seconds. The nucleic acid/amplification reagent solution was then distributed to five assay chambers, such that the assay chambers are fully loaded at T=6:12 (mm:ss).

[0494] The instrument captured an image of the filled assay chambers and then isolated the assay chambers, here by forming a heat stake across the loading channels leading to the assay chambers while under pressure. Pneumatic pressure to the assay chambers was subsequently released and the reaction imaging assembly of the instrument captured another image of the assay chambers to confirm that the contents of the assay chambers do not leak out of the reaction area. At T=9:53, the instrument initiated the amplification reaction and images were collected for an additional 18 minutes. For these test runs, image acquisition time was extended to collect additional amplification information. Total run time, including image processing was approximately 27 minutes. In this initial test, 12 cartridges were run, presenting 10 contrived (CT+/NG+) samples and 2 clean urines. In addition to CT- and NG-specific reagents, one assay well in the cartridge contained primers and probes specific for human beta actin, which is present in human urine, as a positive control. The CT and NG were detected, as expected, in each of the contrived samples. The human beta actin was detected in both contrived and clean urine samples. Amplification results are summarized in Table 1.

Table 1: Sample-to-Answer Test Results

Target	Time to positive (T_p)
1 CFU NG	7.44 ±0.33
1 IFU CT	10.59 ± 0.45
b-actin	6.32 ± 0.41

[0495] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0496] Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

CLAIMS

We claim:

1. A method of testing a sample suspected of containing one or more target pathogens, comprising:

accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens;

advancing the sample suspected of containing the one or more target pathogens to a lysis chamber having at least one lysis reagent therein;

mixing the sample with the at least one lysis agent to generate a lysed sample;

passing the lysed sample through a first porous solid support to capture a nucleic acid on the porous solid support;

releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid;

distributing the enriched nucleic acid to two or more assay chambers;

combining the enriched nucleic acid with one or more amplification reagents;

isolating each one of the two or more assay chambers from each one of all the other two or more assay chambers; and

performing an isothermal amplification reaction within each one of the two or more assay chambers while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

2. A method of testing a sample according to claim 1, wherein the sample is a biological sample obtained from a mammal.

3. A method of testing a sample according to claim 2 wherein the mammal is a person providing a biological sample.

4. The method of testing a sample according to claim 1, wherein the sample is obtained from a food product, a natural non-growth hormone crop sample, a crop sample, a water sample, a non-biological fluid sample or a soil sample.

5. The method of testing a sample according to claim 2, wherein the step of accepting a cartridge step further comprises reading a bar code on the cartridge and determining to proceed with the method of testing.

6. The method of testing a sample according to claim 1, the accepting a cartridge step further comprising: obtaining and analyzing an image of a sample window of the sample port assembly and determining to proceed with the method of testing.

7. The method of testing a sample according to claim 6, wherein the sample in the sample port assembly is in fluid communication with a fill chamber, a metering chamber, and an overflow chamber.

8. The method of testing a sample according to claim 6, wherein the sample window is transparent and formed in at least a portion of a wall of a metering chamber wherein obtaining an image further comprises obtaining an image of the transparent viewing window.

9. The method of testing a sample according to claim on 8, wherein analyzing an image further comprises assessing a height of a sample liquid in the metering chamber via the transparent viewing window.

10. The method of testing a sample according to claim 8, wherein the step of obtaining and analyzing an image further comprises obtaining an image of the metering chamber comprising a buoyant ball and analyzing the image comprises identifying a location of the ball within the metering chamber and determining to proceed with the method based on the location of the ball.

11. The method of testing a sample according to claim 1, the accepting a cartridge step further comprising: obtaining and analyzing an image of a patient ID label and determining to proceed with the method of testing.

12. The method of testing a sample according to claim 1, the accepting a cartridge step further comprising: confirming a rotary valve on the cartridge is in a shipping configuration before proceeding to the advancing the sample step.

13. The method of testing a sample according to claim 1, the accepting a cartridge step further comprising: obtaining a reading from an interference sensor on a valve drive assembly and confirming based on the reading that a rotary valve on the cartridge is not in an operational configuration prematurely.

14. The method of testing a sample according to claim 1, the accepting a cartridge step further comprising: engaging a rotary valve on the cartridge with a valve drive assembly and rotating the rotary valve into an operational configuration.

15. The method of testing a sample according to claim 14, wherein rotating the rotary valve in an operational configuration places a rotary valve gasket into contact with a stator on the cartridge.

16. The method of testing a sample according to claim 1, the step of accepting a cartridge further comprising moving a clamping block for engaging the cartridge with a door support assembly, a pneumatic interface assembly, and a thermal clamp assembly.

17. The method of testing a sample according to claim 16, wherein the moving step is a single continuous movement.

18. The method of testing a sample according to claim 1, the step of accepting a cartridge further comprising moving a frangible seal block having a plurality of frangible seal pins into position to engage one or more frangible seals on the cartridge.

19. The method of testing a sample according to claim 18, wherein moving the frangible seal block simultaneously engages the plurality of frangible seal pins with the one or more frangible seals on the cartridge.

20. The method of testing a sample according to claim 18, wherein moving the frangible seal block sequentially engages the plurality of frangible seal pins with the one or more frangible seals on the cartridge.

21. The method of testing a sample according to claim 18, wherein the step of moving a frangible seal block is performed after performing the step of moving a clamp block

22. The method of testing a sample according to claim 18, wherein the step of moving a frangible seal block is performed initially with the clamp block and ends in a position separate from the clamp block.

23. The method of testing a sample according to claim 1, the step of accepting a cartridge further comprising moving a clamp block and a frangible seal block together for engaging the cartridge.

24. The method of testing a sample according to claim 23, further comprising moving the clamp block together with the frangible seal block until the cartridge is engaged with a door support assembly, a pneumatic interface assembly, and a thermal clamp assembly.

25. The method of testing a sample according to claim 24, further comprising: only driving the frangible seal block assembly to engage one of more frangible seals on the cartridge simultaneously or sequentially.

26. The method of testing a sample according to claim 1, wherein in mixing the sample with the at least one lysis agent, the lysis agent is a mechanical agent.

27. The method of testing a sample according to claim 26, wherein the mechanical agent is ceramic beads, glass beads or steel beads, and the mixing the sample step comprises rotating the stir bar at at least 1000 rpm.

28. The method of testing a sample according to any one of claim 26 or claim 27 further wherein mixing the sample comprises rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent.

29. The method of testing a sample according to any one of claim 26, claim 27 or claim 28, wherein the suspected pathogen is a gram-positive bacterium, a fungus or a plant cell.

30. The method of testing according to claim 1, wherein in the mixing the sample with the at least one lysis agent step, the at least one lysis agent is a chemical lysis agent.

31. The method of testing a sample according to claim 30 wherein the one or more target pathogens is a virus or a gram-negative bacterium and the lysis reagent is a chaotropic agent.

32. The method of testing a sample according to claim 1, wherein prior to passing the lysed sample through the porous solid support, the method further comprises passing the lysed sample through a size-exclusion filter, wherein nucleic acid passes through the filter.

33. The method of testing a sample according to claim 1, wherein the enriched nucleic acid is combined with one or more amplification reagents before the distributing step.

34. The method of testing a sample according to claim 33, wherein the one or more amplification reagents are selected from the group consisting of a DNA polymerase, a reverse transcriptase, a helicase, nucleotide triphosphates (NTPs), a magnesium salt, a potassium salt, an ammonium salt, and a buffer.

35. The method of testing a sample according to claim 34, wherein the one or more amplification reagents further comprise a primer.

36. The method of testing a sample according to claim 35, wherein isothermal amplification is initiated prior to distributing the enriched nucleic acid to the two or more assay chambers.

37. The method of testing a sample according to claim 34, wherein after the distributing step, but prior to perform the isothermal amplification reaction, the method further comprises combining the enriched nucleic acid with a primer set specific to one of the one or more target pathogens.

38. The method of testing a sample according to claim 1, wherein a first assay chamber contains a primer set specific to a first nucleic acid sequence.

39. The method of testing a sample according to claim 38, wherein the first nucleic acid sequence is present in one of the one or more target pathogens.

40. The method of testing a sample according to claim 38, wherein prior to mixing the sample with at least one lysis agent, a process control is added to the sample and the first nucleic acid sequence is present in the process control.

41. The method of testing a sample according to claim 38, wherein prior to passing lysed sample through the porous solid support, a process control is added to the lysed sample and the first nucleic acid sequence is present in the process control.

42. The method of testing a sample according to claim 38, wherein a second assay chamber contains a primer set specific to a second nucleic acid sequent, wherein the second nucleic acid sequence is present in one of the one or more target pathogens.

43. The method of testing a sample according to claim 1, wherein the performing an isothermal amplification reaction step is completed in less than 20 minutes.

44. The method of testing a sample according to claim 43, wherein the performing an isothermal amplification reaction step is completed in less than 15 minutes.

45. The method of testing a sample according to claim 43, wherein the performing an isothermal amplification reaction step is completed in less than 10 minutes.

46. The method of testing a sample according to claim 1, further comprising: providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen.

47. The method of testing a sample according to claim 1, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction.

48. The method of testing a sample according to claim 47, wherein the sample is sputum and the chemical reaction is incubation with a mucolytic agent.

49. The method of testing a sample according to claim 48, wherein the mucolytic agent is dithiothreitol or n-acetylcysteine.

50. The method of testing a sample according to claim 1, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction.

51. The method of testing a sample according to claim 50, wherein the enzymatic reaction is incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase.

52. The method of testing a sample according to claim 50, wherein pretreating comprising incubating the sample with a DNase.

53. The method of testing a sample according to claim 50, wherein pretreating comprises incubating the sample with a protease.

54. The method of testing a sample according to claim 53, wherein the protease is selected from pronase, chymotrypsin, trypsin and pepsin.

55. The method of testing a sample according to claim 1, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment.

56. The method of testing a sample according to claim 55, wherein the physical treatment comprises passing the sample through a size-exclusion filter in a first direction.

57. The method of testing a sample according to claim 56, wherein the target pathogen passes through the filter.

58. The method of testing a sample according to claim 56, wherein the target pathogen does not pass through the filter and is thereby captured on a fill port side of the size-exclusion filter.

59. The method of testing a sample according to claim 58, further comprising passing a volume of suspension buffer through the size-exclusion filter in a second direction, wherein second direction is opposite the first direction, thereby releasing the target pathogen from the fill port side of the filter.

60. The method of testing a sample according to claim 59, wherein the volume of suspension buffer is less than the volume of the sample, and the target pathogen is more concentrated than in the loaded sample.

61. The method of testing a sample according to claim 58, wherein the physical treatment comprises exposing the sample to a capture agent immobilized on a solid substrate.

62. The method of testing a sample according to claim 61, further comprising, after exposure, separating the solid substrate from the sample.

63. The method of testing a sample according to claim 61, wherein the capture agent is a capture antibody.

64. The method of testing a sample according to claim 61, wherein the capture agent is an antibody with affinity for red blood cells.

65. The method of testing a sample according to claim 61, wherein the solid substrate is a magnetic bead, the capture agents has affinity for a class of cells comprising the one or more target pathogens and the method further comprises (1) incubating the magnetic beads with the sample, (2) engaging a magnet to draw the magnetic beads to a location within the sample loading structure, (3) washing away unbound sample, (4) releasing the magnet, and (5) resuspending the magnetic beads and passing the suspension, including target pathogen bound to the magnetic beads, to the lysis chamber.

66. The method of testing a sample according to claim 1, wherein the sample is sputum and the method further comprises, prior to mixing the sample with the at least one lysis reagent, bead beating the sputum to liquify the sample.

67. The method of testing a sample according to claim 66, wherein the bead beating comprises mixing the sputum with ceramic, glass, or steel beads.

68. The method of testing a sample according to claim 66, wherein the bead beating comprises mixing the sputum with ceramic, glass, or steel beads and dithiothreitol.

69. The method of testing a sample according to claim 1, wherein prior to distributing the enriched nucleic acid to the assay wells, the method further comprises passing the enriched nucleic acid through a second porous solid support.

70. The method of testing a sample according to claim 69, wherein the second porous solid support is the same as the first porous solid support.

71. The method of testing a sample according to claim 70, wherein the enriched nucleic acid is mixed with a matrix binding agent prior to passing through the second solid support.

72. The method of testing a sample according to claim 71, wherein matrix binding agent is an alcohol or a salt solution.

73. The method of testing a sample according to claim 69, wherein the second porous solid support is different than the first porous solid support, and the second solid support has an affinity for nucleic acid and the method further comprises releasing the captured nucleic acid from the second solid support to generate a twice-enriched nucleic acid.

74. The method of testing a sample according to claim 69, wherein the second porous solid support is different than the first porous solid support.

75. The method of testing a sample according to claim 1, wherein prior to passing the lysed sample through a first porous solid support, the method further comprises passing the lysed sample through a second porous solid support, wherein the second solid support does not bind

nucleic acid and has affinity for one or more contaminants, thereby removing contaminant from the lysed sample.

76. The method of testing a sample according to claim 1, further comprising releasing the cartridge from engagement with a clamp block and a frangible seal block after completing the performing an isothermal amplification reaction step.

77. The method of testing a sample according to claim 1, further comprising displaying a result produced after the step of performing an isothermal amplification reaction step.

78. The method of testing a sample according to claim 1, further comprising storing in a computer memory a result produced after the step of performing an isothermal amplification reaction step.

79. The method of testing a sample according to claim 1, further comprising maintaining the cartridge in a vertical orientation while performing the steps of testing a sample.

80. The method of testing a sample according to claim 79, wherein the cartridge is inclined no more than 30 degrees while in the vertical orientation.

81. The method of testing a sample according to claim 79, wherein the cartridge is inclined no more than 15 degrees while in the vertical orientation.

82. The method of testing a sample according to claim 1 wherein during the combining the enriched nucleic acid step in each of the two or more assay chambers the enriched nucleic acid combines with a dried reagent contained in each one of the two or more assay chambers.

83. The method of testing a sample according to claim 82 wherein the dried reagent is on a surface of a plug in each one of the two or more assay chambers.

84. The method of testing a sample according to claim 83 wherein the dried reagent is on a surface of the plug formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum used during the performing step.

85. The method of testing a sample according to claim 83 wherein the plug is as in any one of claims 202 to 213 and 214.

86. The method of testing a sample according to claim 1, the distributing step further comprising distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve further wherein the pneumatic signal continues to be introduced while the performing step is performed.

87. The method of testing a sample according to claim 1 wherein performing the isolation step temporarily isolates each one of the two or more assay chambers are from each one of all the other two or more assay chambers.

88. The method testing a sample according to claim 87 wherein the isolation step is performed using a pneumatic signal, a mechanical system to occlude one or more fluid channels to occlude one or more passages or channels of a cartridge.

89. The method of testing a sample according to claim 88 wherein the mechanical system is one of a single pinch valve, a plurality of pinch valves, and a non-heated staker bar.

90. The method of testing a sample according to claim 1 wherein performing the isolation step permanently isolates each one of the two or more assay chambers from each one of all the other two or more assay chambers.

91. The method of testing a sample according to claim 90 wherein after performing the isolation step, a portion of the cartridge is melted or is plastically deformed.

92. The method of testing a sample according to claim 1 wherein after completing the performing step, each one of the two or more assay chambers are isolated from each one of all the other two or more assay chambers.

93. The method of testing a sample according to claim 1, the distributing step further comprising distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve further wherein the pneumatic signal continues to be introduced while performing the isolating step by moving a heat staker into contact with the cartridge to isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers.

94. The method of testing a sample according to claim 93 wherein after performing the isolating step, a single heat stake isolates each one of the two or more assay chambers from each one of all the other two or more assay chambers.

95. The method of testing a sample according to claim 94 further wherein the single heat stake isolates a waste chamber on the cartridge.

96. The method of testing a sample according to claim 1, the isolating step further comprising moving a heat staker into contact with the cartridge to seal each one of the two or more assay chambers from each one of all the other two or more assay chambers.

97. The method of testing a sample according to claim 96, further comprising providing a pneumatic pressure in the cartridge while moving the heat staker into contact with the cartridge.

98. The method of testing a sample according to claim 1, the isolating step further comprising forming a heat stake region in the cartridge to isolate each one of the two or more assay chambers from each one of all the other two or more assay chambers.

99. The method of testing a sample according to any one of claim 96, claim 97 and claim 98 further comprising: obtaining a first image of a level of fluid in each of the one or more assay chambers after the step of distributing the enriched nucleic acid to each one of the two or more assay chambers.

100. The method of claim 85 further comprising obtaining a second image of a level of fluid in each of the one or more assay chambers after the isolating step.

101. The method of testing a sample according to claim 100, further comprising determining the quality of the heat stake by comparing the level of fluid in the first image to the level of fluid in the second image.

102. The method of testing a sample according to claim 1, further comprising rotating a rotary valve on the cartridge prior to performing the advancing the sample step.

103. The method of testing a sample according to claim 102, further comprising advancing the sample to the lysis chamber using a pneumatic signal introduced into a cartridge pneumatic interface.

104. The method of testing a sample according to claim 1, further comprising rotating a rotary valve on the cartridge prior to performing the step of passing the lysed sample through a first porous solid support to capture a nucleic acid on the porous solid support.

105. The method of testing a sample according to claim 104, further comprising passing the lysed sample through the first porous solid support using a pneumatic signal introduced into the rotary valve.

106. The method of testing a sample according to claim 1, further comprising distributing the enriched nucleic acid to two or more assay chambers using a rotary valve on the cartridge and a pneumatic signal introduced into the rotary valve.

107. An apparatus, comprising:
an enclosure;
a fixed support bracket within the enclosure;
a first imaging system mounted on the fixed support bracket within the enclosure adjacent to an opening, the first imaging system configured to collect images from a first imaging area within the enclosure;

a second imaging system mounted on the fixed support bracket within the enclosure configured to collect images from a second imaging area within the enclosure wherein the second imaging area is in non-overlapping relation to the first imaging area;

a moving support bracket within the enclosure and moveable relative to the fixed support bracket, the first imaging system and the second imaging system;

a drive system on the fixed support bracket configured to position the moving support bracket relative to the fixed support bracket; and

an opening positioned in the enclosure to provide access to an interior portion of the enclosure between the fixed support bracket and the moving support bracket.

108. The apparatus of claim 107 wherein the moving support bracket is positioned between the first imaging system and the second imaging system.

109. The apparatus of claim 107 wherein a rotary connector, a pneumatic connector and a multiple pin block are connected to and move with the moving support bracket.

110. The apparatus of claim 109 wherein the multiple pin block is directly connected to the drive system.

111. The apparatus of claim 109 wherein the multiple pin block is configured to move together with the rotary connector and the pneumatic connector and independent of the rotary connector and the pneumatic connector.

112. The apparatus of claim 107 wherein the opening is a slot, wherein the slot is aligned to access an upper rail within the enclosure aligned to an upper portion of the slot and a lower rail within the enclosure aligned to a lower portion of the slot.

113. The apparatus of claim 112 further comprising a loading and ejection mechanism within the enclosure in sliding relation to the lower rail.

114. The apparatus of claim 113 wherein the loading and ejection mechanism moves between a loading position and a loaded position wherein when in the loading position the loading and ejection mechanism is positioned in a forward most position towards the slot and when in the loaded position the loading and ejection mechanism is engaged with a load position sensor.

115. The apparatus of claim 114 wherein the load position sensor provides an electronic indication when the loading and ejection mechanism has translated into the loaded position.

116. The apparatus of claim 107 further comprising a first heater and a second heater mounted on the fixed support bracket.

117. The apparatus of claim 116 wherein the first heater is positioned to heat a portion of the fixed support bracket between the first imaging area and the second imaging area.

118. The apparatus of claim 116 wherein the second heater is positioned to heat a portion of the fixed support bracket only within the second imaging area.

119. The apparatus of claim 107 further comprising a channel in the fixed support bracket and a heat stake assembly positioned to move a heating element through the channel.

120. The apparatus of claim 119 wherein the channel is positioned on the fixed support bracket to allow the heating element to interact within the enclosure between the first imaging area and the second imaging area.

121. The apparatus of claim 119 wherein the channel is positioned within the fixed support bracket such that the heating element may perform a heat staking operation directly adjacent to but outside of the second imaging area.

122. The apparatus of claim 107 wherein the moving support bracket partially blocks the channel when the moving support bracket is positioned at a closest position to the fixed support bracket.

123. An apparatus, comprising:
an enclosure;
a fixed support bracket within the enclosure;
a moving support bracket within the enclosure and moveable relative to the fixed support bracket;
a drive system configured to position the moving support bracket relative to the fixed support bracket;
an opening positioned in the enclosure to provide access to an interior portion of the enclosure between the fixed support bracket and the moving support bracket; and
an upper rail and a lower rail in the enclosure positioned adjacent to the opening wherein a cartridge positioned between the upper rail and the lower rail remains in a vertical position between the fixed support bracket and the moving support bracket.

124. The apparatus of claim 123 further comprising a feature within the upper rail or the lower rail positioned to interfere with the movement of a cartridge improperly aligned with respect to the upper rail and the lower rail.

125. The apparatus of claim 123 further comprising a loading and ejection assembly within the enclosure positioned to engage with a cartridge moving along the upper rail and the lower rail.

126. The apparatus of claim 123 further comprising a latch and pin assembly positioned adjacent to the upper rail adapted to engage a pin with a cartridge moving along the upper rail.

127. The apparatus of claim 123 further comprising a touch screen display on an exterior of the enclosure.

128. The apparatus of claim 123 further comprising a cellular communications module within the enclosure.

129. The apparatus of claim 123 wherein the cellular communication module is adjacent to the opening.

130. The apparatus of claim 123 further comprising: a cartridge heater, a driving magnet system, a chemistry heater, a rehydration motor, a reaction camera and a heat stake assembly coupled to the fixed support bracket and positioned to interact with a corresponding portion of a cartridge positioned between the upper rail and the lower rail.

131. The apparatus of claim 123 further comprising a first imaging system mounted on the fixed support bracket within the enclosure adjacent to the opening, the first imaging system configured to collect images from a first imaging area within the enclosure and a second imaging system mounted on the fixed support bracket within the enclosure configured to collect images from a second imaging area within the enclosure wherein the second imaging area is in non-overlapping relation to the first imaging area.

132. The apparatus of claim 131 wherein the first imaging area includes a label of a cartridge positioned within the enclosure between the upper rail and the lower rail.

133. The apparatus of claim 131 wherein the second imaging area includes one or more assay chambers of a cartridge positioned within the enclosure between the upper rail and the lower rail.

134. The apparatus of claim 123 further comprising a clamp block, a frangible seal block, a valve driver, a pneumatic interface, a thermal clamp, and a driven magnet system coupled to move along with the moving support bracket during operation of the drive system.

135. The apparatus of claim 130 further comprising a plenum adjacent to the chemistry heater and a fan in fluid communication with the plenum.

136. The apparatus of claim 130 the heat stake assembly further comprising a staker blade positioned to move relative to a depth stop frame, the staker blade coupled to a linear actuator motor and a spring with pivot washer.

137. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module; and

a reaction module;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module; and

further wherein the loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation.

138. The integrated diagnostic cartridge of claim 137 further comprising one or more fluid filling conduits arranged to flow into an upper portion of a chamber within a fluidic card of the integrated diagnostics cartridge and one or more fluid outlet conduits arranged to flow out of a lower portion of the chamber within the fluidic card of the integrated diagnostics cartridge.

139. The integrated diagnostic cartridge of claim 138, wherein the chamber is one or more of a lysis chamber, a metering chamber, a wash buffer chamber or an elution buffer chambers.

140. The integrated diagnostic cartridge of claim 139, wherein the chamber further comprises a filter assembly in fluid communication with a fluid outlet conduit of the chamber.

141. The integrated diagnostic cartridge of claim 137, wherein the lysis module comprises a mixing assembly having a vertically oriented lysis chamber containing a lysis agent and a non-magnetized stir bar.

142. The integrated diagnostic cartridge of claim 141, wherein the non-magnetized stir bar is made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system.

143. The integrated diagnostic cartridge according to claim 141, wherein the non-magnetized stir bar is coated with an impermeable material to prevent corrosion by a chemical lysis buffer in the vertically oriented lysis chamber.

144. The integrated diagnostic cartridge of claim 141 wherein, when in use within a diagnostic instrument, the non-magnetized stir bar is disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument, wherein the driving magnet system is configured to rotate the non-magnetized stir bar within the vertically oriented lysis chamber at least 1000 rpm.

145. The integrated diagnostic cartridge of claim 141, further comprising a fluid inlet to the vertically oriented lysis chamber and a fluid outlet to lysis chamber wherein the vertically

oriented lysis chamber is isolated from the other modules on the cartridge by a first frangible seal in fluid communication with the fluid inlet to the vertically oriented lysis chamber and a second frangible seal in fluid communication with the fluid outlet to the vertically oriented lysis chamber.

146. The integrated diagnostic cartridge according to claim 137 further comprising a fluidic card and a cover.

147. The integrated diagnostic cartridge of claim 146, wherein the fluidic card further comprises a first film adhered to a surface of at least a portion of the fluidic card, wherein the first film forms one surface of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module.

148. The integrated diagnostic cartridge of claim 146 further comprising an interference feature on the cover, wherein the interference feature is sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument.

149. The integrated diagnostic cartridge according to claim 148, wherein a thickness of the fluidic card is selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument.

150. The integrated diagnostic cartridge of claim 146 wherein a total sample process volume of the integrated diagnostic cartridge is related to a thickness of the cartridge corresponding to a spacing between the one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module formed in the fluidic card and the first film.

151. The integrated diagnostic cartridge of claim 150, wherein a diagnostic instrument is adapted and configured to accommodate a variation of the thickness of the cartridge by increasing a width of an opening of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument is adapted to accommodate the increased thickness of the cartridge.

152. The integrated diagnostic cartridge of claim 148 further comprising a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing wherein each of the upper spacing and the lower spacing is sized and positioned to engage with the upper rail and lower rail of the diagnostic instrument.

153. The integrated diagnostic cartridge of claim 152 further comprising an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation.

154. The integrated diagnostic cartridge of claim 137 further comprising a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module.

155. The integrated diagnostic cartridge of claim 137, the integrated diagnostic cartridge further comprising a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or an image of a patient identification marking.

156. An integrated diagnostic cartridge, comprising:

a loading module comprising a sample port assembly having a fill chamber, a metering chamber, and an overflow chamber arranged in fluid communication;

a lysis module;

a purification module; and

a reaction module;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

157. The integrated diagnostic cartridge of claim 156, wherein the metering chamber comprises a transparent viewing window for observing the height of a sample within the metering chamber.

158. The integrated diagnostic cartridge of claim 157 further comprising a ball float in the metering chamber adapted for use with the transparent viewing window.

159. The integrated diagnostic cartridge of claim 156, wherein the fill chamber comprises a cap operable to provide access to the fill chamber.

160. The integrated diagnostic cartridge of claim 159, wherein the cap is positioned for interaction with a closing apparatus of a diagnostic instrument.

161. The integrated diagnostic cartridge of claim 156, wherein, the cartridge is in a vertical orientation when in use within a diagnostic instrument and a fluid channel connects an outlet at a lower portion of the fill chamber with an inlet to the metering chamber located in an upper portion of the metering chamber.

162. The integrated diagnostic cartridge of claim 156, wherein the metering chamber comprises a transparent viewing window.

163. The integrated diagnostic cartridge of claim 162 further comprising a buoyant ball within the metering chamber, said buoyant ball adapted to appear adjacent to the transparent viewing window permitting an assessment of the height of the sample liquid in the metering chamber.

164. The integrated diagnostic cartridge of claim 156, wherein the metering chamber comprises a buoyant ball for assessing a height of a sample liquid in the metering chamber.

165. An integrated diagnostic cartridge, comprising:
a loading module;
a lysis module comprising a mixing assembly having a lysis chamber containing a lysis agent and a non-magnetized stir bar;
a purification module; and
a reaction module;
wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

166. The integrated diagnostic cartridge of claim 165, wherein the non-magnetized stir bar is made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system.

167. The integrated diagnostic cartridge of claim 166 wherein the metal comprises a ferritic stainless steel or a duplex stainless steel.

168. The integrated diagnostic cartridge of claim 166 wherein the non-magnetized stir bar is made from a metal selected from the group consisting of a carbon steel, a mild carbon steel, a low alloy steel, a tool steel, a metal alloy contain nickel, a metal alloy containing cobalt, a non-austenitic stainless steel, a ferritic grade of stainless steel including 430 steel, Atlas CR12 steel, 444 steel, F20S steel, a duplex grade of steel including 2205 steel, 2304 steel, 2101 steel, 2507 steel and a martensitic grade of steel such as 431 steel, 416 steel, 420 steel and 440C steel wherein the metal has a magnetic permeability to be responsive to a rotating magnetic field produced within the mixing chamber.

169. The integrated diagnostic cartridge of claim 168 wherein the metal has a magnetic permeability between 500-1,000,000.

170. The integrated diagnostic cartridge according to any one of claims 165-169, wherein the non-magnetized stir bar is coated with an impermeable material to prevent corrosion by a chemical lysis buffer in lysis chamber.

171. The integrated diagnostic cartridge of claim 170, wherein the impermeable material is PTFE, parylene C, parylene D, a functionalized perfluoropolyether (PFPE), Xylan Fluoropolymer, epoxy, or urethane.

172. The integrated diagnostic cartridge of claim 165 wherein, when in use within a diagnostic instrument, the non-magnetized stir bar is disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument, wherein the driving magnet system is configured to rotate the non-magnetized stir bar within the lysis chamber at at least 1000 rpm.

173. The integrated diagnostic cartridge of claim 165, wherein the lysis agent is a mechanical agent.

174. The integrated diagnostic cartridge of claim 173, wherein the mechanical agent is ceramic beads, glass beads or steel beads.

175. The integrated diagnostic cartridge of claim 165, wherein the lysis agent is a chemical agent.

176. The integrated diagnostic cartridge of claim 175, wherein the chemical agent is an anionic detergent, a cationic detergent, a non-ionic detergent or a chaotropic agent.

177. The integrated diagnostic cartridge of claim 176, wherein the cartridge is configured for testing of one or more target pathogens that is a virus or a gram-negative bacterium.

178. The integrated diagnostic cartridge of claim 165, further comprising a fluid inlet in fluid communication with the lysis chamber and a fluid outlet in fluid communication with the lysis chamber and a filter assembly in fluid communication with the fluid outlet of the lysis chamber.

179. The integrated diagnostic cartridge of claim 165, further comprising a fluid inlet to the lysis chamber and a fluid outlet to lysis chamber wherein the lysis chamber is isolated from the other modules on the cartridge by a first frangible seal in fluid communication with the fluid inlet to the lysis chamber and a second frangible seal in fluid communication with the fluid outlet of the lysis chamber.

180. The integrated diagnostic cartridge of claim 178 or claim 179 further comprising: a process control chamber having an inlet, an outlet and a plug comprising a process control wherein the process control chamber is in fluid communication with the lysis chamber inlet.

181. An integrated diagnostic cartridge, comprising:
a loading module;
a lysis module;
a purification module comprising a rotary valve comprising:
a. a stator comprising a stator face and a plurality of passages, each passage comprising a port at the stator face;

b. a rotor operably connected to the stator and comprising a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel comprises a porous solid support; and

c. a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal; and

a reaction module;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

182. The integrated diagnostic cartridge of claim 181, wherein the rotary valve further comprises a gasket between the stator face and the rotor valving face, and wherein the stator comprises a displaceable spacer for preventing the gasket from sealing against at least one of the rotor and stator, and wherein, when the spacer is displaced the gasket seals the rotor and stator together in a fluid-tight manner.

183. The integrated diagnostic cartridge of claim 182, wherein, when the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument displaces the spacer and seals the rotor and stator together in a fluid-tight manner.

184. The integrated diagnostic cartridge of claim 183, wherein a rotation movement performed by the rotor driver of the diagnostic instrument displaces the spacer and seals the rotor and the stator together in a fluid-tight manner.

185. The integrated diagnostic cartridge of claim 181 further comprising at least one pair of ridges and spaces on a retention ring and at least one pair of ridges and spaces on the rotor wherein while the at least one pair of ridges and spaces of the retention ring is engaged with the at least one pair of ridges and spaces of the rotor sealing of the rotor and stator is prevented wherein, relative movement between the at least one pair of ridges and spaces on the retention ring and the at least one pair of ridges and spaces on the rotor seals the rotor and stator together in a fluid-tight manner.

186. The integrated diagnostic cartridge of claim 185, wherein, when the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument produces the relative movement between the at least two pairs of ridges and spaces on the retention ring and the rotor that seals the rotor and stator together in a fluid-tight manner.

187. The integrated diagnostic cartridge of claim 186, wherein a rotation movement of less than one full rotation of the rotor performed by the rotor driver of the diagnostic instrument seals the rotor and stator together in a fluid-tight manner.

188. The integrated diagnostic cartridge of any one of claims 185, 186 and 187 further comprising a gasket interposed at the rotor-stator interface.

189. The integrated diagnostic cartridge of claim 181, wherein the rotary valve is maintained in a storage condition while a threaded portion of a retention ring is engaged with a threaded portion of the rotor wherein relative motion between the threaded portion of the retention ring and the threaded portion of the rotor seals the rotor and stator together in a fluid-tight manner.

190. The integrated diagnostic cartridge of claim 189 wherein, when the cartridge is positioned within a diagnostic instrument, engagement with a rotor driver of the diagnostic instrument produces the relative movement between the threaded portion of the retention ring and the threaded portion of the rotor.

191. The integrated diagnostic cartridge of claim 190, wherein a rotation movement of less than one full rotation of the rotor performed by the rotor driver of the diagnostic instrument seals the rotor and stator together in a fluid-tight manner.

192. The integrated diagnostic cartridge of any one of claim 189, claim 190 and claim 191 further comprising: a gasket interposed at the rotor-stator interface.

193. The integrated diagnostic cartridge of claim 181, the purification module further comprising: a waste collection element, a wash buffer reservoir and an elution buffer reservoir.

194. The integrated diagnostic cartridge of claim 181 further comprising a pneumatic interface in fluidic communication with at least the purification module.

195. The integrated diagnostic cartridge of claim 181 wherein the porous solid support is polymeric.

196. The integrated diagnostic cartridge of claim 181 wherein the porous solid support is selected from the group consisting of alumina, silica, celite, ceramics, metal oxides, porous glass, controlled pore glass, carbohydrate polymers, polysaccharides, agarose, Sepharose™, Sephadex™, dextran, cellulose, starch, chitin, zeolites, synthetic polymers, polyvinyl ether, polyethylene, polypropylene, polystyrene, nylons, polyacrylates, polymethacrylates, polyacrylamides, polymaleic anhydride, membranes, hollow fibers and fibers, and any combination thereof.

197. The integrated diagnostic cartridge of claim 181 wherein the rotor valving face comprises a gasket interposed at the rotor-stator interface.

198. The integrated diagnostic cartridge of claim 197 the gasket further comprising a fluid connector or a fluid selector comprising a volume dimensioned to provide an aliquot of liquid when filled.

199. The integrated diagnostic cartridge of claim 181 wherein the rotor comprises a plurality of flow channels, each flow channel comprising an inlet, an outlet, and a porous solid support.

200. The integrated diagnostic cartridge of claim 181 the rotor valving face further comprising a fluid connector or a fluid selector comprising a volume dimensioned to provide an aliquot of liquid when filled.

201. The integrated diagnostic cartridge of any one of claim 181 to claim 200, the purification module further comprising: a waste collection element, a wash buffer reservoir and an elution buffer reservoir.

202. An integrated diagnostic cartridge, comprising:
a loading module;
a lysis module;
a purification module; and
a reaction module comprising a plurality of individual assay chambers, wherein at least one wall in each one of the plurality of individual assay chambers is provided by a plug comprising:
a body with a bottom surface;
a central opening in the body; and
a dried reagent on the bottom surface, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum;
wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

203. The integrated diagnostic cartridge of claim 202, wherein the bottom surface of the plug body comprises a cavity in the bottom surface with the dried reagent within the cavity, and wherein the plug has a plug thickness between a central opening bottom and the plug body bottom, and further wherein a depth of the cavity is less than 90% of the plug thickness, is less than 70% of the plug thickness or is less than 50% of the plug thickness.

204. The integrated diagnostic cartridge of claim 202, wherein the plug has a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths.

205. The integrated diagnostic cartridge according to any one of claim 202, claim 203 and claim 204, wherein the dried reagent is selected from the group consisting of nucleic acid synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, monomers, detection reagents, catalysts or combinations thereof.

206. The integrated diagnostic cartridge of claim 202 further comprising: a cartridge perimeter, wherein each one of the plurality of individual assay chambers is in communication with an air chamber and each air chamber is closer to the cartridge perimeter than the plug in each one of the plurality of individual assay chambers.

207. The integrated diagnostic cartridge of claim 202 further comprising: a reaction area perimeter, wherein each one of the plurality of individual assay chambers is in communication with an air chamber and further wherein each plug in each one of the plurality of individual assay chambers is within the reaction area perimeter and each air chamber is outside of the reaction area perimeter.

208. The integrated diagnostic cartridge of claim 202 further comprising: a cartridge perimeter and a reaction area perimeter wherein each one of the plurality of individual assay chambers is in communication with an air chamber and each air chamber is closer to the cartridge perimeter than the plug in each one of the plurality of individual assay chambers and is located outside of the reaction area perimeter and each one of the plurality of individual assay chambers is within the reaction area perimeter.

209. The integrated diagnostic cartridge according to claim 202, wherein the body of the plug protrudes into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber.

210. The integrated diagnostic cartridge according to claim 202 further comprising at least one fluid inlet conduit to each one of the plurality of individual assay chambers of the reaction module wherein each one of the at least one fluid inlet conduits further comprises a heat staked region.

211. The integrated diagnostic cartridge according to claim 210 wherein a heat stake in the heat staked region fluidically isolates the reaction module from the loading module, the lysis module, and the purification module.

212. The integrated diagnostic cartridge according to any one of claim 202 to claim 211, wherein the dried reagent is a continuous film adhered to the plug bottom surface.

213. The integrated diagnostic cartridge according to any one of claim 202 to claim 212, wherein the dried reagent is a lyophilized reagent.

214. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module; and

a reaction module comprising one or more assay chambers, wherein each assay chamber comprises:

a. a tapered inlet;

b. a tapered outlet;

c. a plug comprising a bottom surface and a central opening in the body, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of an ultraviolet spectrum, a blue spectrum, a green spectrum and a red spectrum;

d. two curved boundaries, wherein each curved boundary extends from the tapered inlet to the tapered outlet such that together, the two curved boundaries and the plug enclose a volume of the assay chamber; and

e. a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved boundaries, the shoulders extending from each of the curved boundaries and the plug.

215. The integrated diagnostic cartridge of claim 214, the plug as in any one of claim 203 to claim 213.

216. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module; and

a reaction module comprising:

a. a common fluid pathway, and

b. a plurality of independent, continuous fluidic pathways connected to the common fluid pathway, wherein each independent, continuous fluidic pathway comprising:

i. an assay chamber, and

ii. a pneumatic compartment;

1. wherein the assay chamber is connected to the common fluid pathway, the assay chamber having a fluid volume defined in part by a plug having a dried reagent thereon; and

2. the pneumatic compartment, having a pneumatic volume, is connected to the common fluid pathway via the assay chamber;

wherein, each fluidic pathway of the plurality of independent, continuous fluidic pathways is a closed system excluding the connection between the assay chamber and common fluid source, wherein each assay chamber further comprises:

c. a double tapered chamber, the double tapered chamber comprising:

iii. a tapered inlet in fluidic communication with a terminus of the entry conduit of the fluidic pathway,

iv. a tapered outlet in fluidic communication with a terminus of the pneumatic compartment, and

v. two curved boundaries, wherein each curved boundary extends from the tapered inlet to the tapered outlet such that together, the two curved boundaries enclose the volume of the assay chamber;

d. a shoulder extending from each curved boundary wherein the plug contacts each shoulder such that a boundary of the assay chamber is provided by the two curved boundaries, the shoulders extending from each of the curved boundaries and the plug.

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

217. The integrated diagnostic cartridge of claim 216, wherein the two curved boundaries are formed in a monolithic substrate or a fluidic card of the cartridge.

218. The integrated diagnostic cartridge according to any one of claim 202 to claim 217, wherein the body of the plug protrudes into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber.

219. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module; and

a reaction module comprising a reagent storage component comprising a capsule capable of holding a liquid or solid sample, said capsule comprising an opening, a closed end and a wall extending from the closed end to the opening, wherein the capsule is oval-shaped and the wall is rounded, and wherein the closed end and wall define an interior volume having a substantially smooth surface;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

220. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module; and

a reaction module comprising a capsule capable of holding a liquid or a solid sample,

said capsule comprising

an inner surface extending from the bottom of said capsule to an oval-shaped opening at the top of the capsule, wherein said inner surface is substantially smooth and comprises a concave shape extending from the bottom of the capsule; and

a planar layer affixed around the oval-shaped opening of said capsule and oriented in the same plane as the oval-shaped opening of said capsule, wherein said planar layer comprises a top surface and a bottom surface, said top surface aligned with the inner surface of said capsule at said oval-shaped opening to provide a continuous surface;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module.

221. An integrated diagnostic cartridge of claim 219 or claim 220 wherein said capsule is capable of holding a volume from approximately 50 µL to approximately 200 µL or wherein said oval-shaped opening is contained within an area of 9 mm x 9 mm.

222. An integrated diagnostic cartridge of claim 219 or claim 220 wherein said capsule comprises a dried reagent according to any one of claim 205, claim 212 or claim 213.

223. The integrated diagnostic cartridge according to any one of claim 137 to claim 222 further comprising a fluidic card and a cover.

224. The integrated diagnostic cartridge of claim 223, wherein at least two of the loading module, the lysis module, the purification module and the reaction module are formed in or supported by the fluidic card.

225. The integrated diagnostic cartridge of claim 223, wherein at least two of the loading module, the lysis module, the purification module and the reaction module are formed in or supported by the cover.

226. The integrated diagnostic cartridge of claim 223, the fluidic card further comprising a slot positioned to engage with a latch and pin assembly of a diagnostic instrument to secure the integrated diagnostic cartridge in a testing position within the diagnostic instrument.

227. The integrated diagnostic cartridge of claim 223 further comprising an interference feature on the cover, wherein the interference feature is sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument.

228. The integrated diagnostic cartridge according to any one of claim 223 to claim 227, wherein a thickness of the fluidic card is selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument.

229. The integrated diagnostic cartridge of any one of claim 137 to claim 228 wherein a total sample process volume of the integrated diagnostic cartridge is provided by increasing the thickness of the cartridge.

230. The integrated diagnostic cartridge of claim 229, wherein a diagnostic instrument is adapted and configured to accommodate the increased thickness of the cartridge by increasing a width of an opening of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument is adapted to accommodate the increased thickness of the cartridge.

231. The integrated diagnostic cartridge of any one of claim 137 to claim 230 further comprising a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing wherein each of the upper spacing and the lower spacing is sized and positioned to engage with an upper rail and a lower rail of the instrument.

232. The integrated diagnostic cartridge of claim 231 further comprising an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation.

233. The integrated diagnostic cartridge of any one of claim 137 to claim 232 further comprising a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module.

234. The integrated diagnostic cartridge of any one of claim 137 to claim 233 further comprising a label section.

235. The integrated diagnostic cartridge of any one of claim 137 to claim 234 further comprising one or more machine readable marking indicating the sample type to be used in the cartridge or target pathogen to be detected.

236. The integrated diagnostic cartridge of any one of claim 137 to claim 235 further comprising a pneumatic interface.

237. The integrated diagnostic cartridge of any one of claim 137 to claim 236 wherein prior to loading the cartridge into a diagnostic instrument a lysis chamber in the cartridge contains a lysis buffer.

238. The integrated diagnostic cartridge of any one of claim 137 to claim 237, the integrated diagnostic cartridge further comprising a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or a patient identification marking.

239. The integrated diagnostic cartridge of claim 217 or claim 218 further comprising a film adhered to a surface of the monolithic substrate, wherein the film forms one wall of the assay chamber.

240. The integrated diagnostic cartridge of any one of claim 137 to claim 239, further comprising a first film adhered to a surface of at least a portion of the cartridge, wherein the first film forms one wall of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module.

241. The integrated diagnostic cartridge of claim 240, further comprising a second film adhered to the first film, wherein the second film has a higher melting temperature than the first film.

242. The integrated diagnostic cartridge of claim 241 further comprising a heat staked region formed in each of the fluidic pathways using the first film or the second film wherein the heat staked region seals off the common fluid pathway from the assay chamber and the pneumatic chamber.

243. The integrated diagnostic cartridge of claim 242 further comprising a raised platform within each of the plurality of independent, continuous fluidic pathways the raised platform positioned between an inlet to the assay chamber and the common fluid pathway wherein the heat staked region is formed using a portion of the raised platform.

244. An integrated diagnostic cartridge, comprising:

a loading module having a fill chamber within the cartridge having a volume sufficient to hold a sample, a fluid inlet in fluid communication with the fill chamber, a fluid outlet in fluid communication with fill chamber;

a lysis module;

a purification module; and

a reaction module;

wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module;

further wherein the loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation; and

further wherein when the cartridge is in a horizontal sample loading orientation the fluid inlet accesses the fill chamber via an upper surface of the cartridge and when the cartridge is in a vertical sample processing orientation the fluid inlet is positioned adjacent to an upper portion of the fill chamber and the fluid outlet is arranged for the sample to flow out of a lower portion of the fill chamber.

245. The integrated diagnostic cartridge of claim 244 further comprising one or more fluid filling conduits arranged to flow into an upper portion of a vertically oriented chamber within a fluidic card of the integrated diagnostics cartridge and one or more fluid outlet conduits arranged to flow out of a lower portion of the vertically oriented chamber within the fluidic card of the integrated diagnostics cartridge.

246. The integrated diagnostic cartridge of claim 245, wherein the vertically oriented chamber further comprises a filter assembly in fluid communication with a fluid outlet conduit of the vertically oriented chamber.

247. The integrated diagnostic cartridge of claim 244, wherein the lysis module comprises a mixing assembly having a vertically oriented lysis chamber containing a lysis agent and a non-magnetized stir bar.

248. The integrated diagnostic cartridge of claim 247, wherein the non-magnetized stir bar is made from a metal having a magnetic permeability to be responsive to a rotating magnetic field induced between a drive magnetic element and a driven magnetic element of a magnetic drive system.

249. The integrated diagnostic cartridge according to claim 247, wherein the non-magnetized stir bar is coated with an impermeable material to prevent corrosion by a chemical lysis buffer in the vertically oriented lysis chamber.

250. The integrated diagnostic cartridge of claim 247 wherein, when in use within a diagnostic instrument, the non-magnetized stir bar is disposed between a driving magnet system and a driven magnet system of a magnetic mixing assembly in the diagnostic instrument, wherein the driving magnet system is configured to rotate the non-magnetized stir bar within the vertically oriented lysis chamber at least 1000 rpm.

251. The integrated diagnostic cartridge of claim 247, further comprising a fluid inlet to the vertically oriented lysis chamber and a fluid outlet to lysis chamber wherein the vertically oriented lysis chamber is isolated from the other modules on the cartridge by a first frangible

seal in fluid communication with the fluid inlet to the vertically oriented lysis chamber and a second frangible seal in fluid communication with the fluid outlet to the vertically oriented lysis chamber.

252. The integrated diagnostic cartridge according to claim 244 further comprising a fluidic card and a cover.

253. The integrated diagnostic cartridge of claim 252, wherein the fluidic card further comprises a first film adhered to a surface of at least a portion of the fluidic card, wherein the first film forms one surface of one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module.

254. The integrated diagnostic cartridge of claim 252 further comprising an interference feature on the cover, wherein the interference feature is sized and positioned to interact with one of an upper rail or a lower rail of a loading apparatus of a diagnostic instrument.

255. The integrated diagnostic cartridge according to claim 254, wherein a thickness of the fluidic card is selected for sliding arrangement within an upper rail and a lower rail of a loading apparatus of the diagnostic instrument.

256. The integrated diagnostic cartridge of claim 252 wherein a total sample process volume of the integrated diagnostic cartridge is related to a thickness of the cartridge corresponding to a spacing between the one or more chambers, compartments, or fluid conduits of the loading module, the lysis module, the purification module and the reaction module formed in the fluidic card and the first film.

257. The integrated diagnostic cartridge of claim 256, wherein a diagnostic instrument is adapted and configured to accommodate a variation of the thickness of the cartridge by increasing a width of a loading slot of the diagnostic instrument to accommodate the increased thickness of the cartridge or a displacement range of a cartridge clamping system of the diagnostic instrument is adapted to accommodate the increased thickness of the cartridge.

258. The integrated diagnostic cartridge of claim 254 further comprising a cartridge front face and a cartridge rear face forming an upper spacing and a lower spacing wherein each of the upper spacing and the lower spacing is sized and positioned to engage with the upper rail and lower rail of the diagnostic instrument.

259. The integrated diagnostic cartridge of claim 258 further comprising an interference feature within the upper spacing or the lower spacing positioned to ensure the cartridge engages with the upper rail and the lower rail in a desired orientation.

260. The integrated diagnostic cartridge of claim 244 further comprising a plurality of frangible seal chambers in fluid communication with at least one or more of the loading module, the lysis module, the purification module or the reaction module.

261. The integrated diagnostic cartridge of claim 244, the integrated diagnostic cartridge further comprising a machine-readable code adapted and configured to identify the cartridge to a diagnostic instrument or an image of a patient identification marking.

262. An integrated diagnostic cartridge, comprising:

a loading module;

a lysis module;

a purification module comprising a rotary valve comprising:

a. a stator comprising a stator face and a plurality of passages, each passage comprising a port at the stator face;

b. a rotor operably connected to the stator and comprising a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel comprises a porous solid support; and

c. a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal; and

a reaction module comprising a plurality of individual assay chambers, wherein at least one surface in each one of the plurality of individual assay chambers is provided by a plug comprising:

a body with a bottom surface;

a central opening in the body; and

a dried reagent on the bottom surface, wherein the body is formed from a material transmissive to excitation wavelengths and emission wavelengths in at least one of a red spectrum, a blue spectrum and a green spectrum,

further wherein the loading module is in fluidic communication with the lysis module and the purification module is in fluidic communication with the reaction module; and

wherein the loading module, the lysis module, the purification module and the reaction module are arranged for use while the cartridge is in a vertical orientation.

263. The integrated diagnostic cartridge of claim 262, wherein the bottom surface of the plug body comprises a cavity in the bottom surface with the dried reagent within the cavity, and wherein the plug has a plug thickness between a central opening bottom and the plug body bottom, and further wherein a depth of the cavity is less than 90% of the plug thickness, is less than 70% of the plug thickness or is less than 50% of the plug thickness.

264. The integrated diagnostic cartridge of claim 262, wherein the plug has a polished or smooth finish facilitating the transmissivity of the excitation wavelengths and the emission wavelengths.

265. The integrated diagnostic cartridge according to claim 262, wherein the dried reagent is selected from the group consisting of nucleic acid synthesis reagents, nucleic acids, nucleotides, nucleobases, nucleosides, monomers, detection reagents, catalysts or combinations thereof.

266. The integrated diagnostic cartridge according to claim 262, wherein the body of the plug protrudes into the monolithic substrate of the assay chamber at a depth such that the assay chamber volume can be readily changed by altering the depth at which the body of the plug protrudes into the monolithic substrate of the assay chamber.

267. The integrated diagnostic cartridge according to claim 262 further comprising at least one fluid inlet conduit to each one of the plurality of individual assay chambers of the reaction module wherein each one of the at least one fluid inlet conduits further comprises a heat staked region.

268. The integrated diagnostic cartridge according to claim 267 wherein a heat stake in the heat staked region fluidically isolates the reaction module from the loading module, the lysis module, and the purification module.

269. The integrated diagnostic cartridge according to claim 244, the purification module further comprising: a rotary valve comprising:

a. a stator comprising a stator face and a plurality of passages, each passage comprising a port at the stator face;
b. a rotor operably connected to the stator and comprising a rotational axis, a rotor valving face, and a flow channel having an inlet and an outlet at the rotor valving face, wherein the flow channel comprises a porous solid support; and
c. a retention element biasing the stator and the rotor together at a rotor-stator interface to form a fluid tight seal.

270. The integrated diagnostic cartridge of claim 269, wherein the rotary valve further comprises a gasket between the stator face and the rotor valving face, and wherein the stator comprises a displaceable spacer for preventing the gasket from sealing against at least one of the rotor and stator, and wherein, when the spacer is displaced the gasket seals the rotor and stator together in a fluid-tight manner.

271. The integrated diagnostic cartridge of claim 270, wherein, when the cartridge is positioned within a diagnostic instrument, engagement with a valve drive assembly of the diagnostic instrument displaces the spacer and seals the rotor and stator together in a fluid-tight manner.

272. The integrated diagnostic cartridge of claim 269, the purification module further comprising: a waste collection element, a wash buffer reservoir and an elution buffer reservoir.

273. The integrated diagnostic cartridge of claim 269 further comprising a pneumatic interface in fluidic communication with at least the purification module.

274. A method of testing a sample suspected of containing one or more target pathogens, comprising:

accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens;

advancing the sample suspected of containing the one or more target pathogens to a lysis chamber within the cartridge having at least one lysis reagent therein;

mixing the sample with the at least one lysis agent to generate a lysed sample;

passing the lysed sample through a porous solid support within the cartridge to capture a nucleic acid on the porous solid support;

releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid;

introducing the enriched nucleic into a rehydration chamber within the cartridge containing one or more dried reagents;

after introducing the analyte/reagent solution into a metering channel, mixing the contents of the rehydration chamber to produce an analyte/reagent solution;

distributing the analyte/reagent solution to two or more assay chambers within the cartridge after performing the mixing step;

combining the analyte/reagent solution with one or more amplification reagents after performing the distributing step;

sealing each one of the two or more assay chambers within the cartridge containing analyte/reagent solution from each one of all the other two or more assay chambers within the cartridge containing analyte/reagent solution and a waste chamber; and

performing an isothermal amplification reaction within each one of the two or more assay chambers in the cartridge while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

275. The method of testing a sample according to claim 274, wherein in mixing the sample with the at least one lysis agent, the lysis agent is a mechanical agent.

276. The method of testing a sample according to claim 275, wherein the mechanical agent is ceramic beads, glass beads or steel beads, and the mixing the sample step comprises rotating a stir bar within the lysis chamber at at least 1000 rpm.

277. The method of testing a sample according to claim 276, wherein mixing the sample comprises rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent.

278. The method of testing according to claim 274, wherein the at least one lysis agent is a chemical lysis agent.

279. The method of testing a sample according to claim 278, wherein the one or more target pathogens is a virus or a gram-negative bacterium and the lysis reagent is a chaotropic agent.

280. The method of testing a sample according to claim 274, wherein prior to passing the lysed sample through the porous solid support, the method further comprises passing the lysed sample through a size-exclusion filter, wherein nucleic acid passes through the filter.

281. The method of testing a sample according to claim 274, wherein the enriched nucleic acid is combined with one or more amplification reagents before the distributing step and further wherein the one or more amplification reagents comprise a primer.

282. The method of testing a sample according to claim 281, wherein the performing of the isothermal amplification reaction step is initiated prior to the distributing the enriched nucleic acid to the two or more assay chambers step.

283. The method of testing a sample according to claim 274, wherein after the distributing step, but prior to performing the isothermal amplification reaction step, the method further comprises combining the enriched nucleic acid with a primer set specific to one of the one or more target pathogens.

284. The method of testing a sample according to claim 274, wherein a first assay chamber contains a primer set specific to a first nucleic acid sequence.

285. The method of testing a sample according to claim 284, wherein the first nucleic acid sequence is present in one of the one or more target pathogens.

286. The method of testing a sample according to claim 284, wherein prior to mixing the sample with at least one lysis agent, a process control is added to the sample and the first nucleic acid sequence is present in the process control.

287. The method of testing a sample according to claim 284, wherein prior to passing lysed sample through the porous solid support, a process control is added to the lysed sample and the first nucleic acid sequence is present in the process control.

288. The method of testing a sample according to claim 284, wherein a second assay chamber contains a primer set specific to a second nucleic acid sequence, wherein the second nucleic acid sequence is present in one of the one or more target pathogens.

289. The method of testing a sample according to claim 274, wherein the performing an isothermal amplification reaction step is completed in less than 15 minutes.

290. The method of testing a sample according to claim 274, further comprising: providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen.

291. The method of testing a sample according to claim 274, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction.

292. The method of testing a sample according to claim 291, wherein the sample is sputum and the chemical reaction is incubation with a mucolytic agent.

293. The method of testing a sample according to claim 274, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction.

294. The method of testing a sample according to claim 293, wherein the enzymatic reaction is incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase.

295. The method of testing a sample according to claim 274, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment.

296. The method of testing a sample according to claim 295, wherein the physical treatment comprises passing the sample through a size-exclusion filter in a first direction.

297. The method of testing a sample according to claim 295, wherein the physical treatment comprises exposing the sample to a capture agent immobilized on a solid substrate.

298. The method of testing a sample according to claim 297, further comprising, after exposure, separating the solid substrate from the sample.

299. The method of testing a sample according to claim 297, wherein the capture agent is an antibody with affinity for red blood cells.

300. The method of testing a sample according to claim 274, wherein the sample is sputum and the method further comprises, prior to mixing the sample with the at least one lysis reagent, bead beating the sputum to liquify the sample.

301. The method of testing a sample according to claim 300, wherein the bead beating comprises mixing the sputum with ceramic, glass, or steel beads.

302. The method of testing a sample according to claim 274, wherein prior to distributing the enriched nucleic acid to the assay chambers, the method further comprises passing the enriched nucleic acid through a second porous solid support.

303. A method of testing a sample suspected of containing one or more target pathogens, comprising:

accepting a cartridge having a sample port assembly containing the sample suspected of containing the one or more target pathogens;

advancing the sample suspected of containing the one or more target pathogens to a lysis chamber within the cartridge having at least one lysis reagent therein;

mixing the sample with the at least one lysis agent to generate a lysed sample;

passing the lysed sample through a porous solid support within the cartridge to capture a nucleic acid on the porous solid support;

releasing the captured nucleic acid from the first porous solid support to generate an enriched nucleic acid;

introducing the enriched nucleic into a rehydration chamber within the cartridge containing one or more dried reagents to generate an analyte/reagent solution;

after introducing the analyte/reagent solution into a metering channel, mixing the contents of the rehydration chamber to homogenize an analyte/reagent solution;

distributing the analyte/reagent solution to two or more assay chambers within the cartridge after performing the mixing step;

combining the analyte/reagent solution with one or more amplification reagents after performing the distributing step to generate an amplification solution;

sealing each one of the two or more assay chambers within the cartridge containing amplification solution from each one of all the other two or more assay chambers within the cartridge containing amplification solution and a waste chamber; and

performing an isothermal amplification reaction within each one of the two or more assay chambers in the cartridge while simultaneously detecting amplification product, wherein presence of an amplification product is an indication of a presence, an absence or a quantity of the target pathogen in the sample suspected of containing the target pathogen.

304. The method of testing a sample according to claim 303, wherein in mixing the sample with the at least one lysis agent, the lysis agent is a mechanical agent.

305. The method of testing a sample according to claim 304, wherein the mechanical agent is ceramic beads, glass beads or steel beads, and the mixing the sample step comprises rotating a stir bar within the lysis chamber at at least 1000 rpm.

306. The method of testing a sample according to claim 305, wherein mixing the sample comprises rotating the stir bar or the ceramic, glass or steel beads along with a chemical lysis agent.

307. The method of testing according to claim 303, wherein the at least one lysis agent is a chemical lysis agent.

308. The method of testing a sample according to claim 307, wherein the one or more target pathogens is a virus or a gram-negative bacterium and the lysis reagent is a chaotropic agent.

309. The method of testing a sample according to claim 303, wherein prior to passing the lysed sample through the porous solid support, the method further comprises passing the lysed sample through a size-exclusion filter, wherein nucleic acid passes through the filter.

310. The method of testing a sample according to claim 303, wherein a first assay chamber contains a primer set specific to a first nucleic acid sequence.

311. The method of testing a sample according to claim 310, wherein the first nucleic acid sequence is present in one of the one or more target pathogens.

312. The method of testing a sample according to claim 310, wherein prior to mixing the sample with at least one lysis agent, a process control is added to the sample and the first nucleic acid sequence is present in the process control.

313. The method of testing a sample according to claim 310, wherein prior to passing lysed sample through the porous solid support, a process control is added to the lysed sample and the first nucleic acid sequence is present in the process control.

314. The method of testing a sample according to claim 310, wherein a second assay chamber contains a primer set specific to a second nucleic acid sequence, wherein the second nucleic acid sequence is present in one of the one or more target pathogens.

315. The method of testing a sample according to claim 303, wherein the performing an isothermal amplification reaction step is completed in less than 15 minutes.

316. The method of testing a sample according to claim 303, further comprising: providing a result containing a determination made during the performing step relating to the presence, the absence or the quantity of the target pathogen in the sample suspected of containing the target pathogen.

317. The method of testing a sample according to claim 303, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a chemical reaction.

318. The method of testing a sample according to claim 317, wherein the sample is sputum and the chemical reaction is incubation with a mucolytic agent.

319. The method of testing a sample according to claim 303, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with an enzymatic reaction.

320. The method of testing a sample according to claim 319, wherein the enzymatic reaction is incubation of the sample with a nuclease, a protease, an amylase, a glycosylase, or a lipase.

321. The method of testing a sample according to claim 303, wherein the method further comprises, prior to advancing the sample to a lysis chamber, pretreating the sample with a physical treatment.

322. The method of testing a sample according to claim 321, wherein the physical treatment comprises passing the sample through a size-exclusion filter in a first direction.

323. The method of testing a sample according to claim 321, wherein the physical treatment comprises exposing the sample to a capture agent immobilized on a solid substrate.

324. The method of testing a sample according to claim 323, further comprising, after exposure, separating the solid substrate from the sample.

325. The method of testing a sample according to claim 323, wherein the capture agent is an antibody with affinity for red blood cells.

326. The method of testing a sample according to claim 303, wherein the sample is sputum and the method further comprises, prior to mixing the sample with the at least one lysis reagent, bead beating the sputum to liquify the sample.

327. The method of testing a sample according to claim 326, wherein the bead beating comprises mixing the sputum with ceramic, glass, or steel beads.

328. The method of testing a sample according to claim 303, wherein prior to distributing the analyte/reagent solution to the two or more assay chambers, the method further comprises passing the analyte/reagent solution through a second porous solid support.

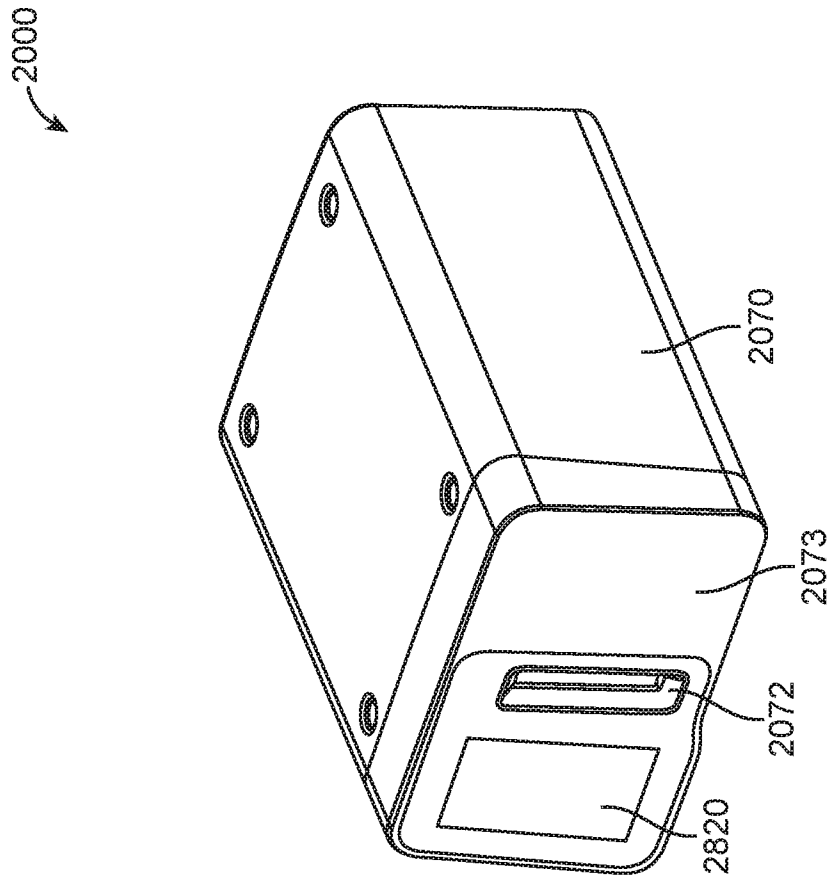


FIG. 1

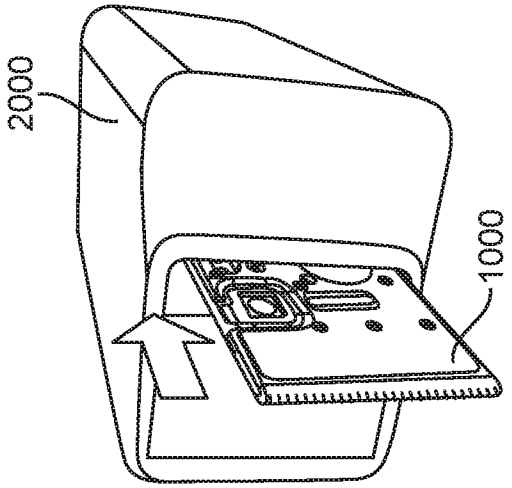


FIG. 3

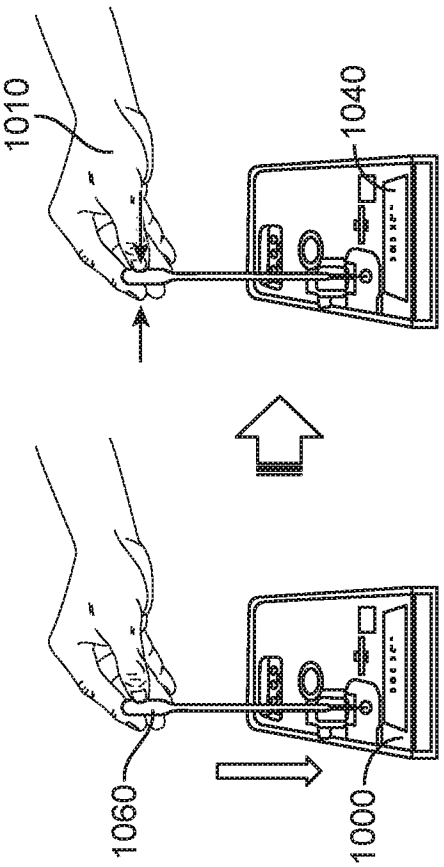


FIG. 2A

FIG. 2B

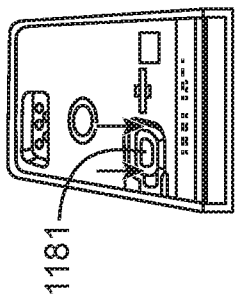


FIG. 2C

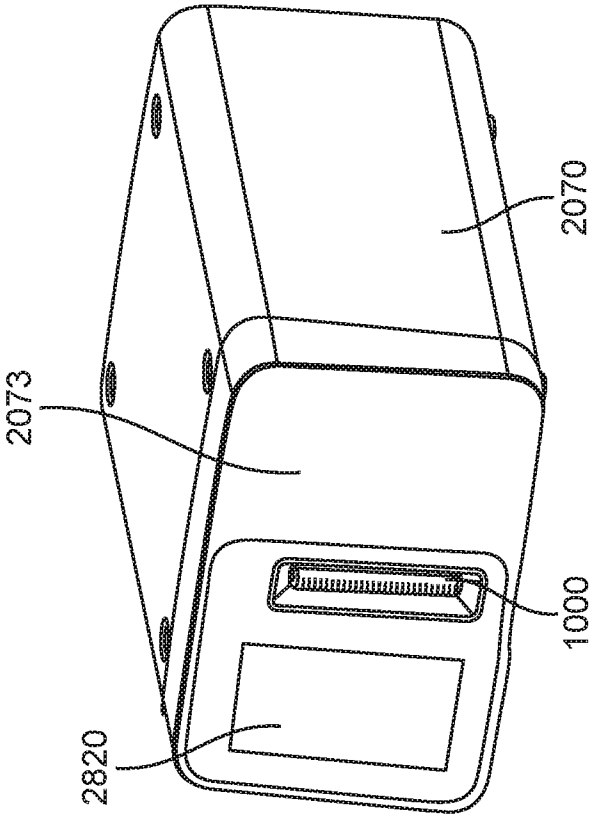

2073
2070
1000
2820

FIG. 4B

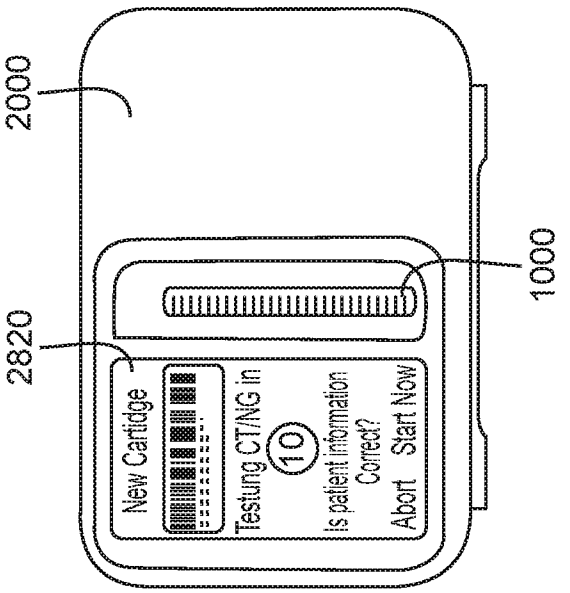

2000
2820
1000
New Cartidge
Testing CT/NG in
10
Is patient information
Correct?
Abort Start Now

FIG. 4A

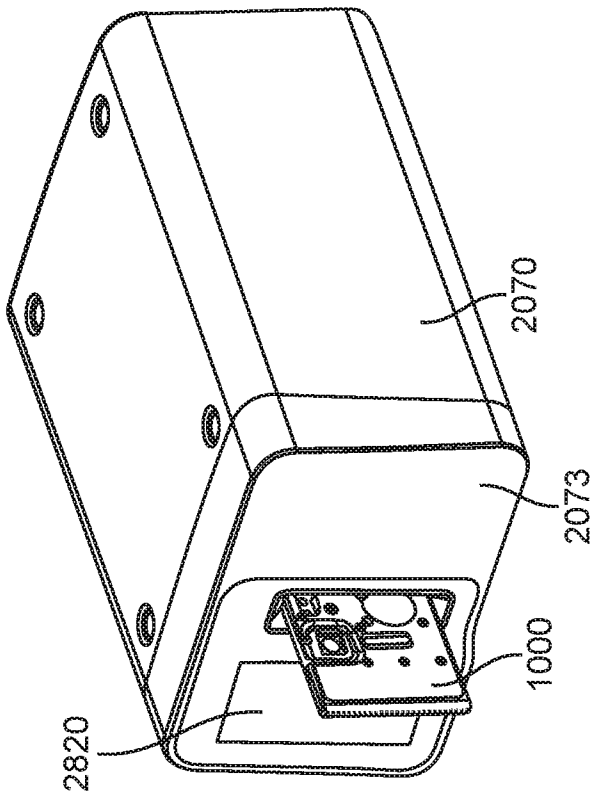


FIG. 5

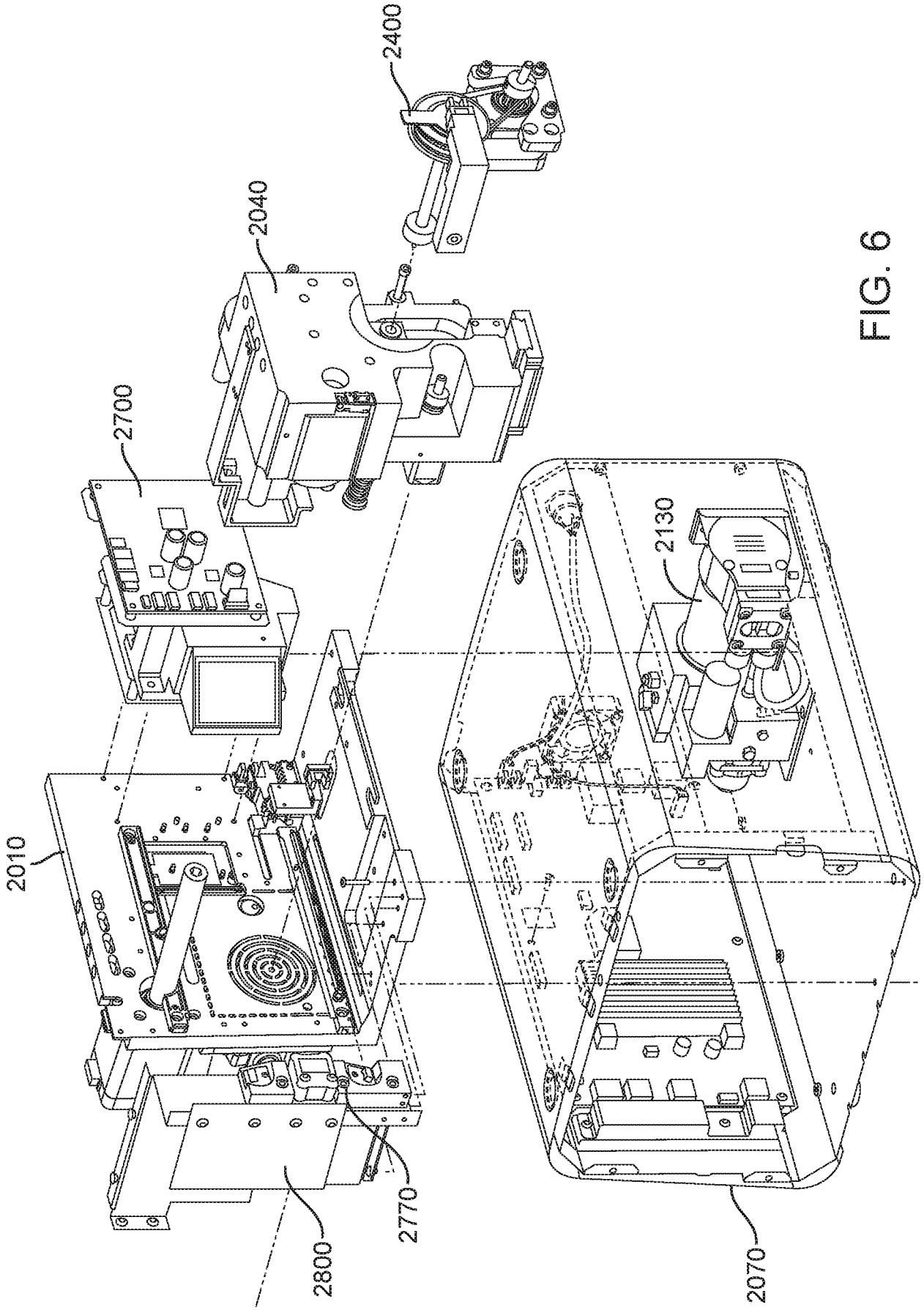


FIG. 6

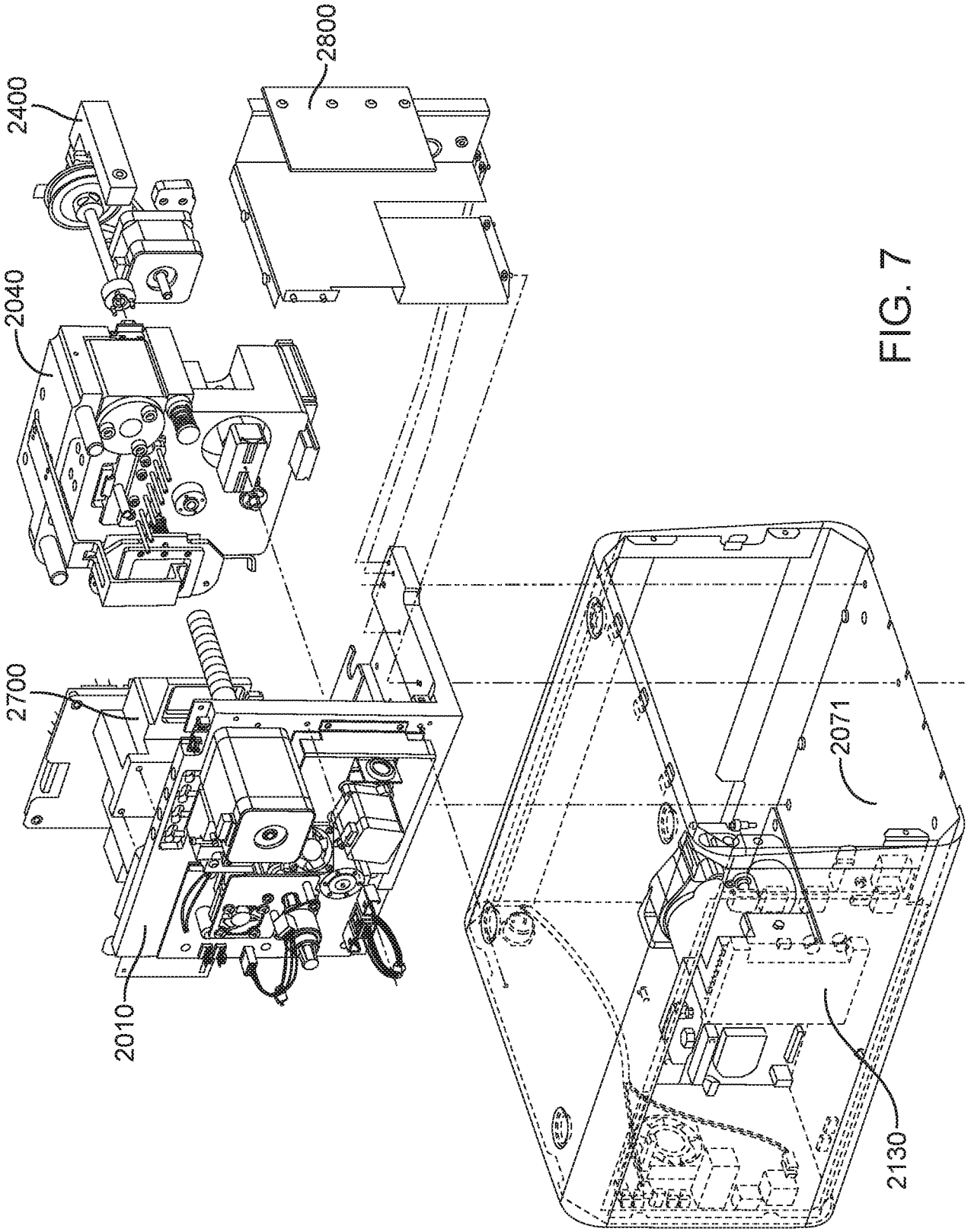


FIG. 7

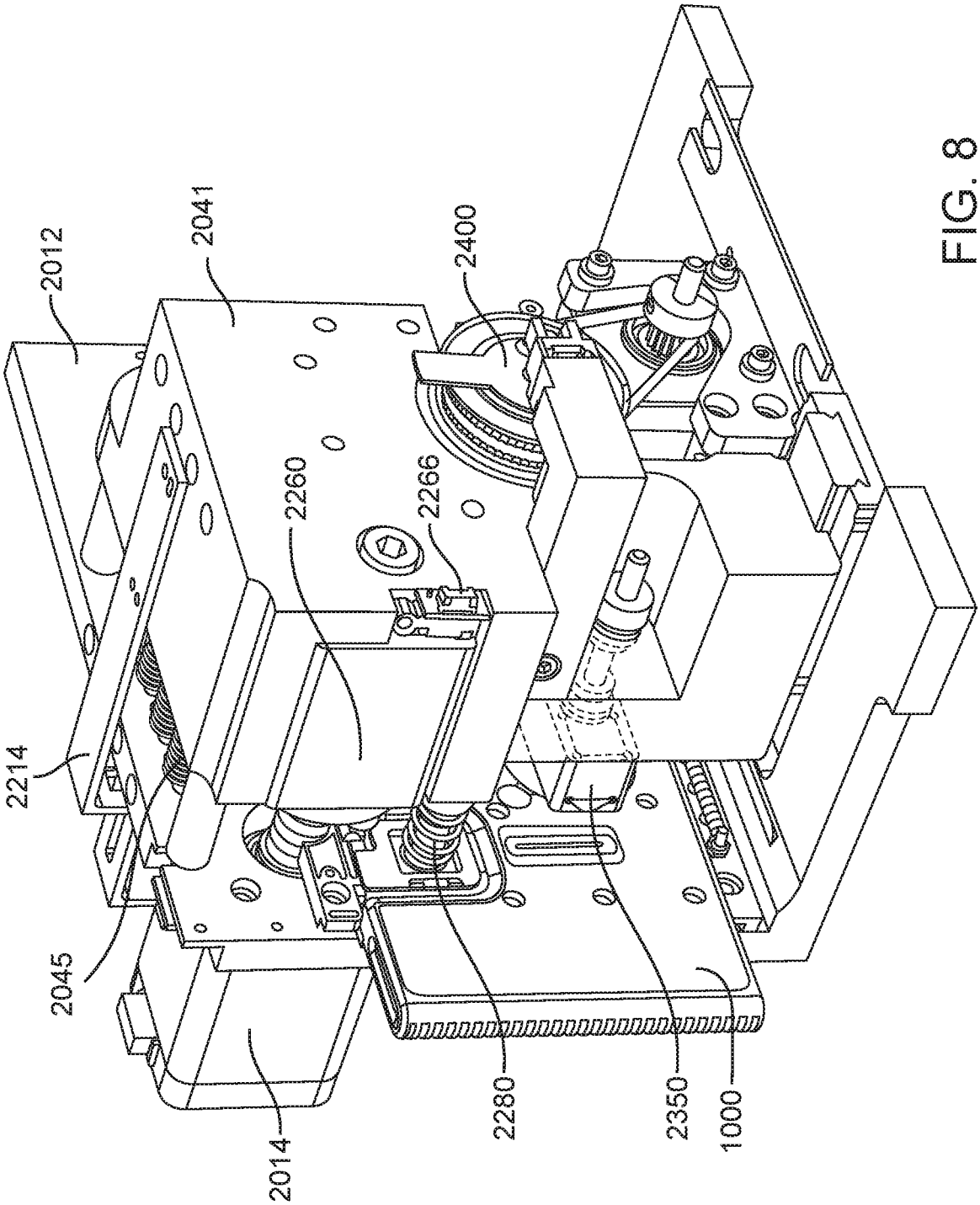


FIG. 8

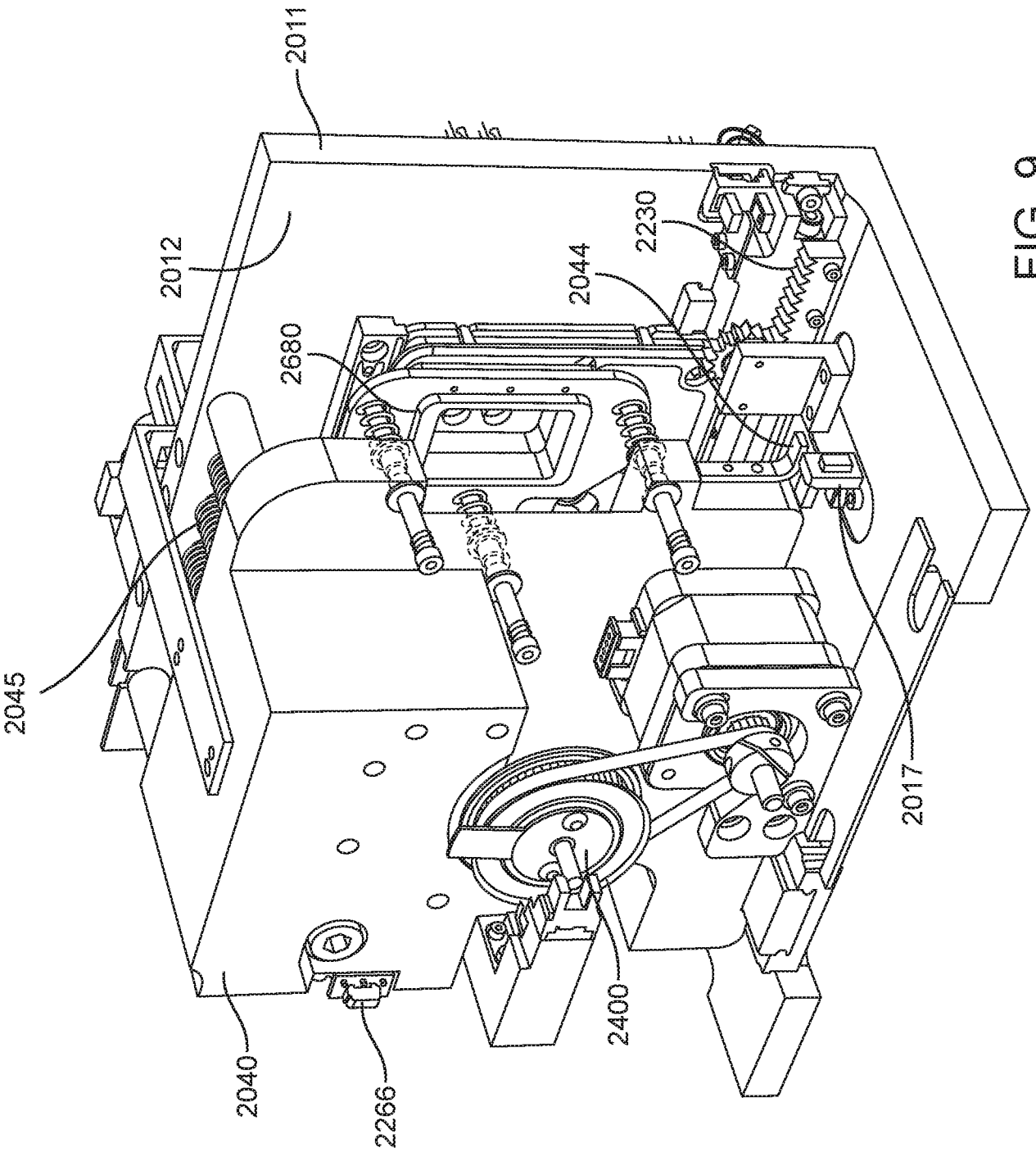


FIG. 9

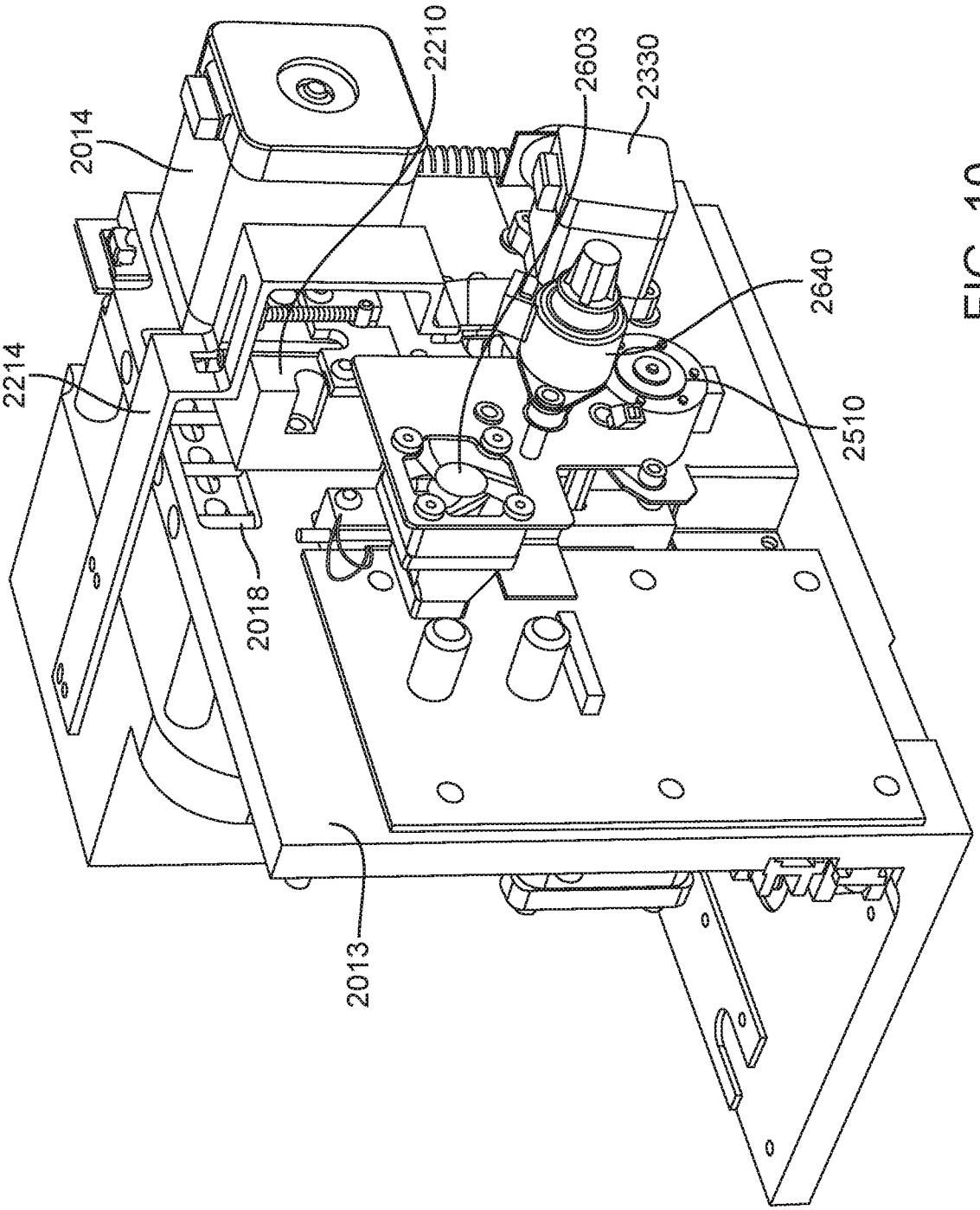
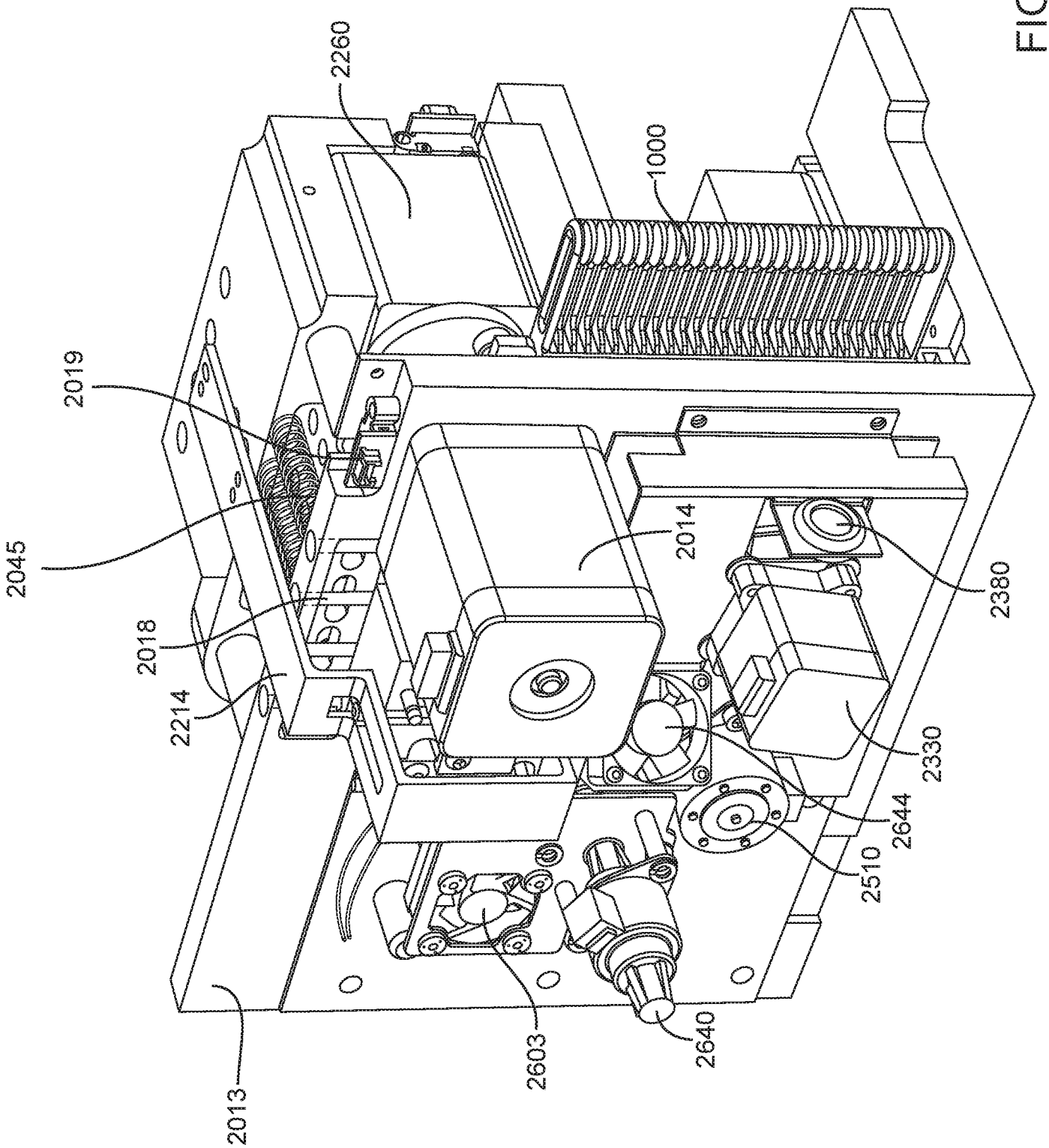


FIG. 10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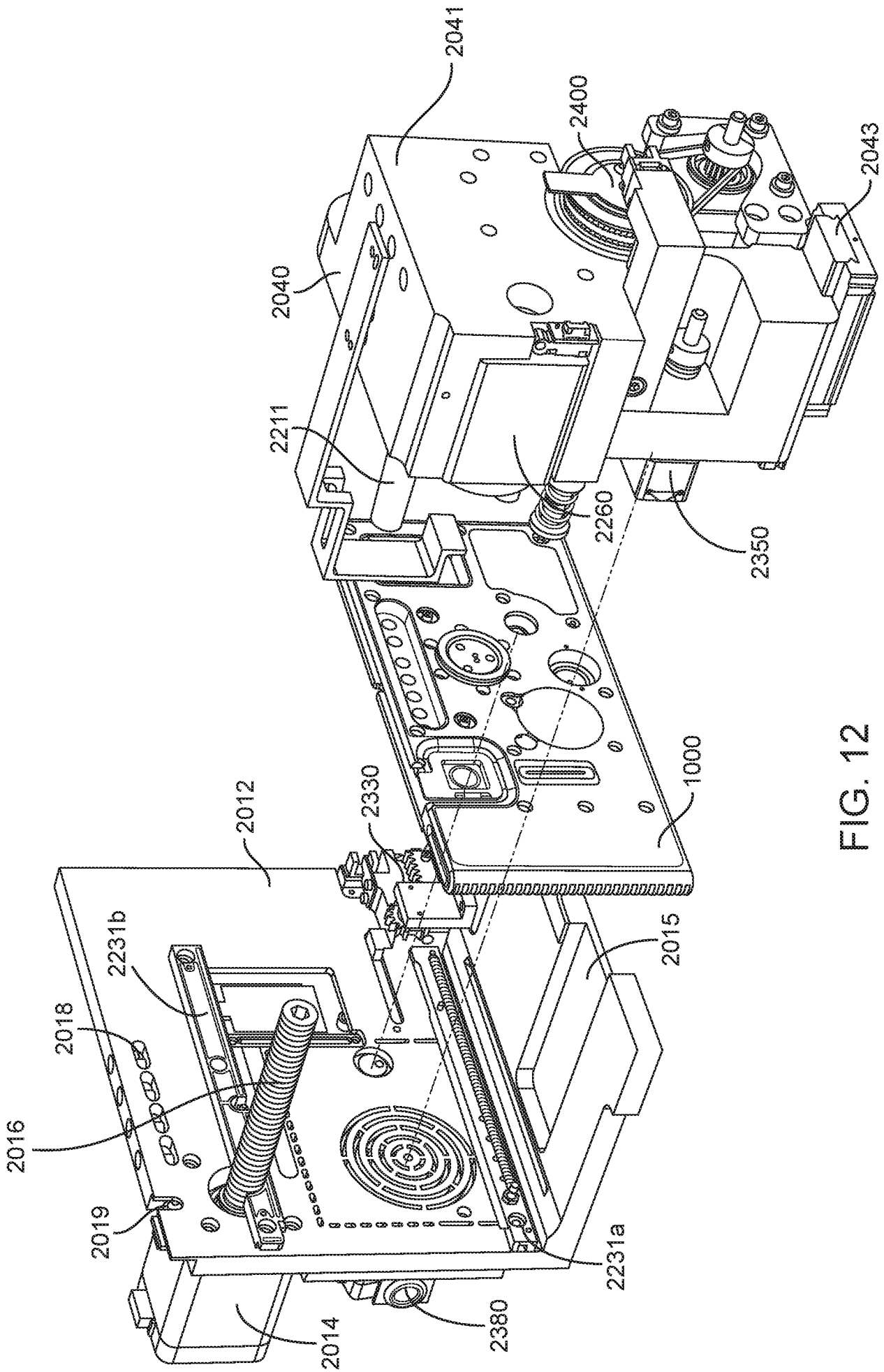
2644
2510
2603
2640
2013

FIG. 11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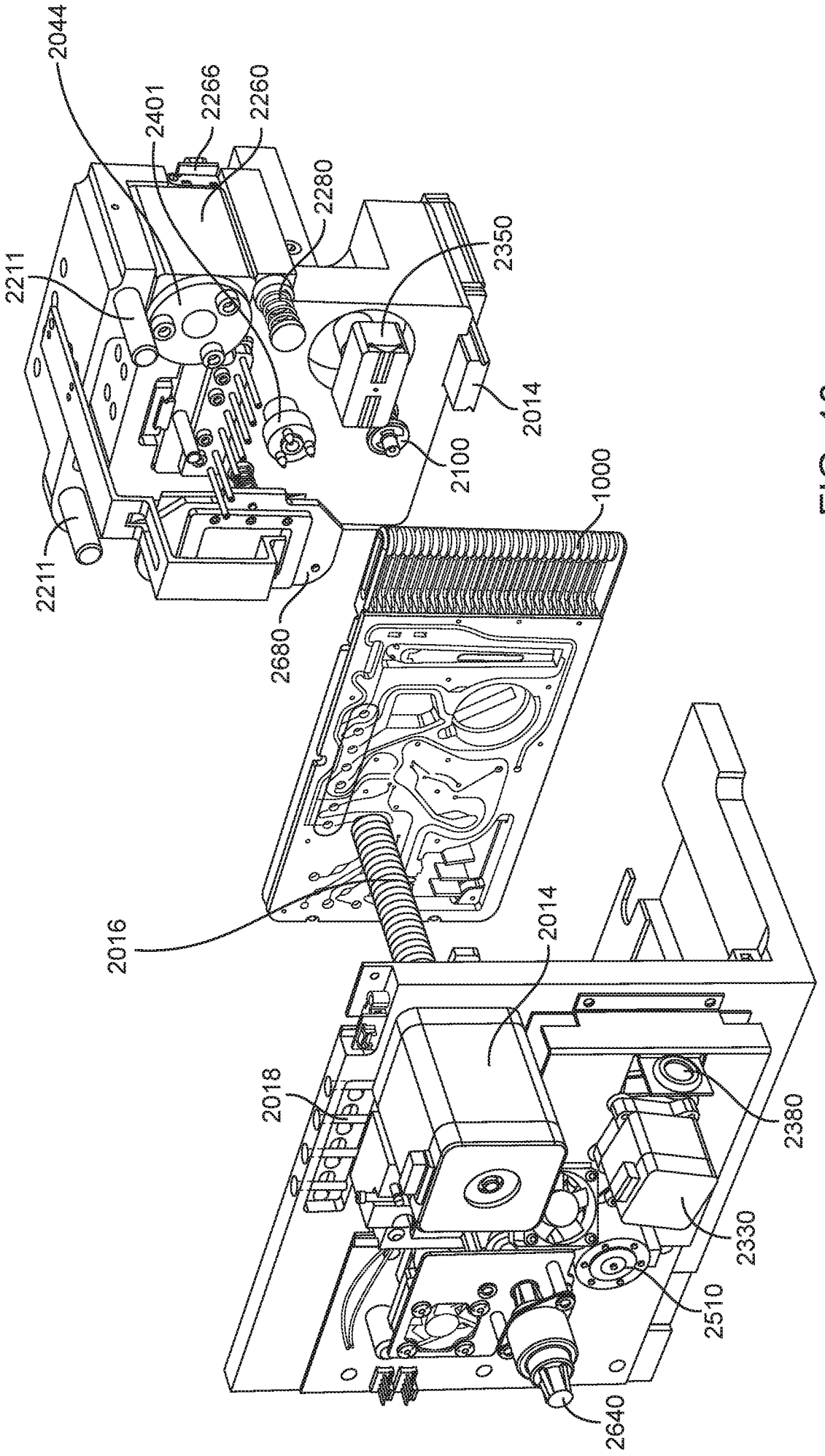
1000
2330
2012
2231b
2018
2016
2019
2015
2231a
2014
2380

FIG. 12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2211
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2100
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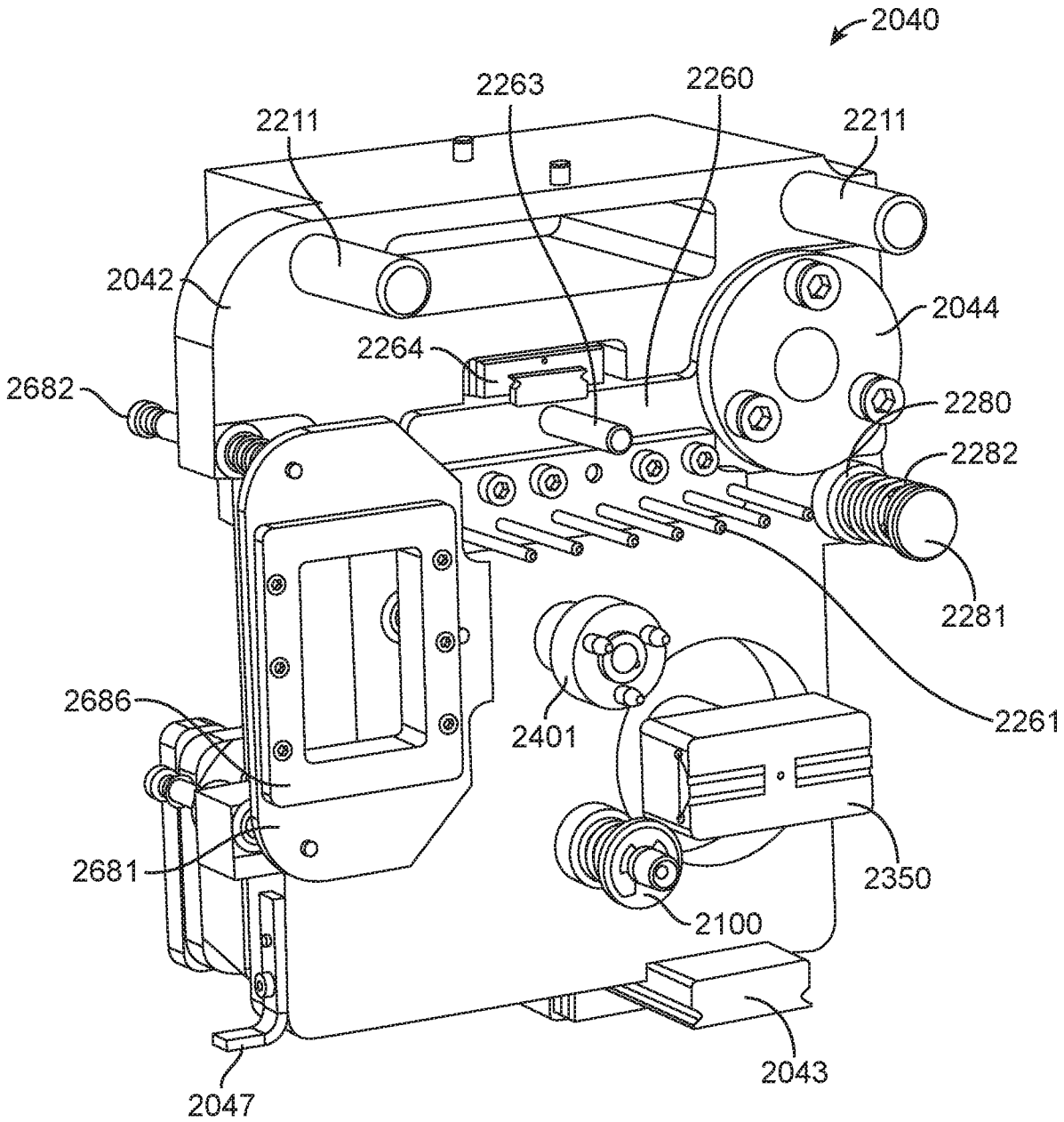
2211
2680
2016
2014
2018
2380
2330
2510
2640

FIG. 13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2211
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2044
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2280
2282
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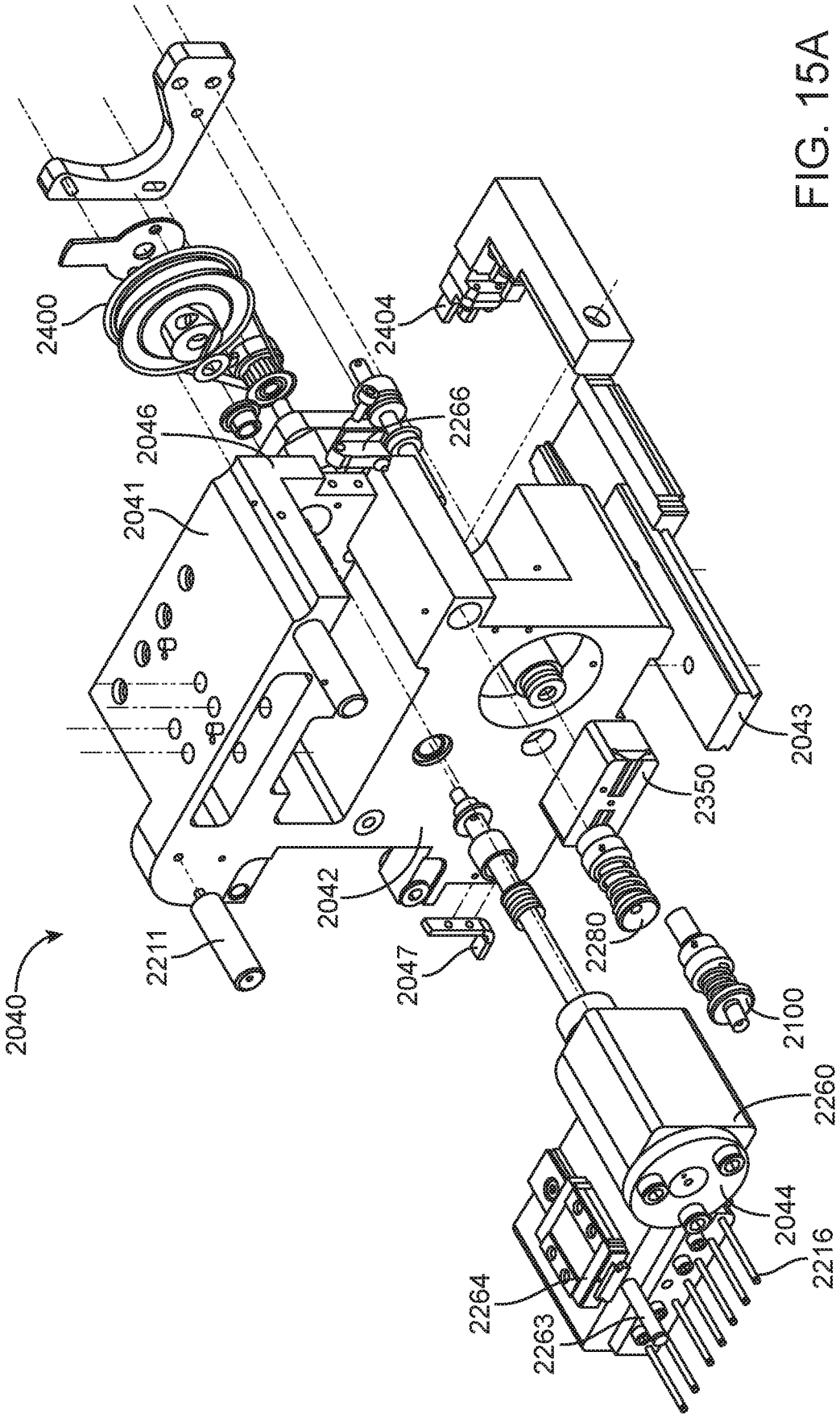
2261
2686
2401
2681
2350
2100
2043
2047

FIG. 14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2404
2046
2266
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2043
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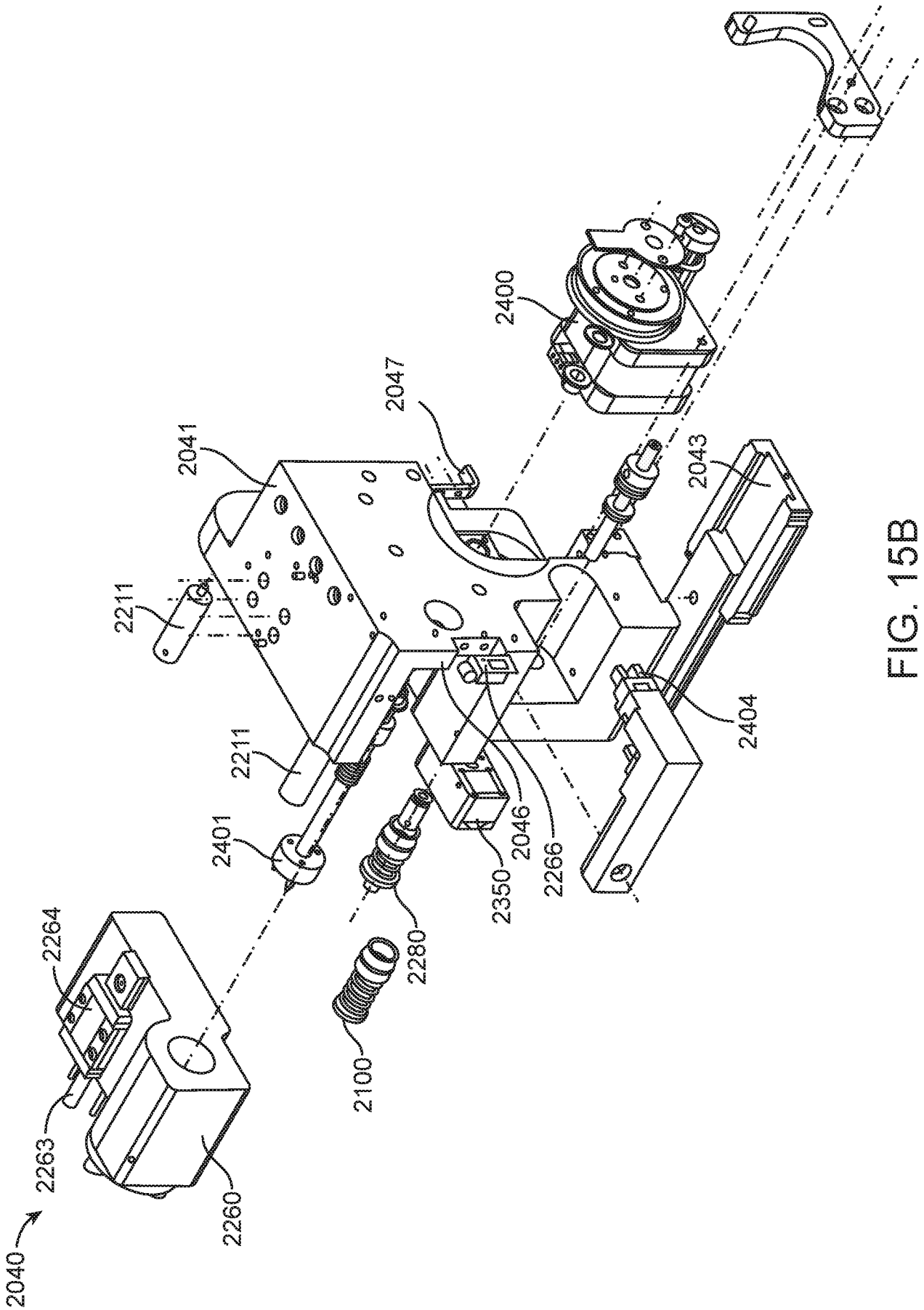
2040
2260
2044
2216
2264
2263

FIG. 15A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2211
2041
2047
2400
2043
2404
2280
2100
2350
2046
2266

FIG. 15B

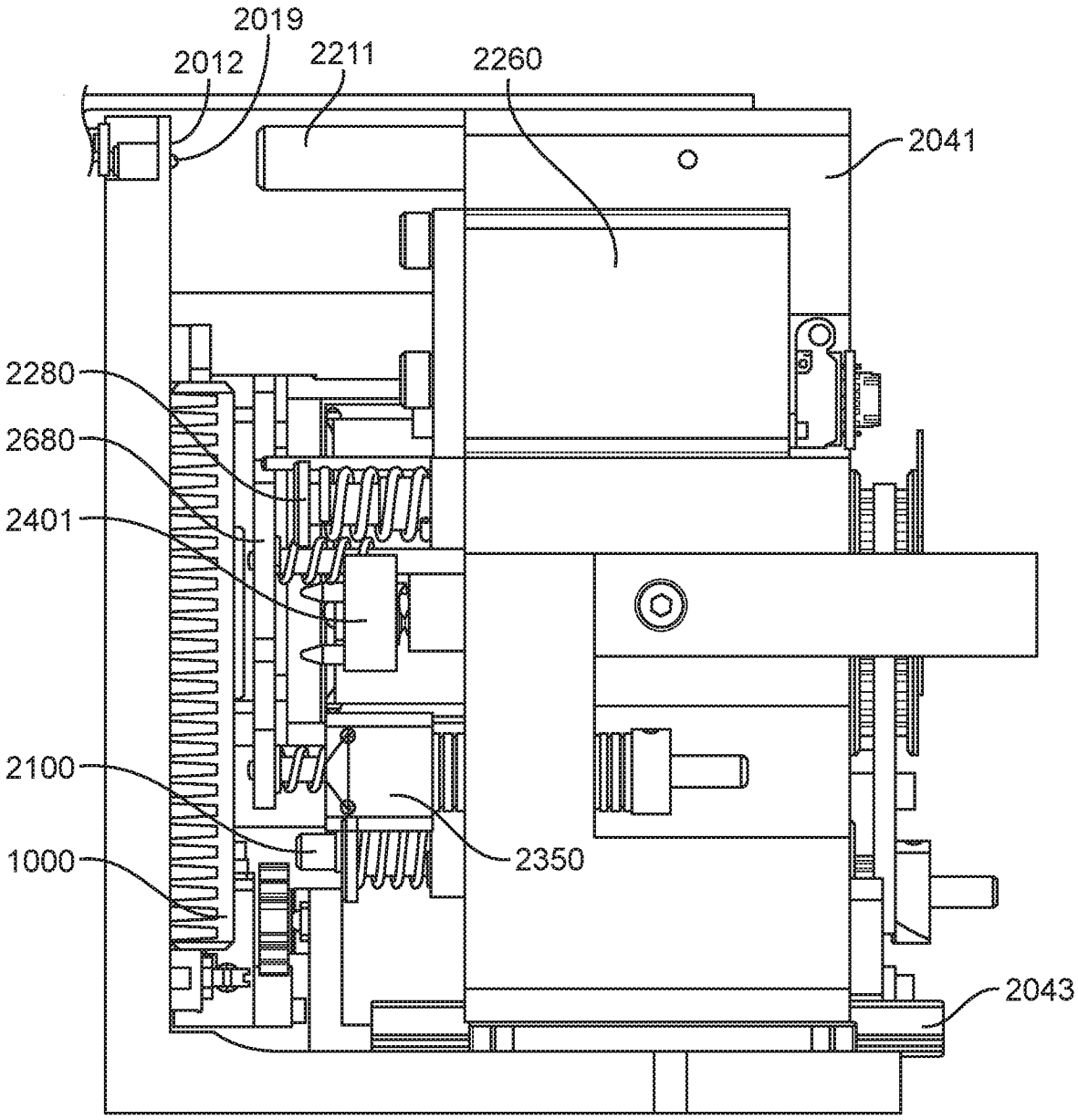


FIG. 16A

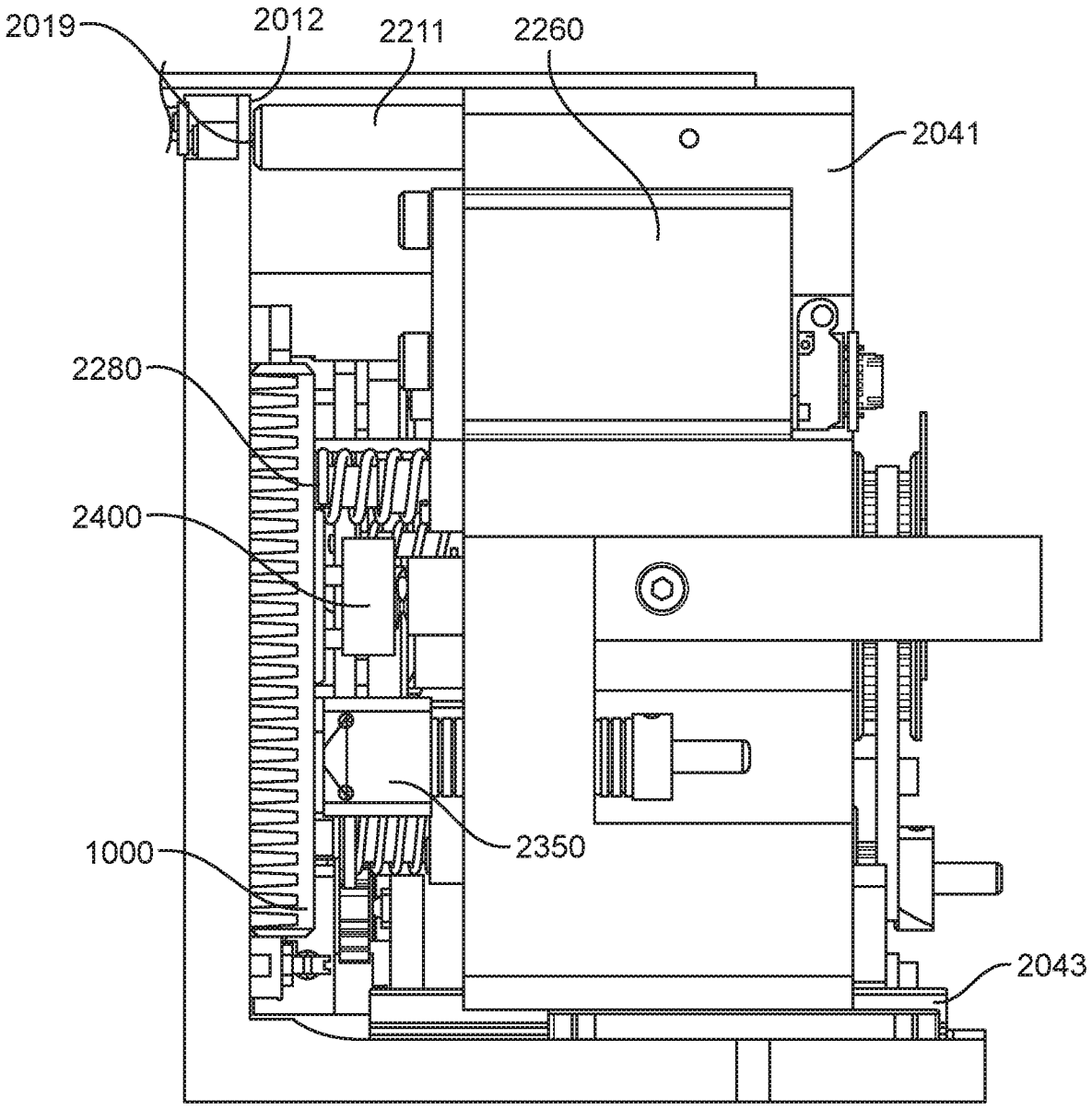


FIG. 16B

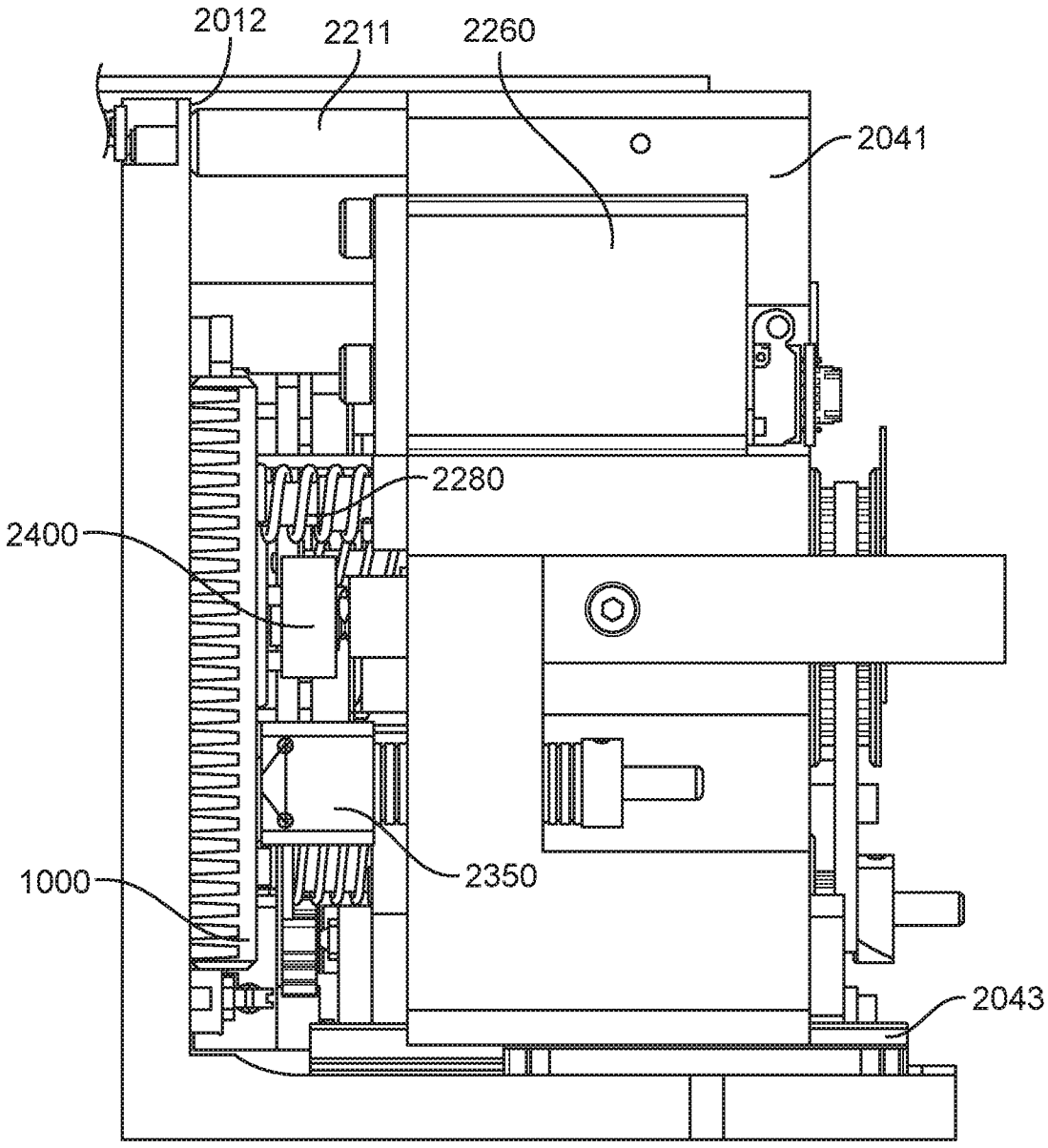


FIG. 16C

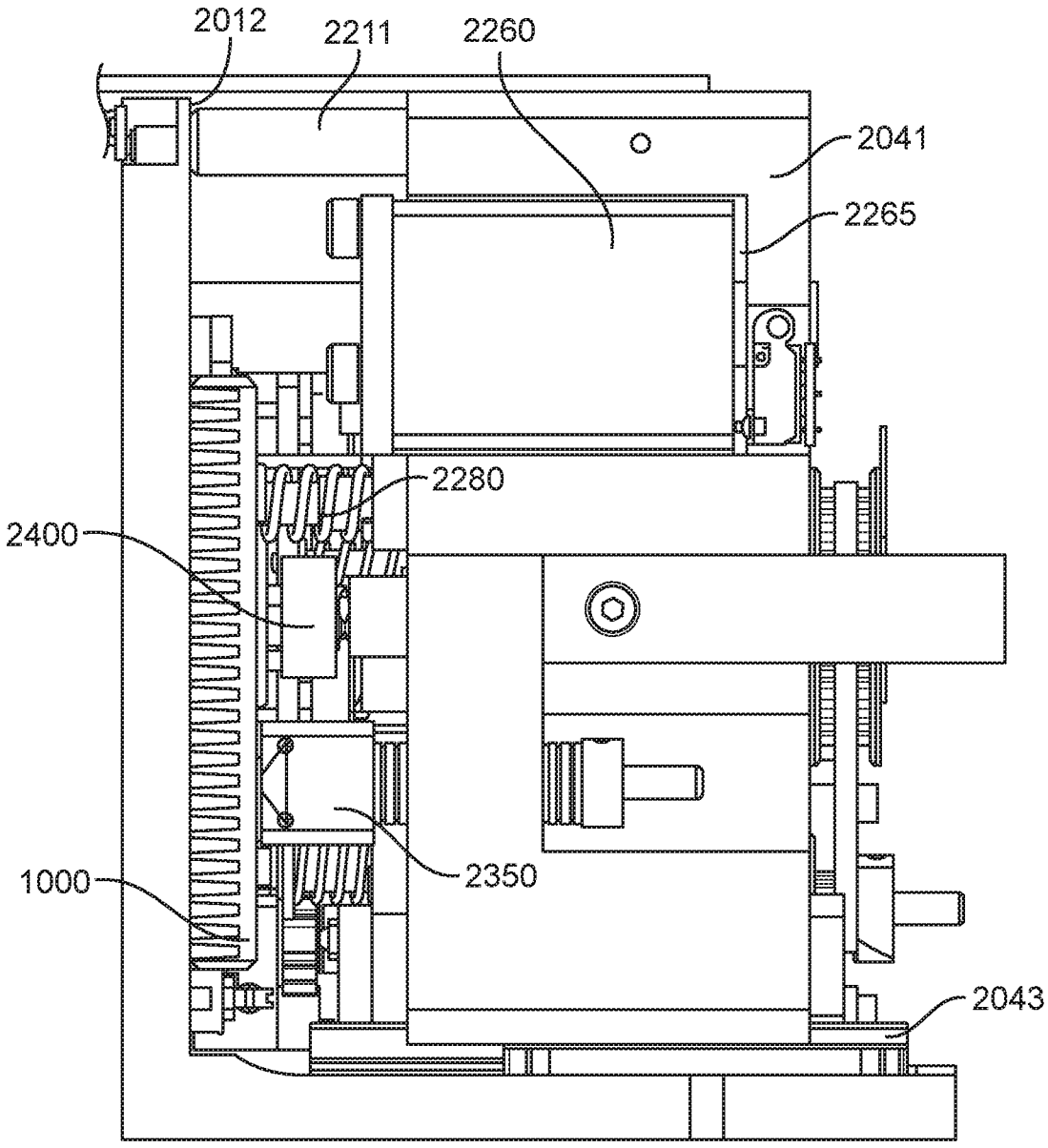


FIG. 16D

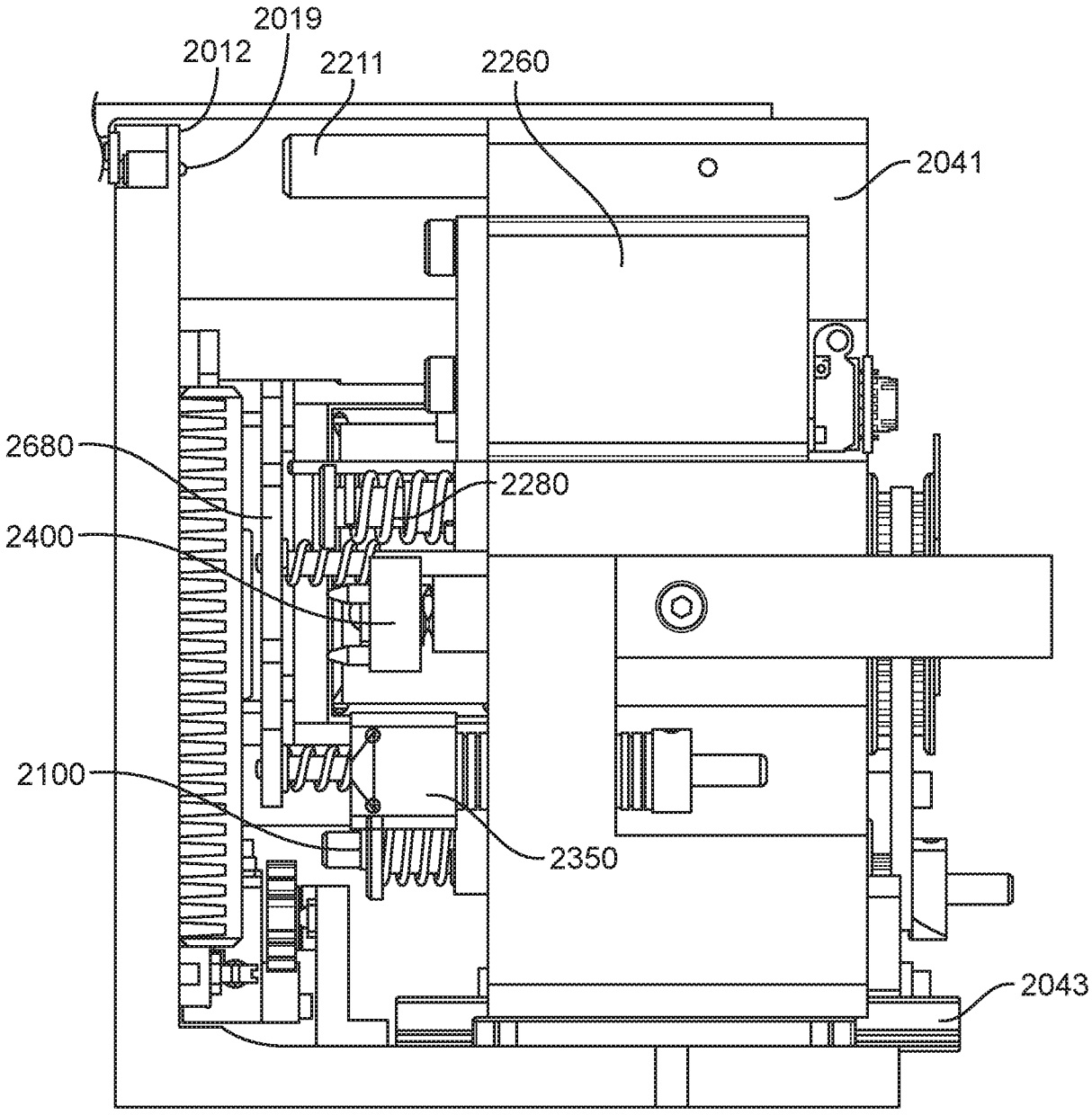


FIG. 16E

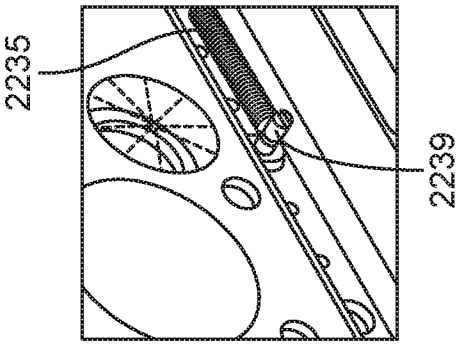


FIG. 17C

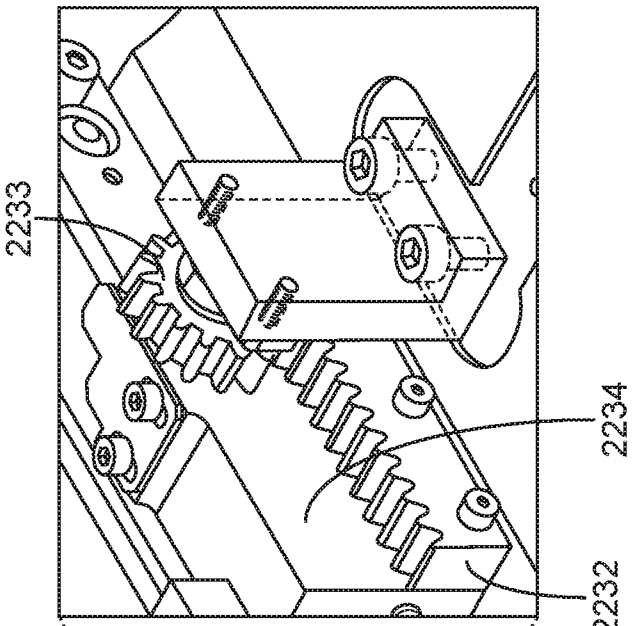


FIG. 17B

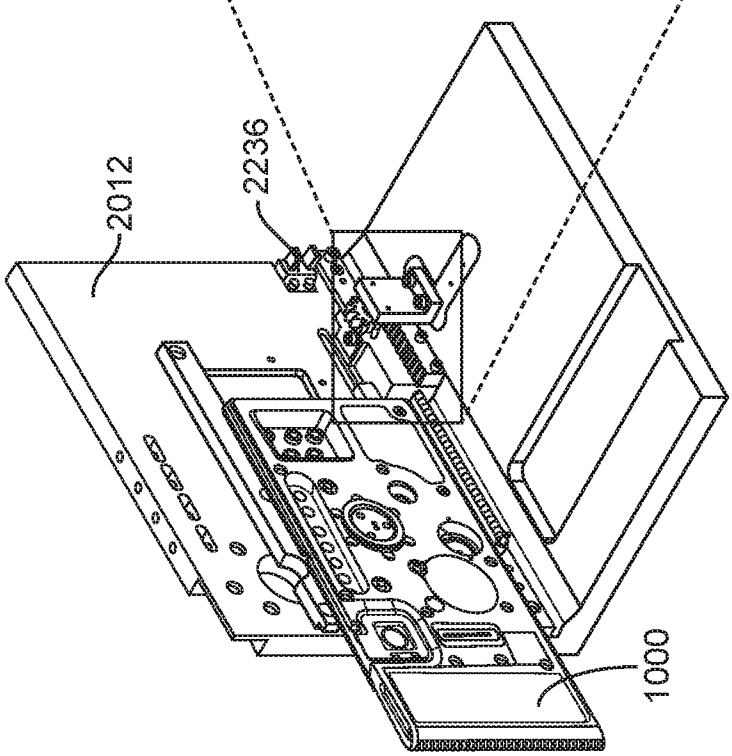


FIG. 17A

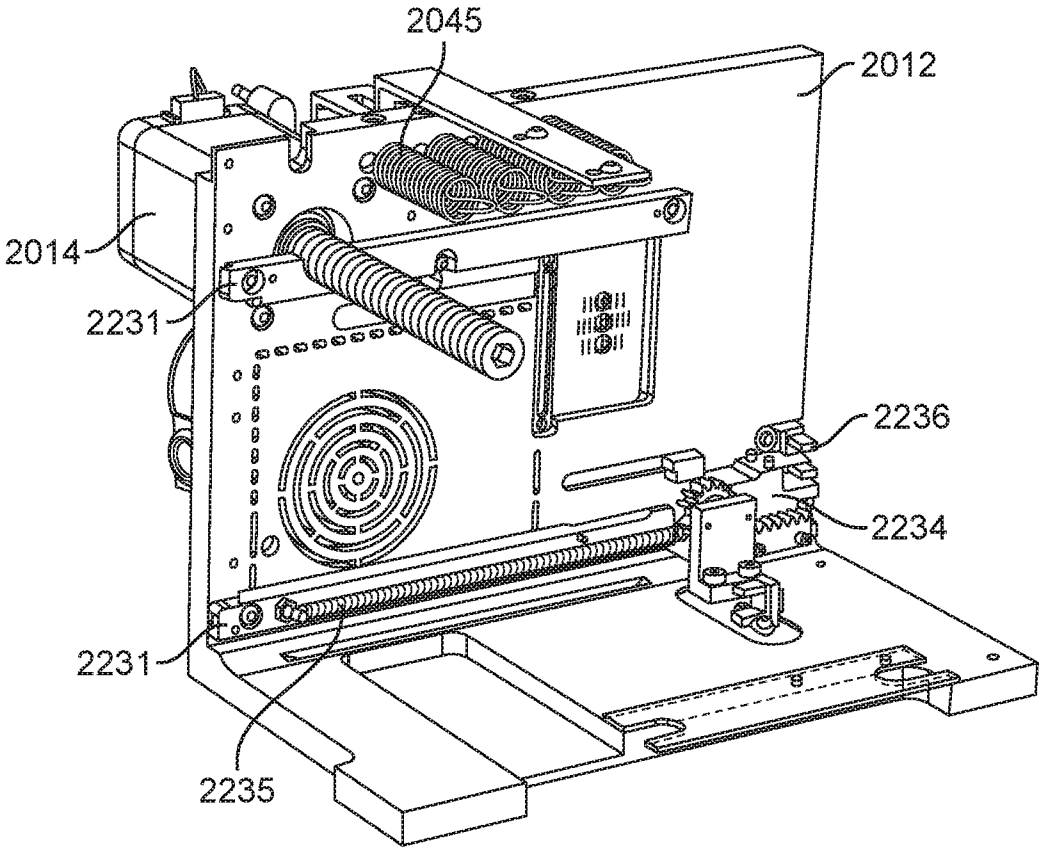


FIG. 18A

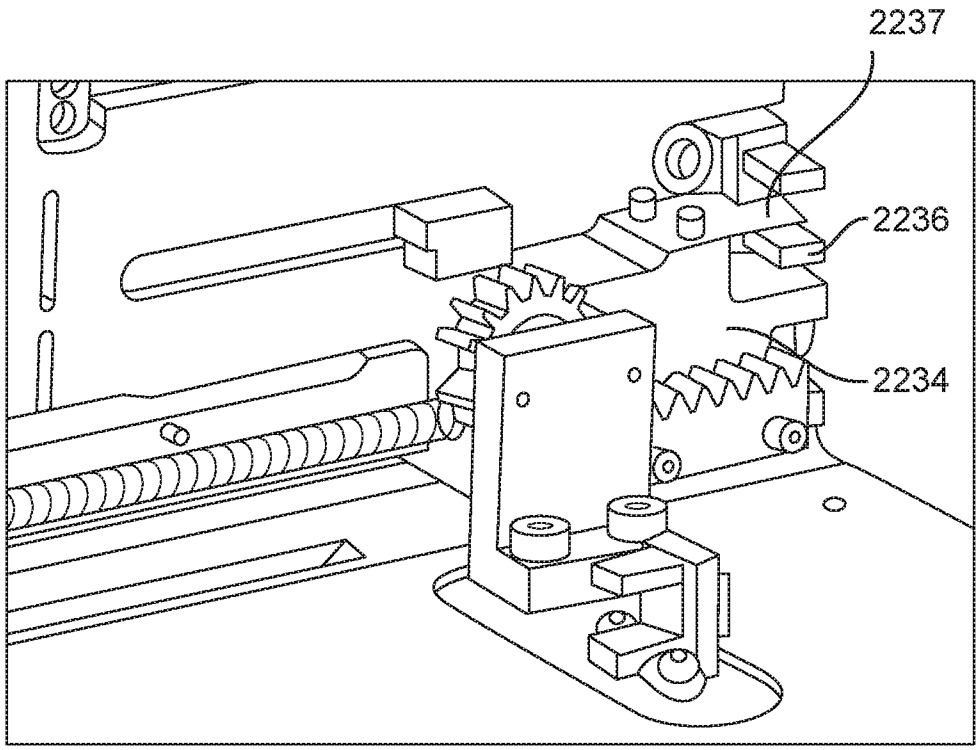


FIG. 18B

FIG. 19B

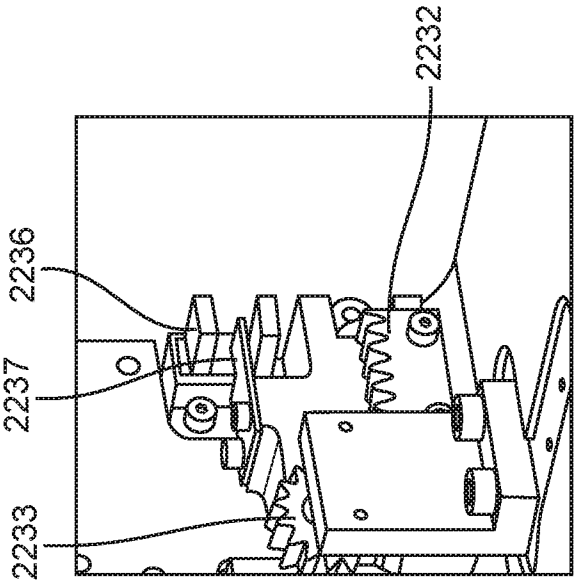
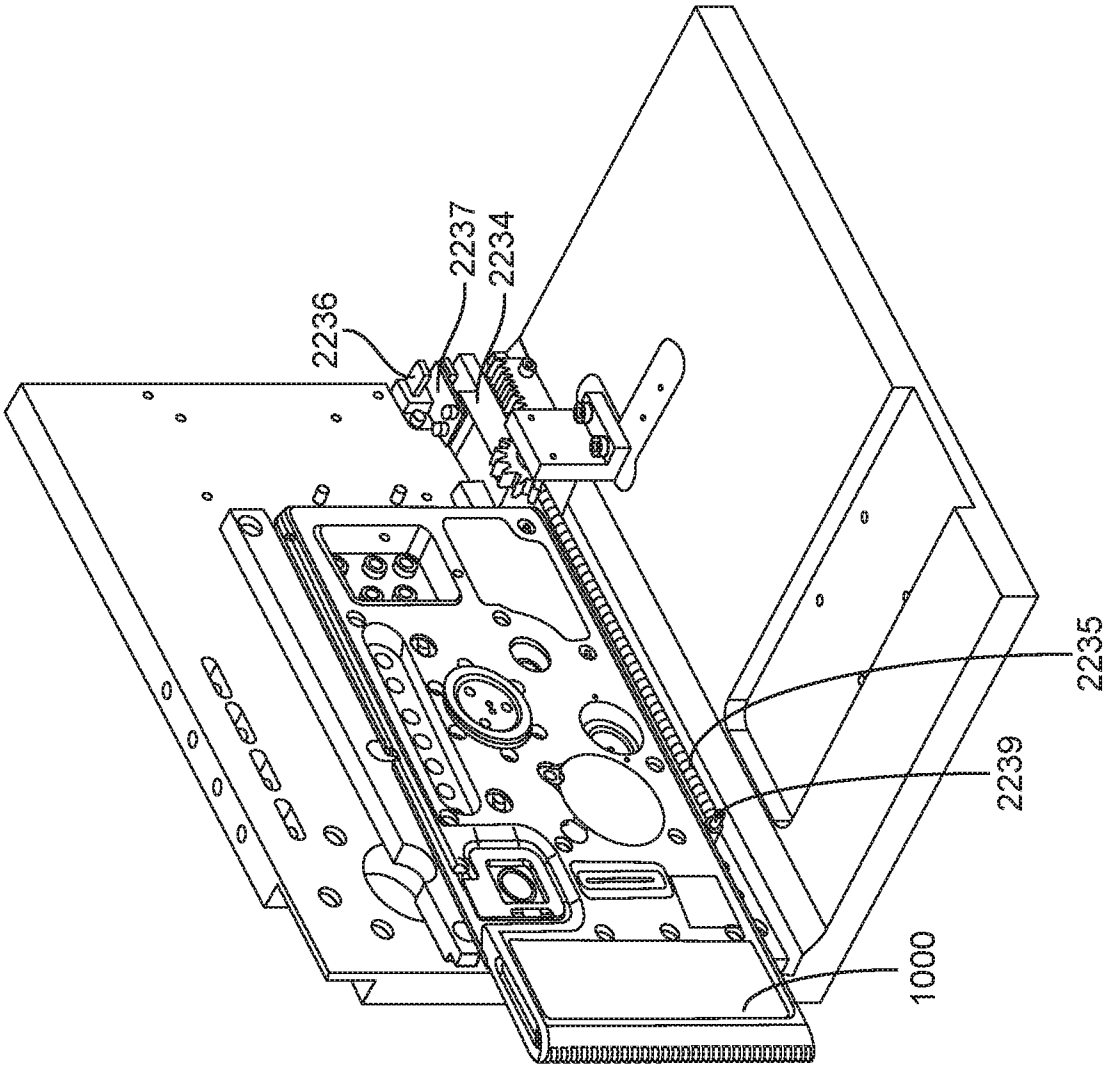


FIG. 19A



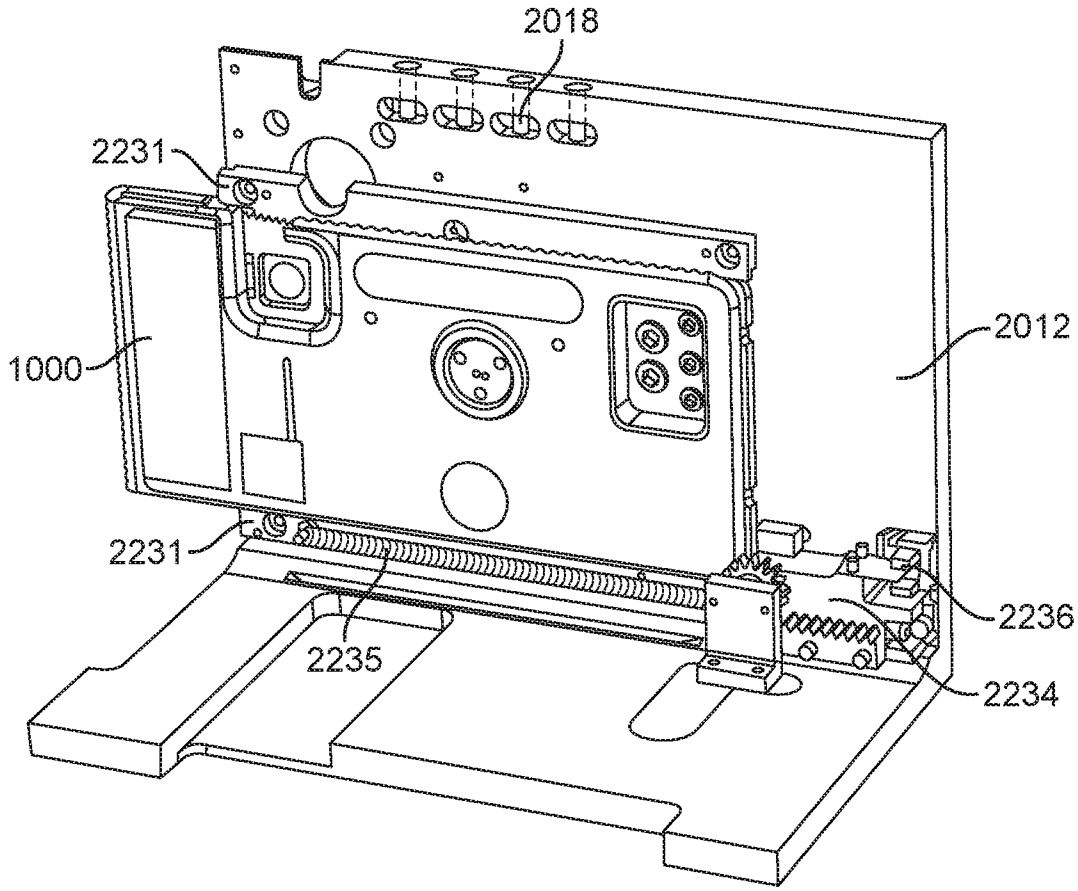


FIG. 19C

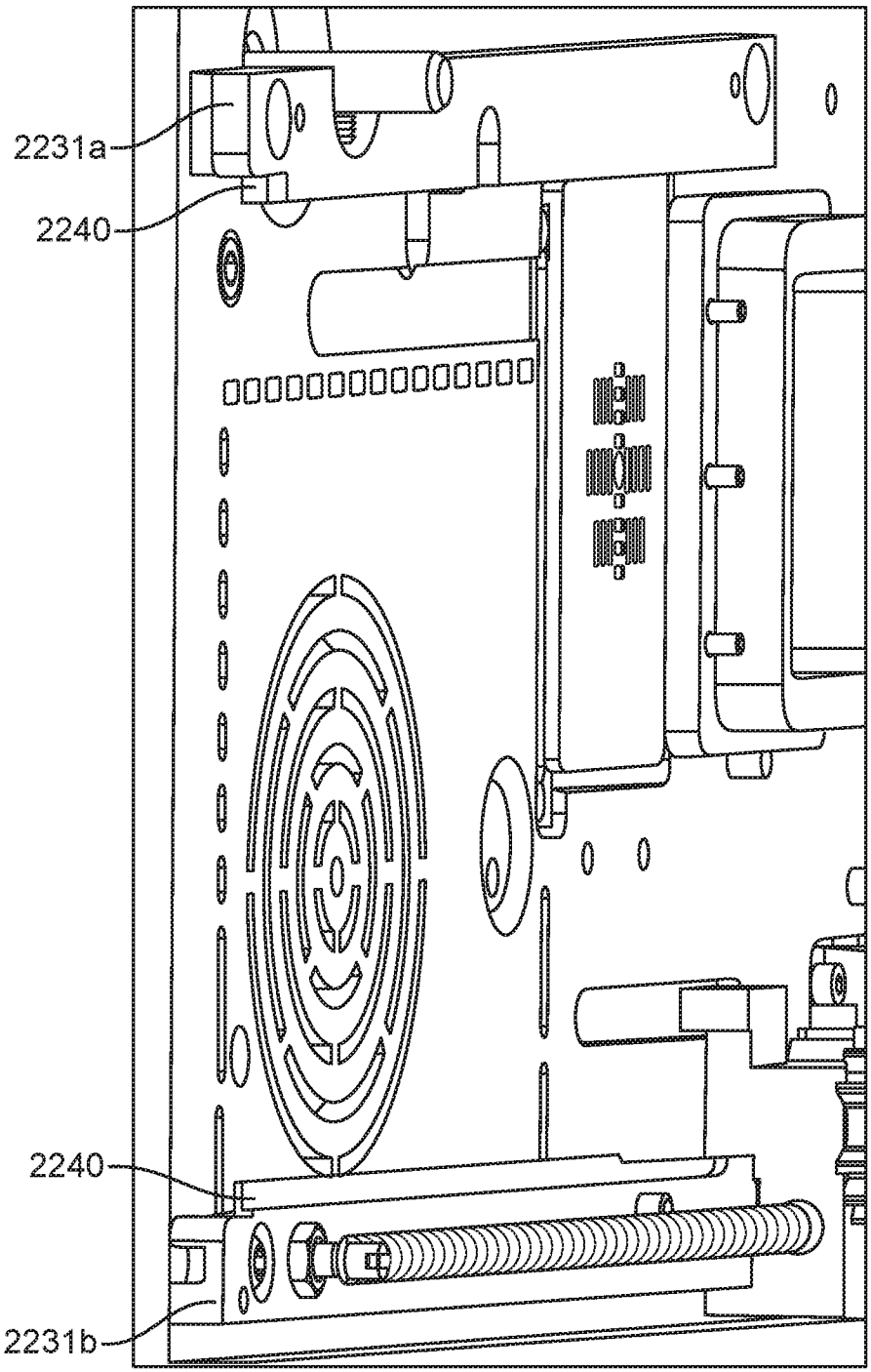


FIG. 20

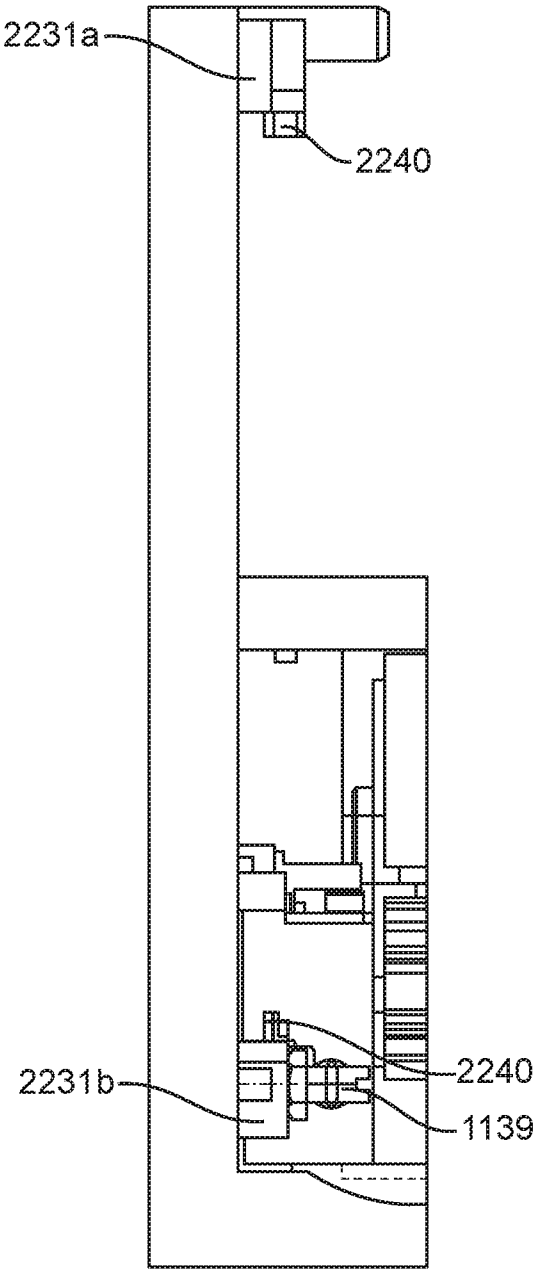


FIG. 21

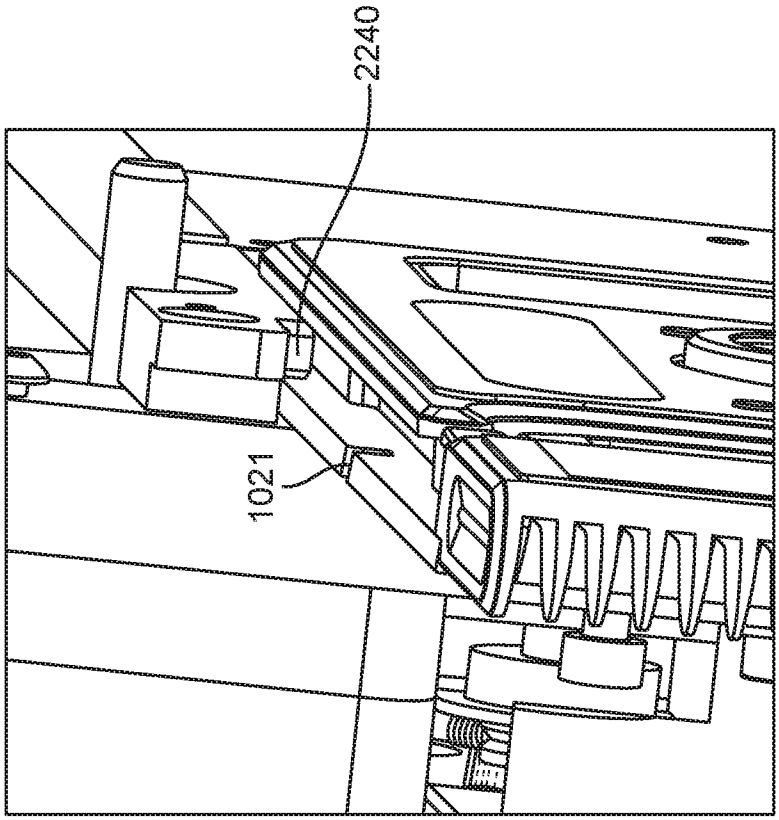


FIG. 22B

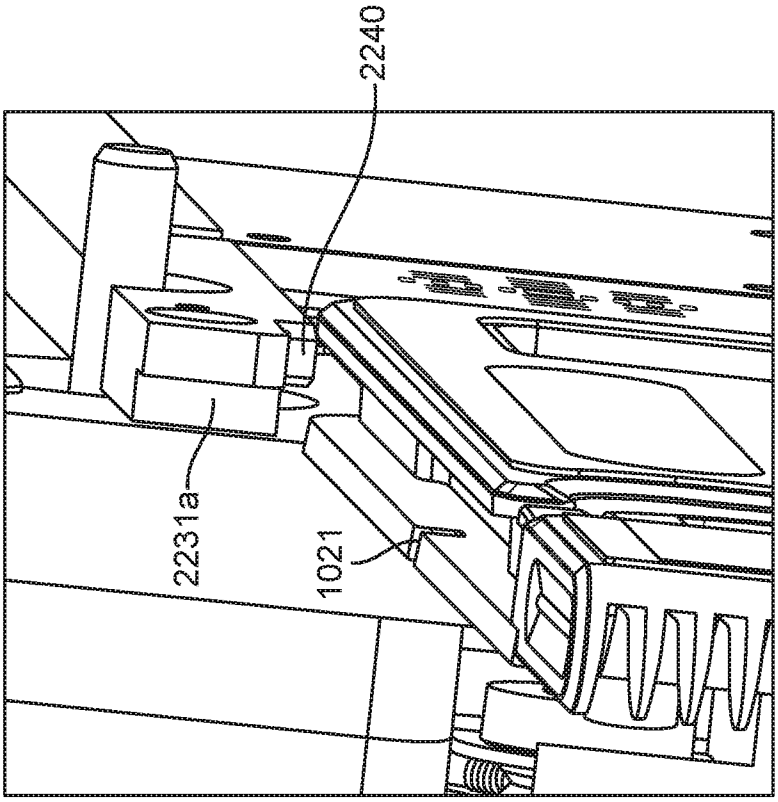


FIG. 22A

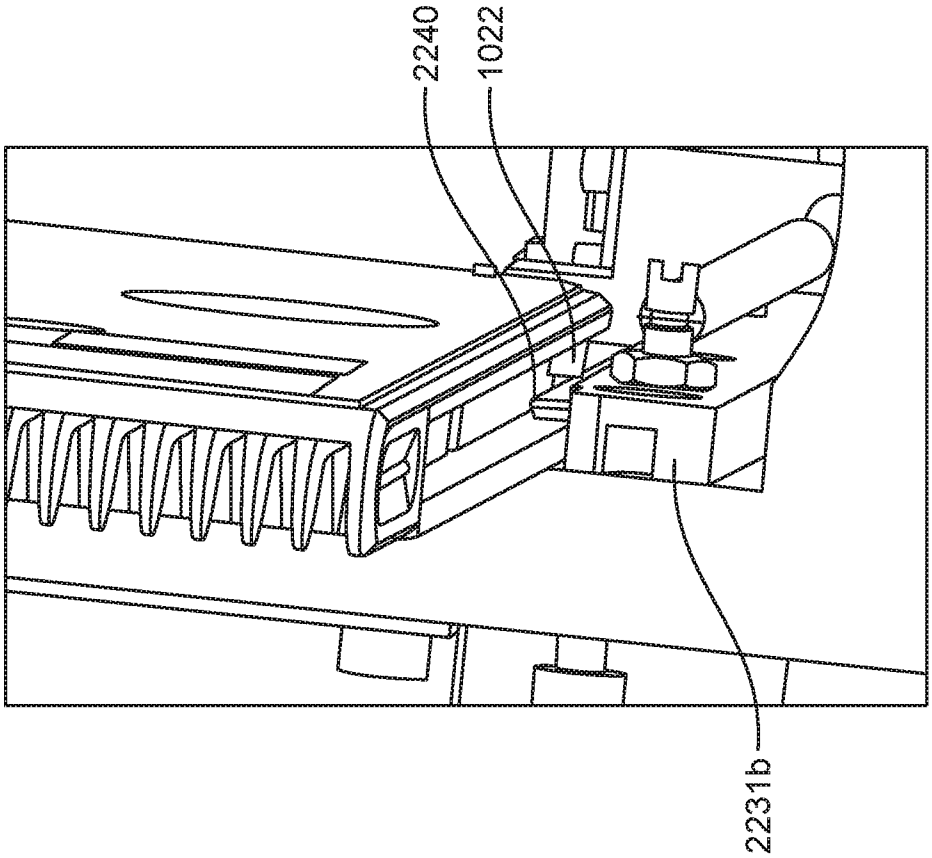


FIG. 23B

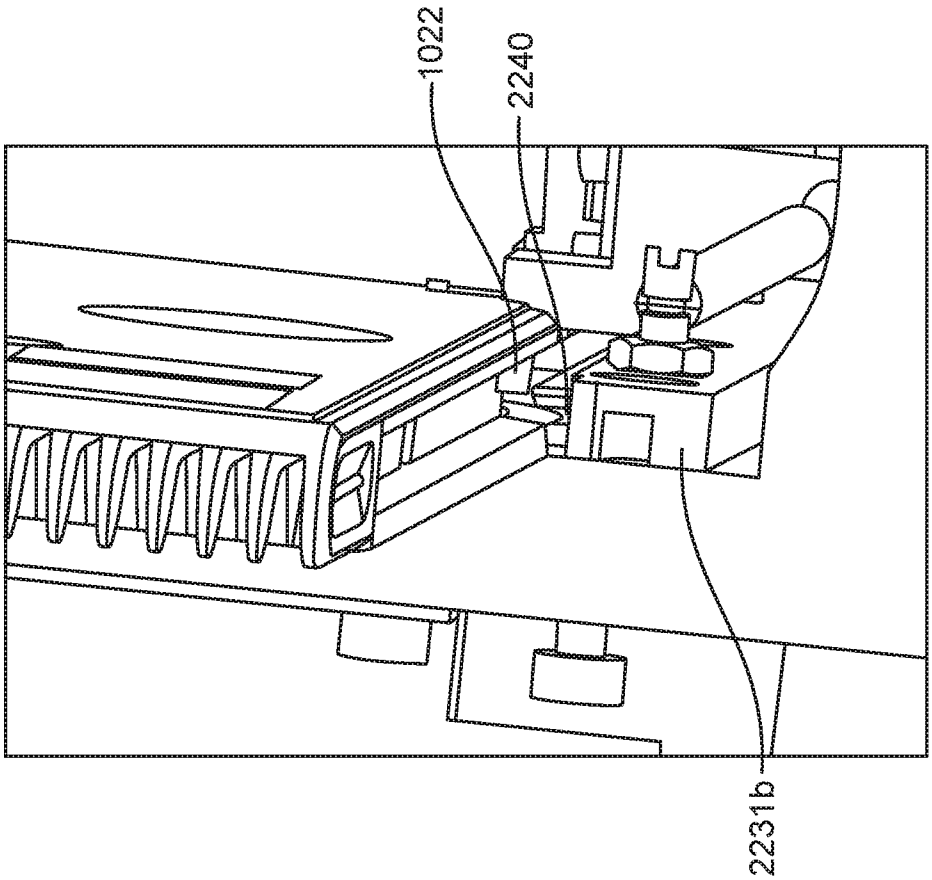
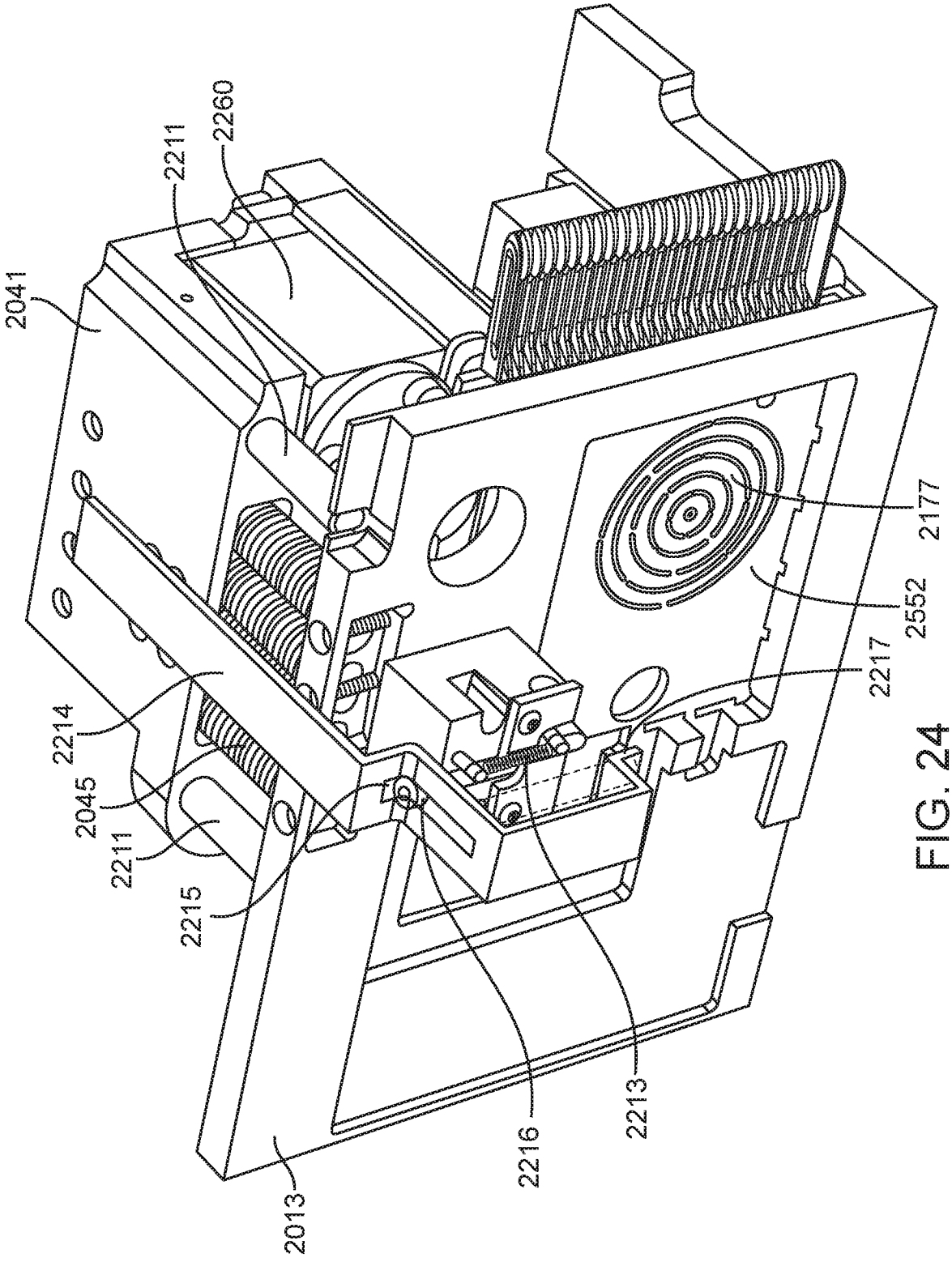


FIG. 23A


2041
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2260
2177
2552
2217
2214
2045
2211
2215
2216
2213
2013

FIG. 24

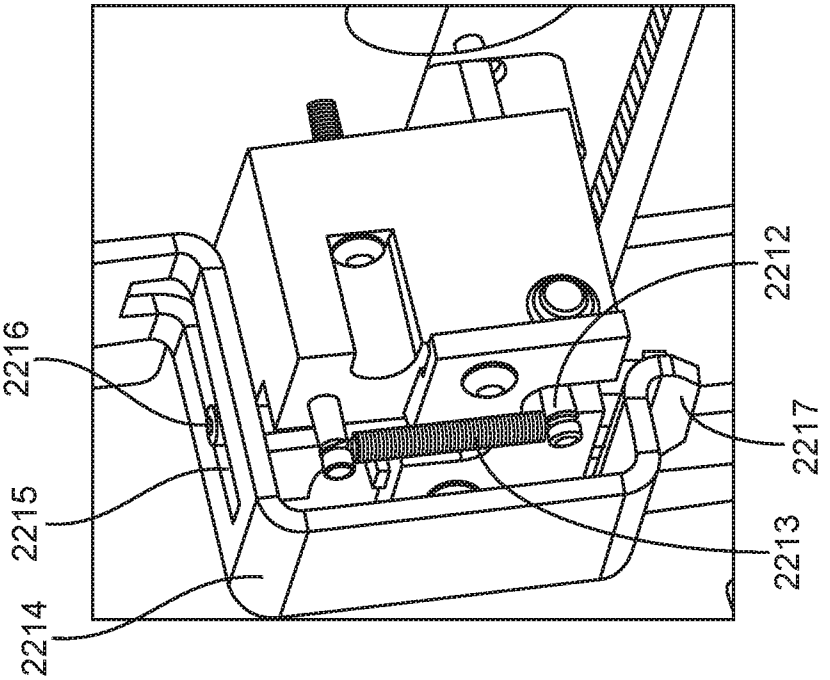


FIG. 25B

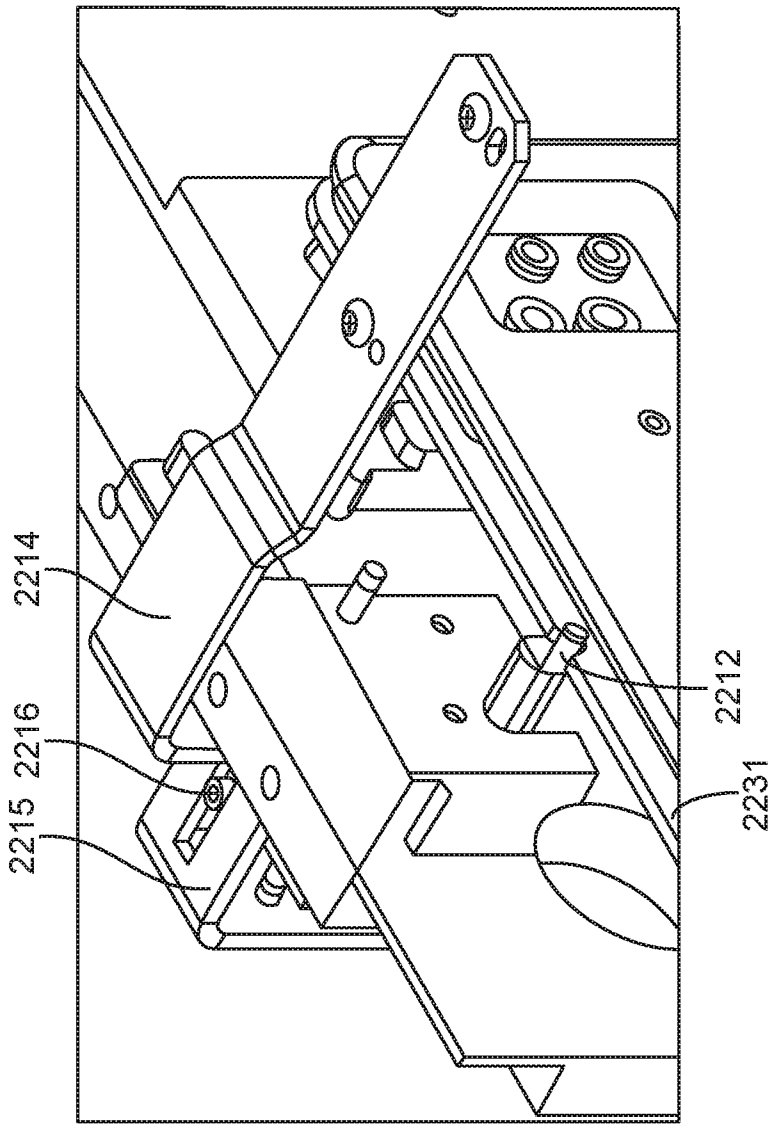


FIG. 25A

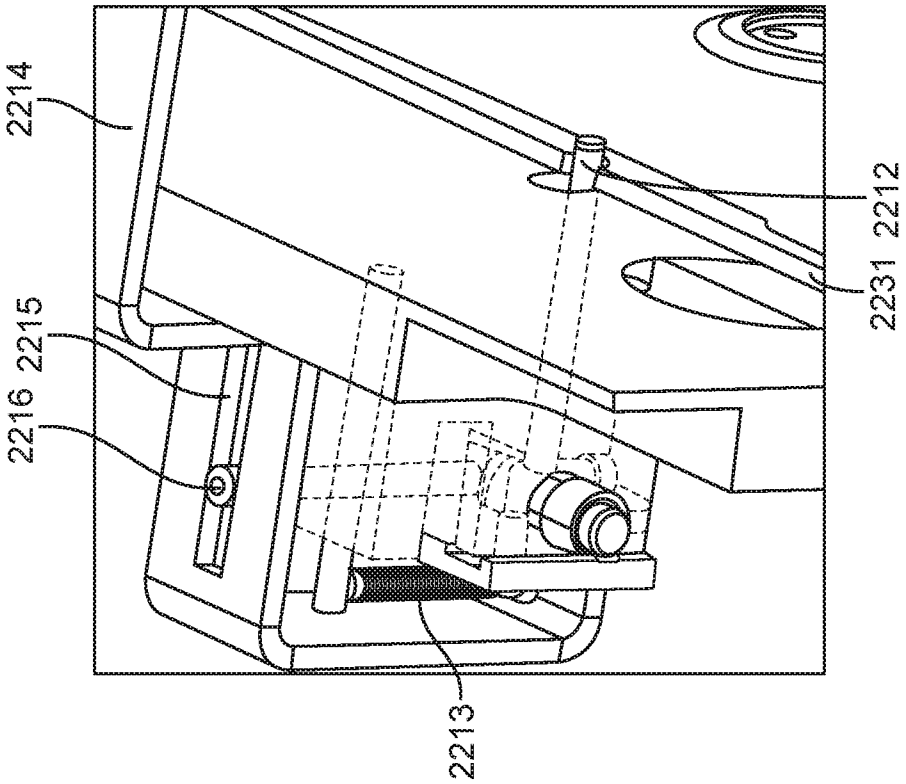
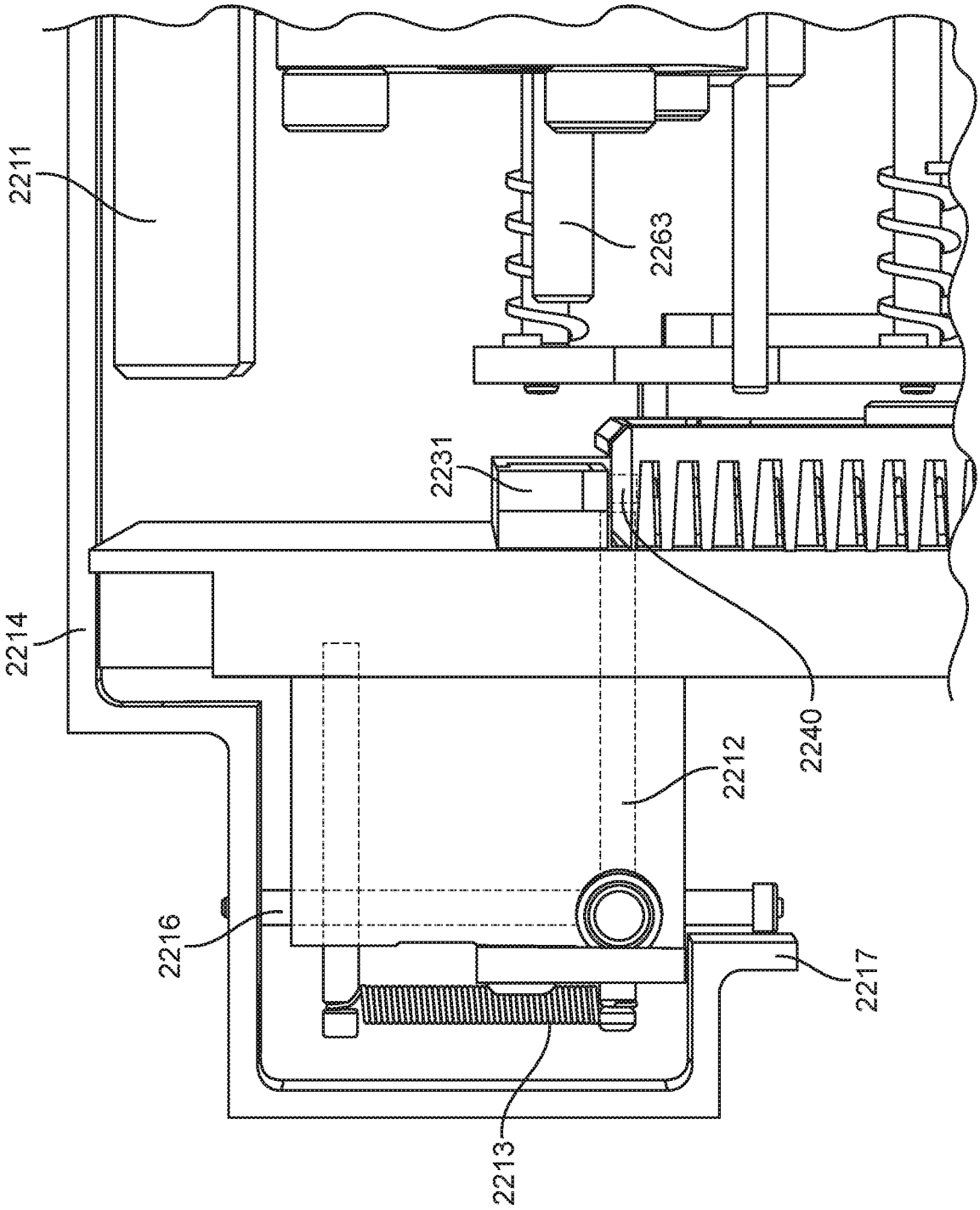


FIG. 25C

FIG. 25D



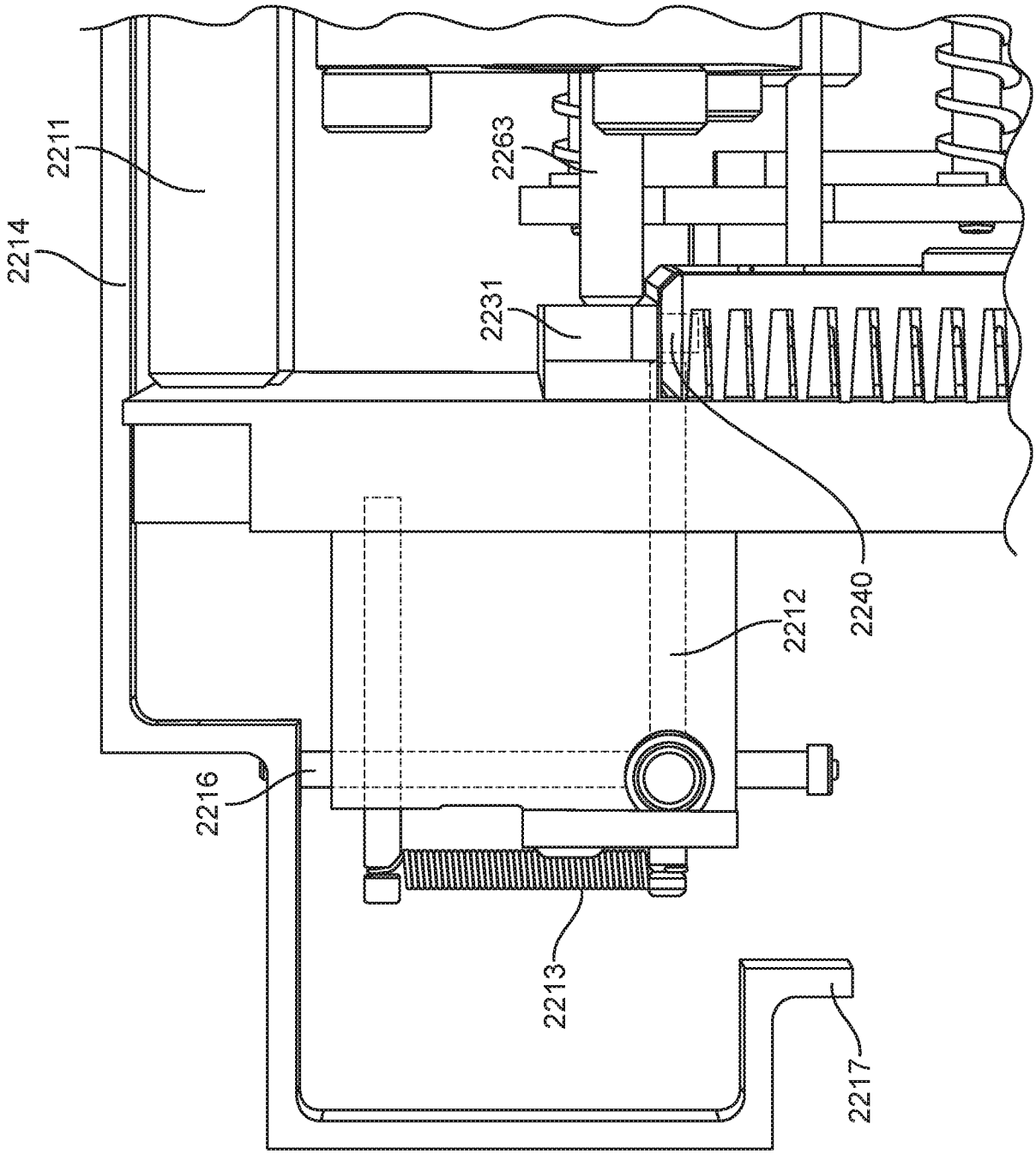


FIG. 26A

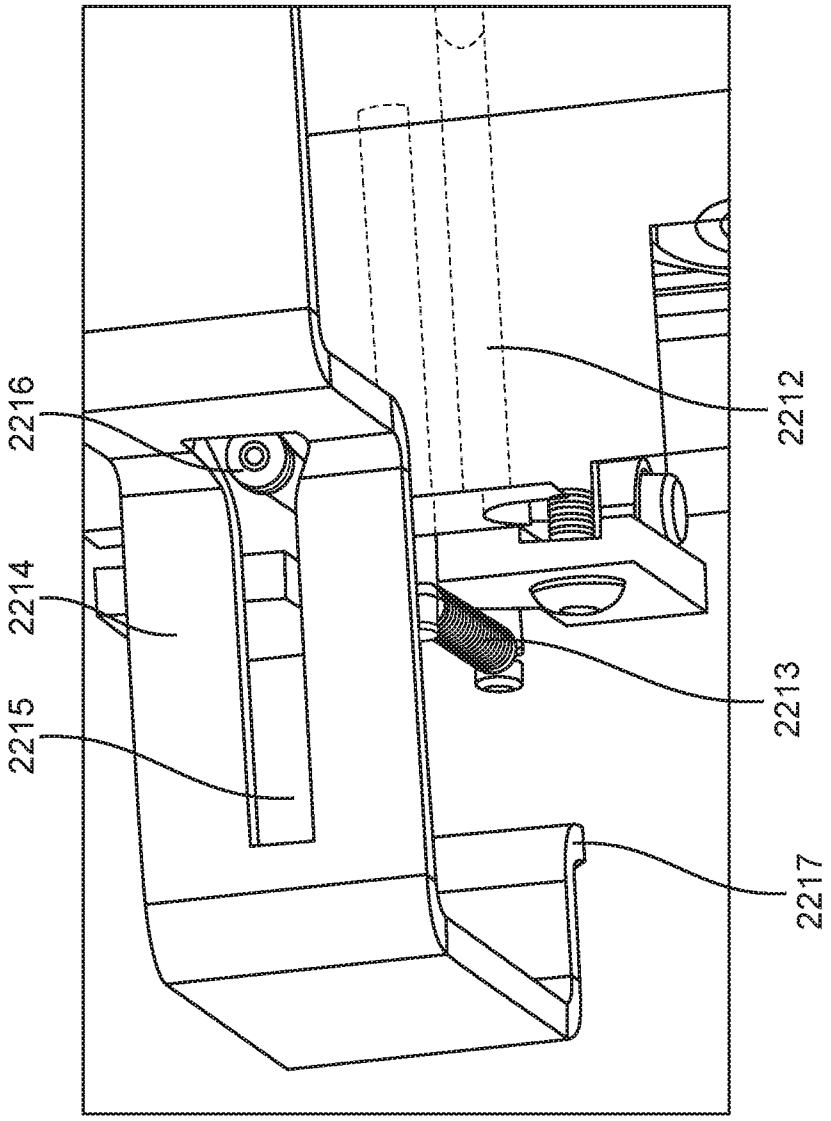


FIG. 26B

FIG. 27

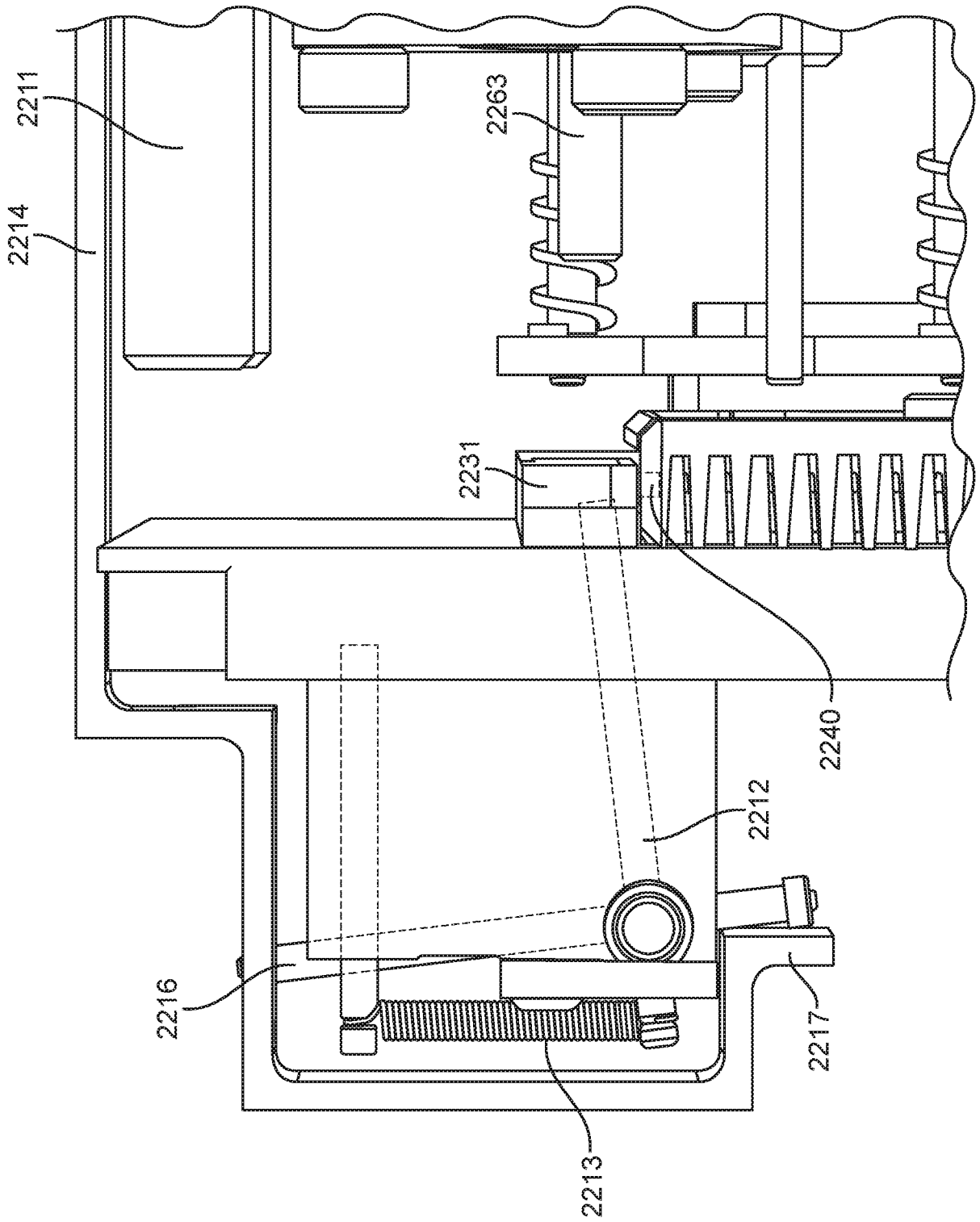
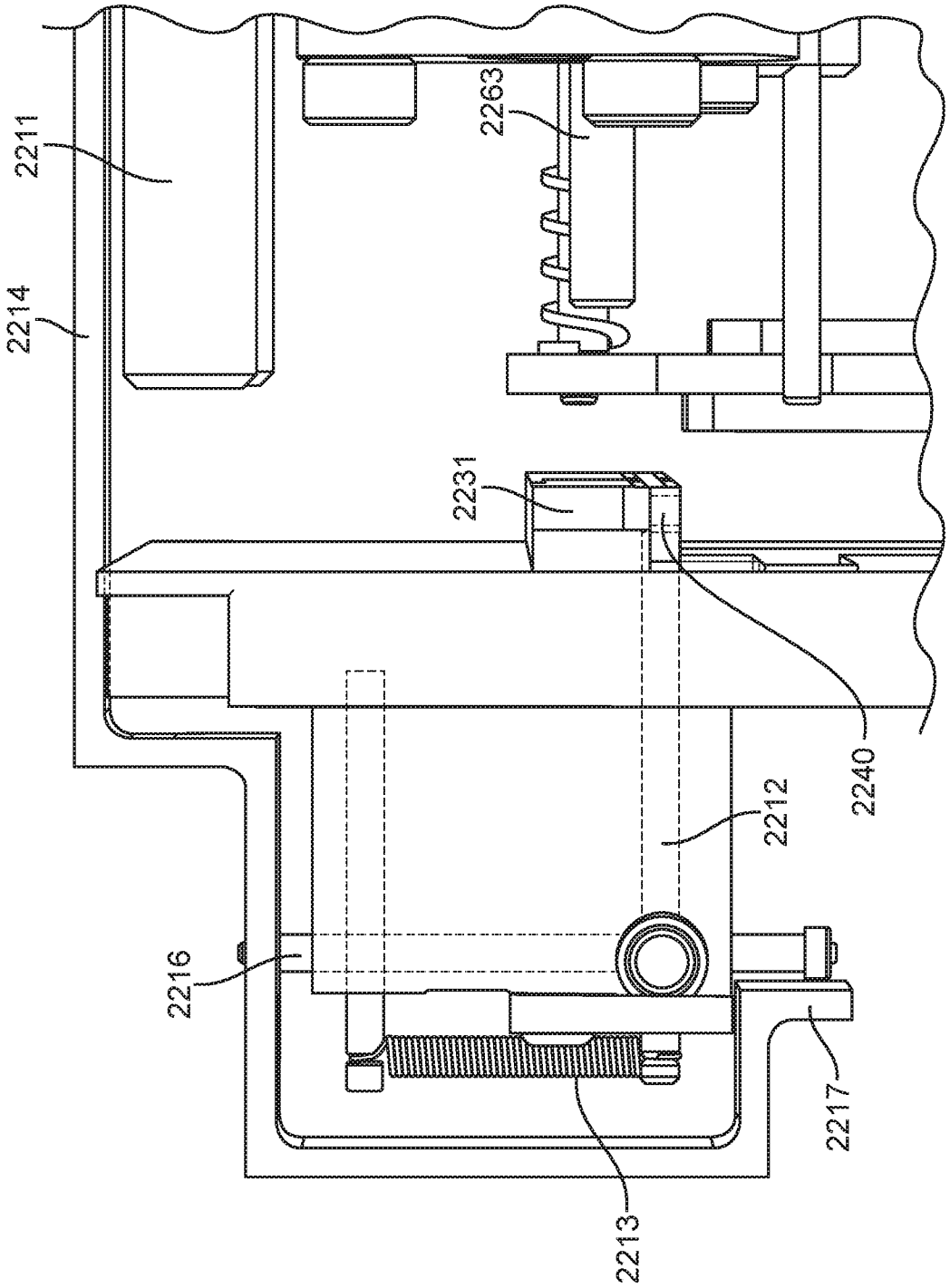


FIG. 28



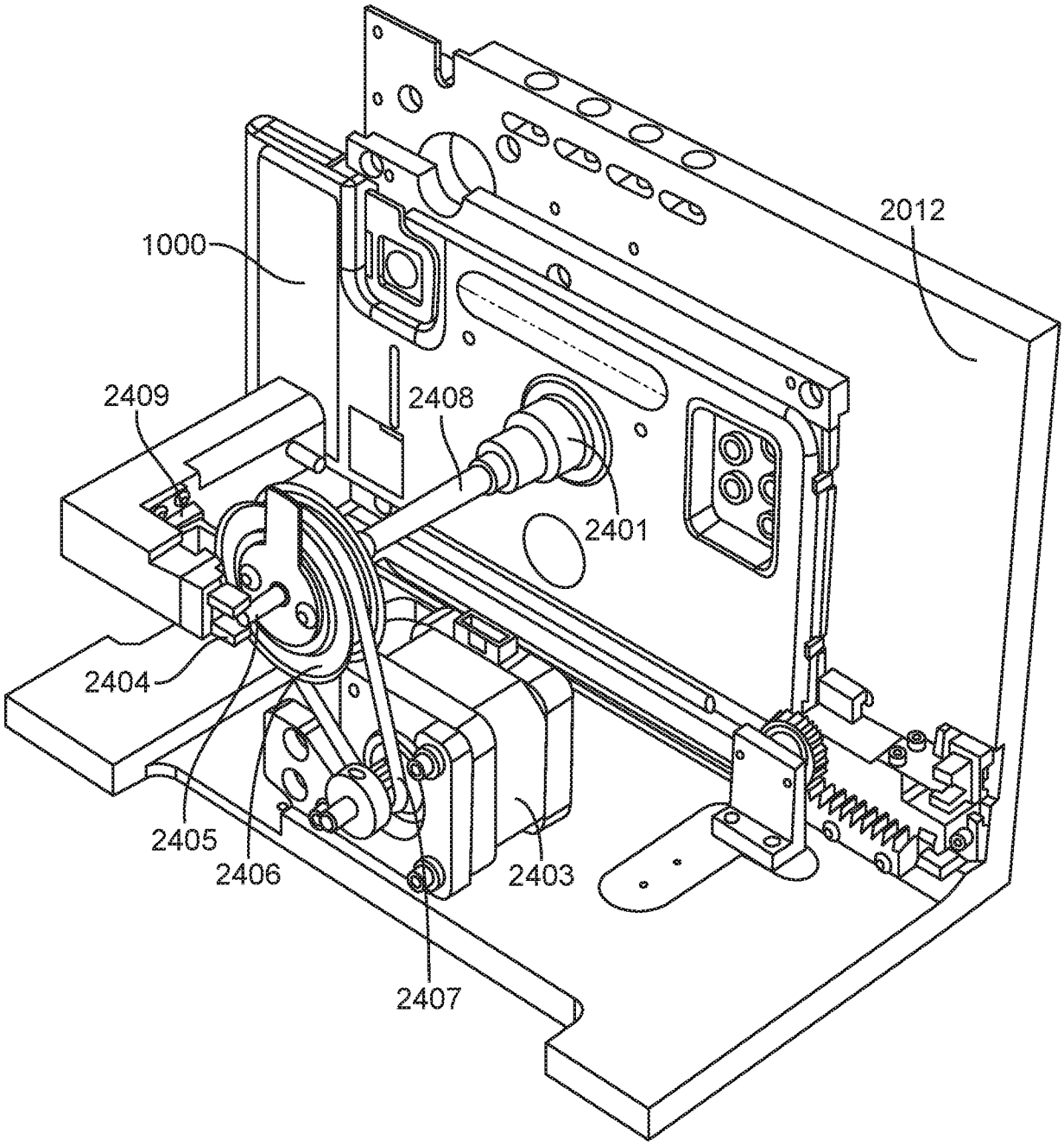


FIG. 29

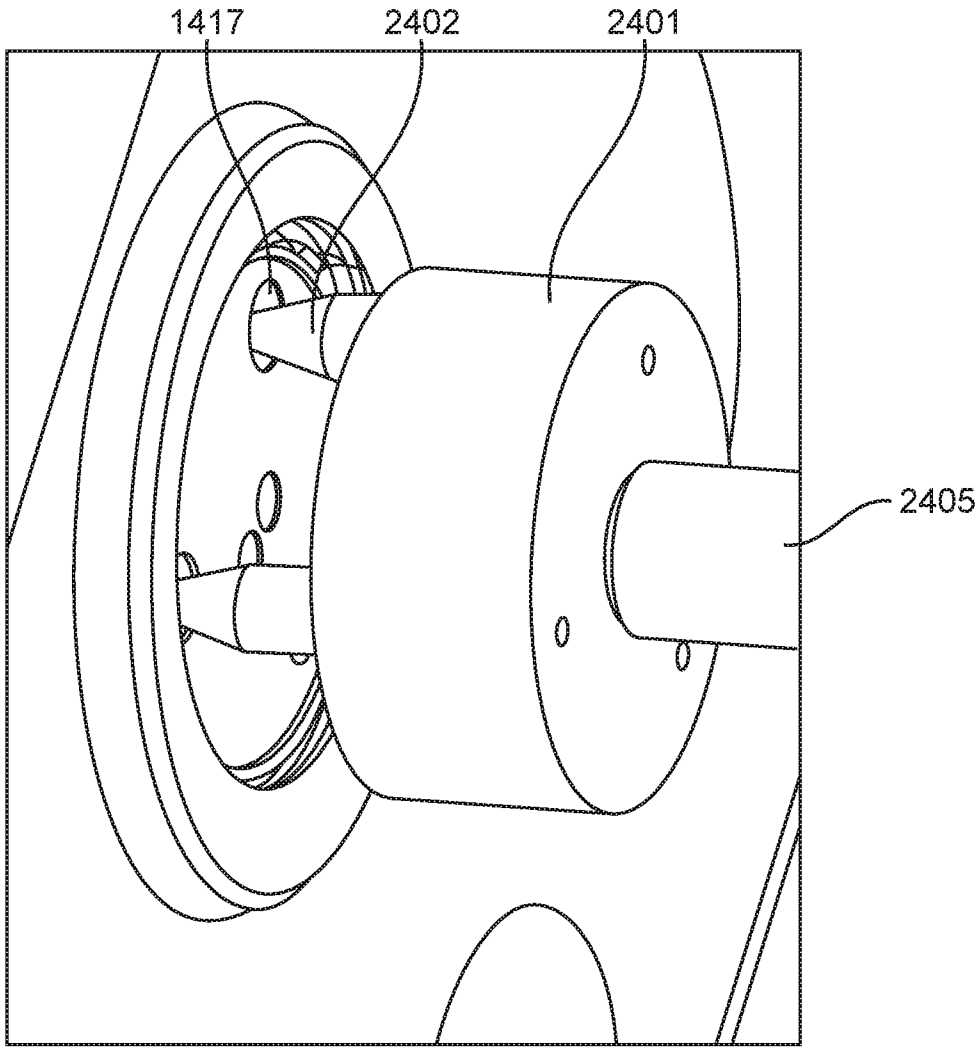
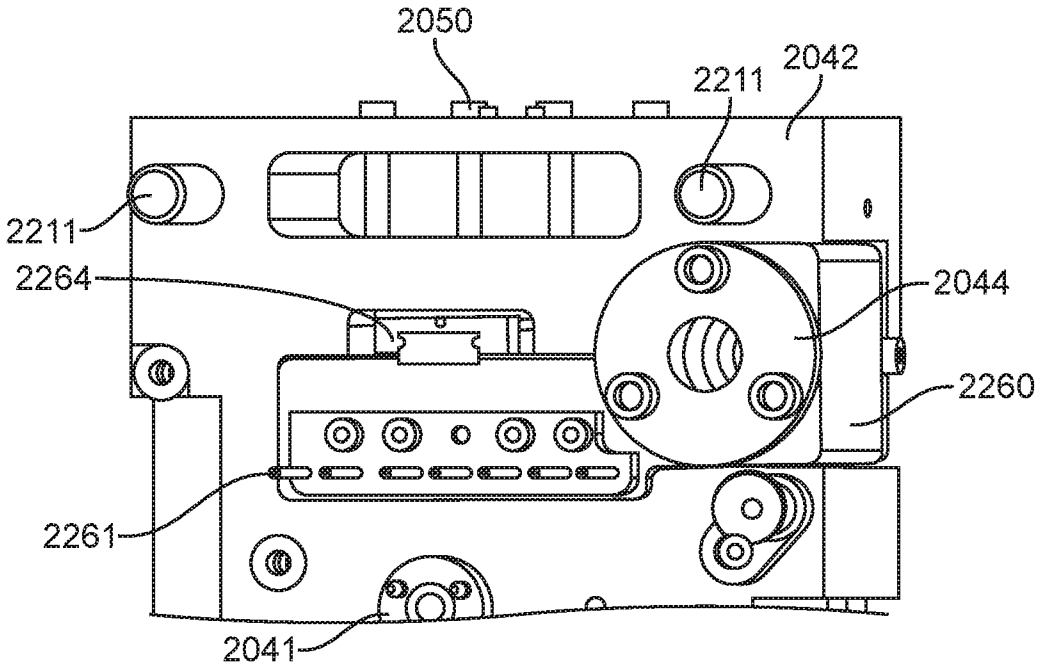


FIG. 30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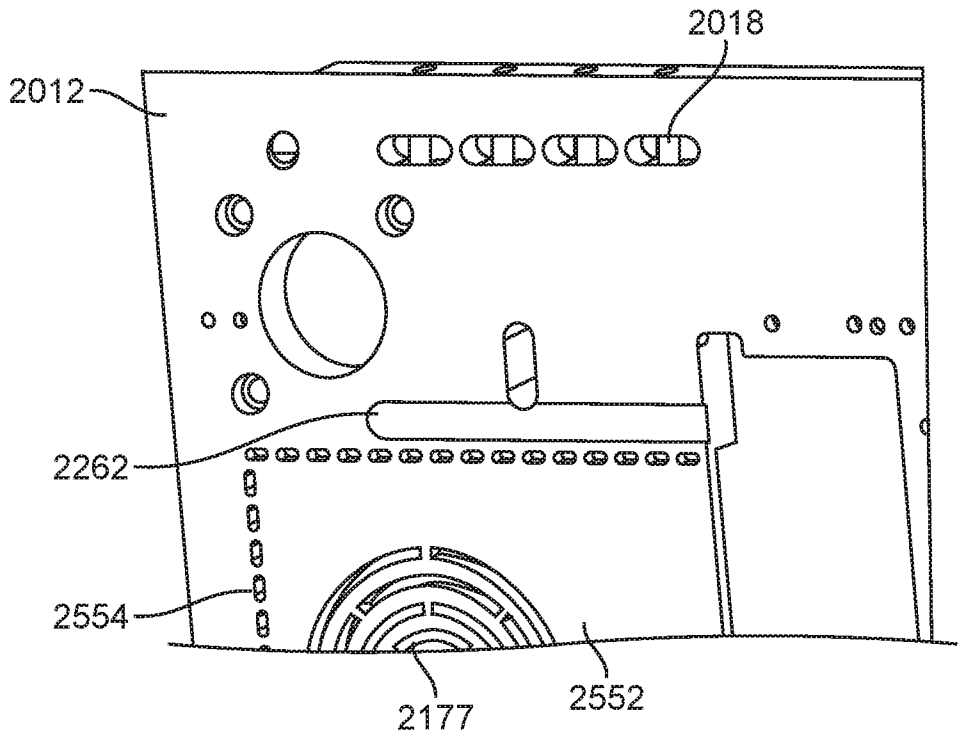
2264
2044
2260
2261
2041

FIG. 31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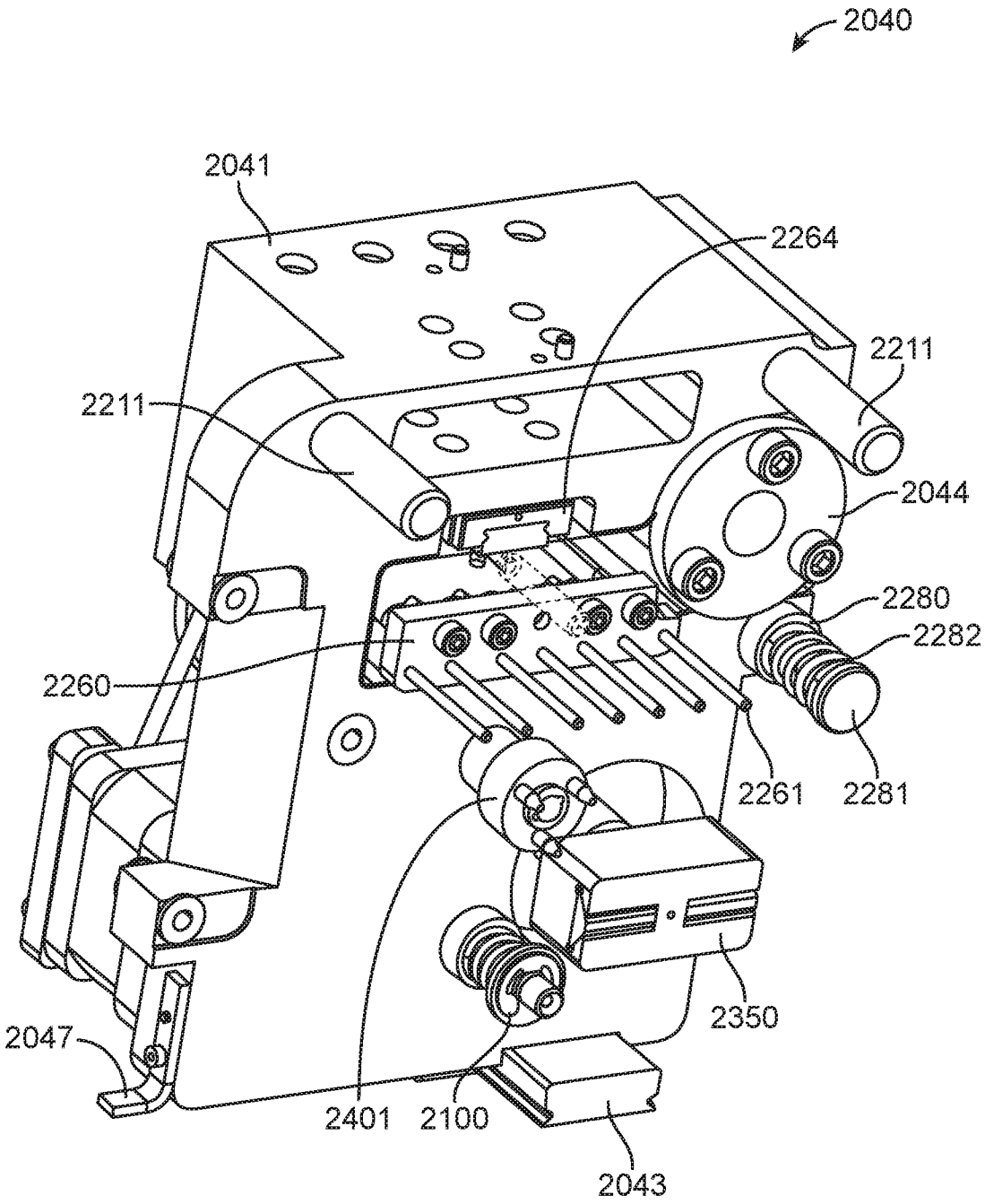
2012
2262
2554
2177
2552

FIG. 32


2040
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2261
2281
2350
2047
2401
2100
2043

FIG. 33

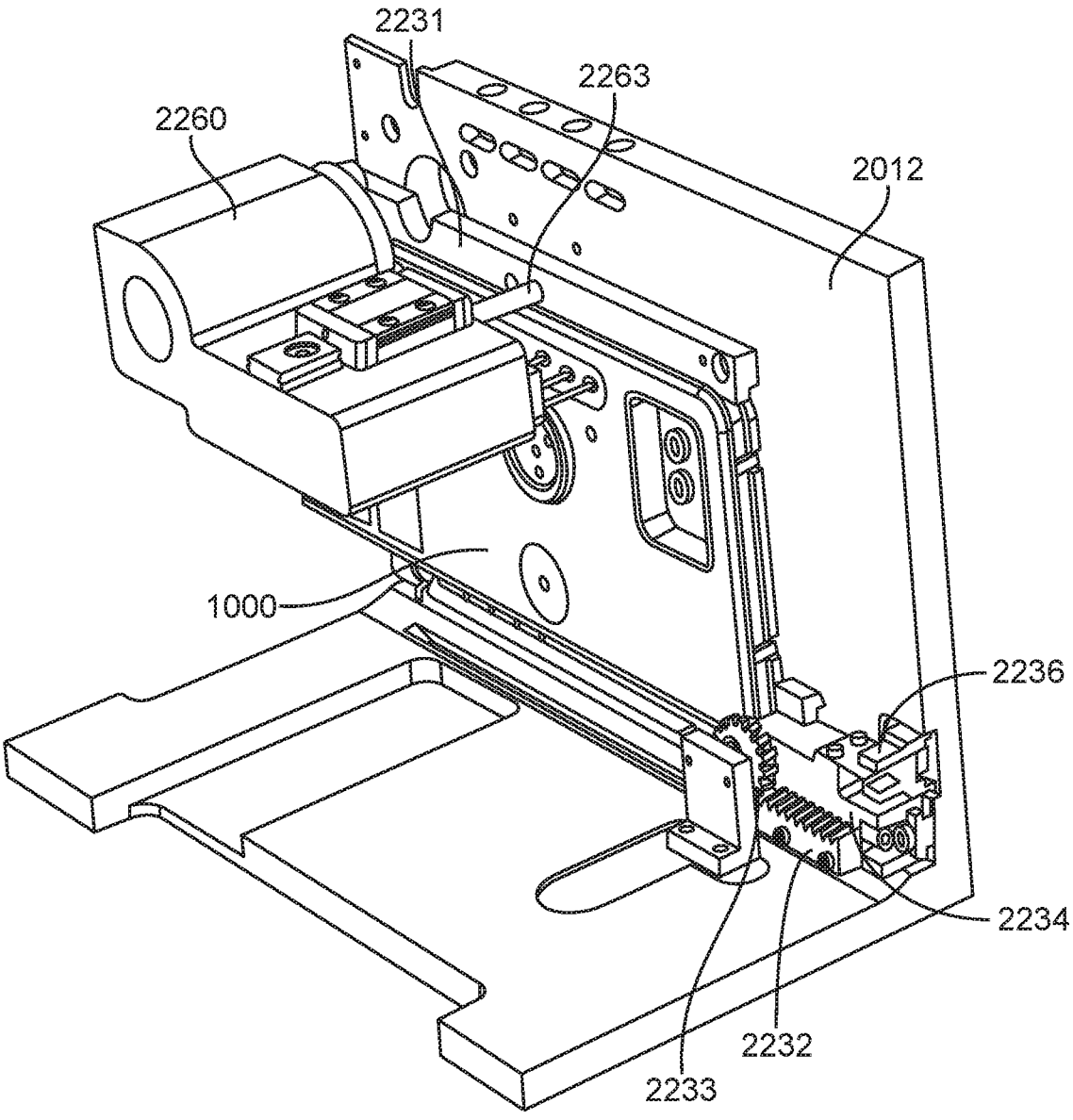


FIG. 34

FIG. 35

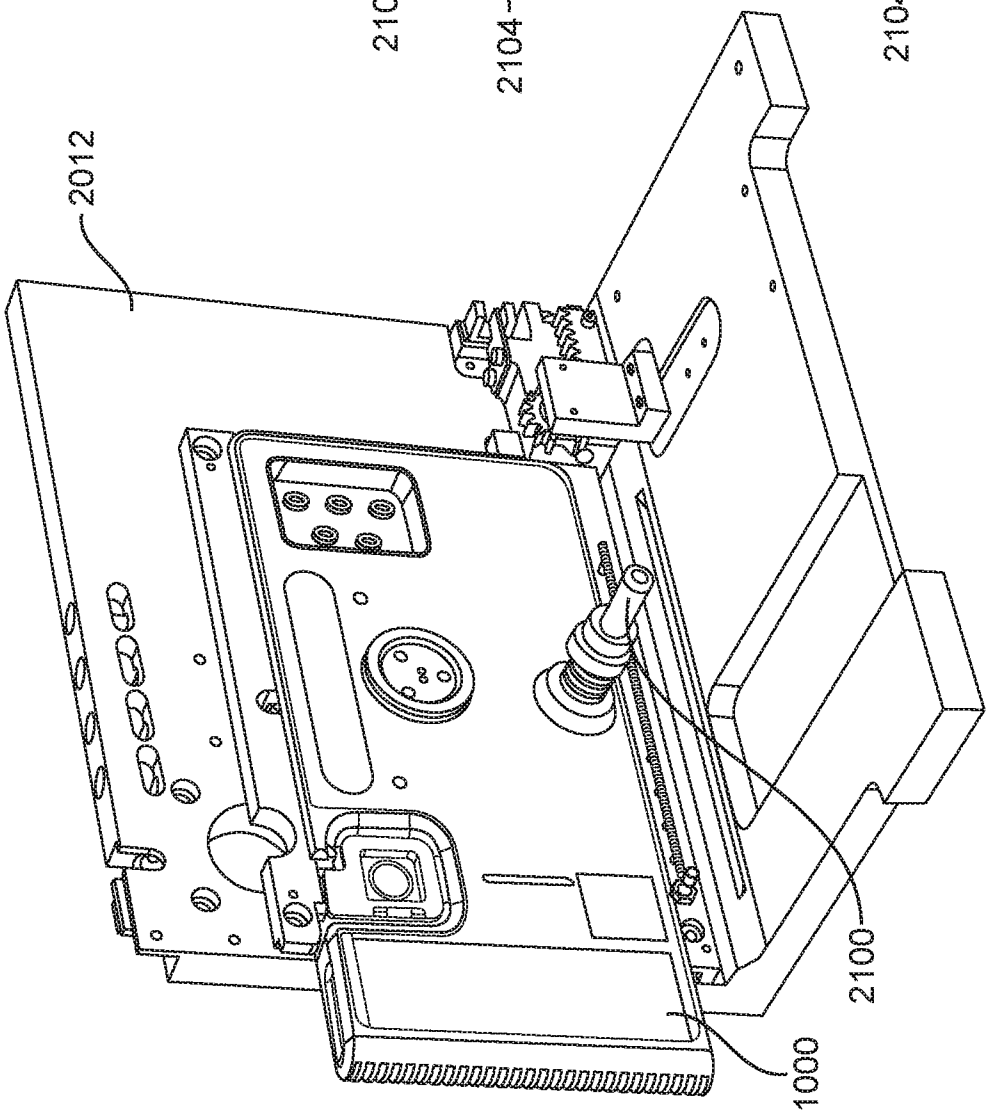


FIG. 36A

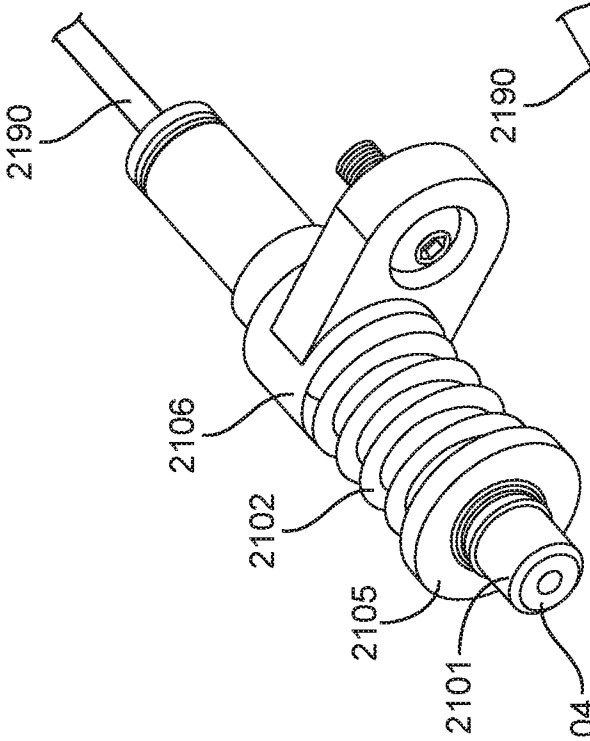
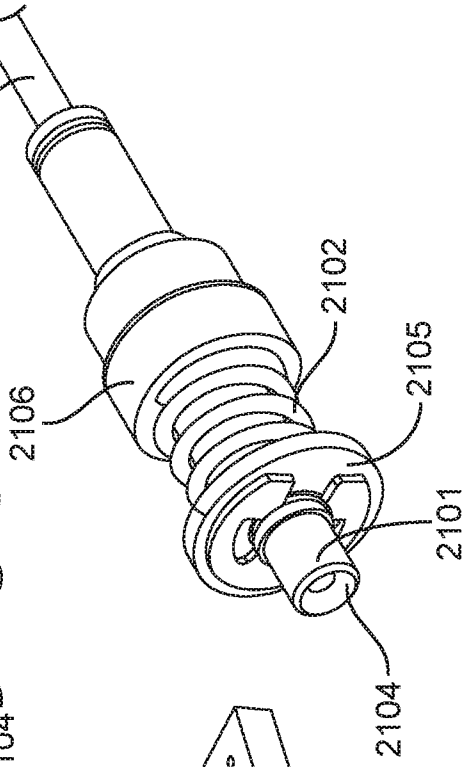


FIG. 37A



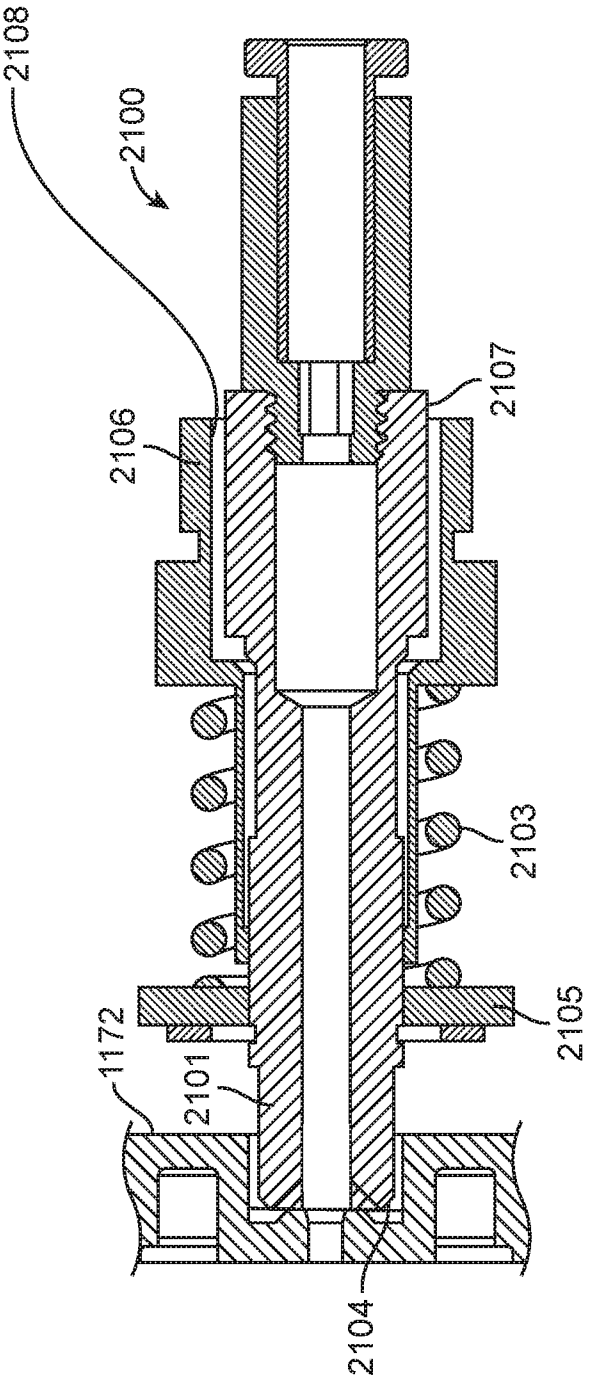


FIG. 36B

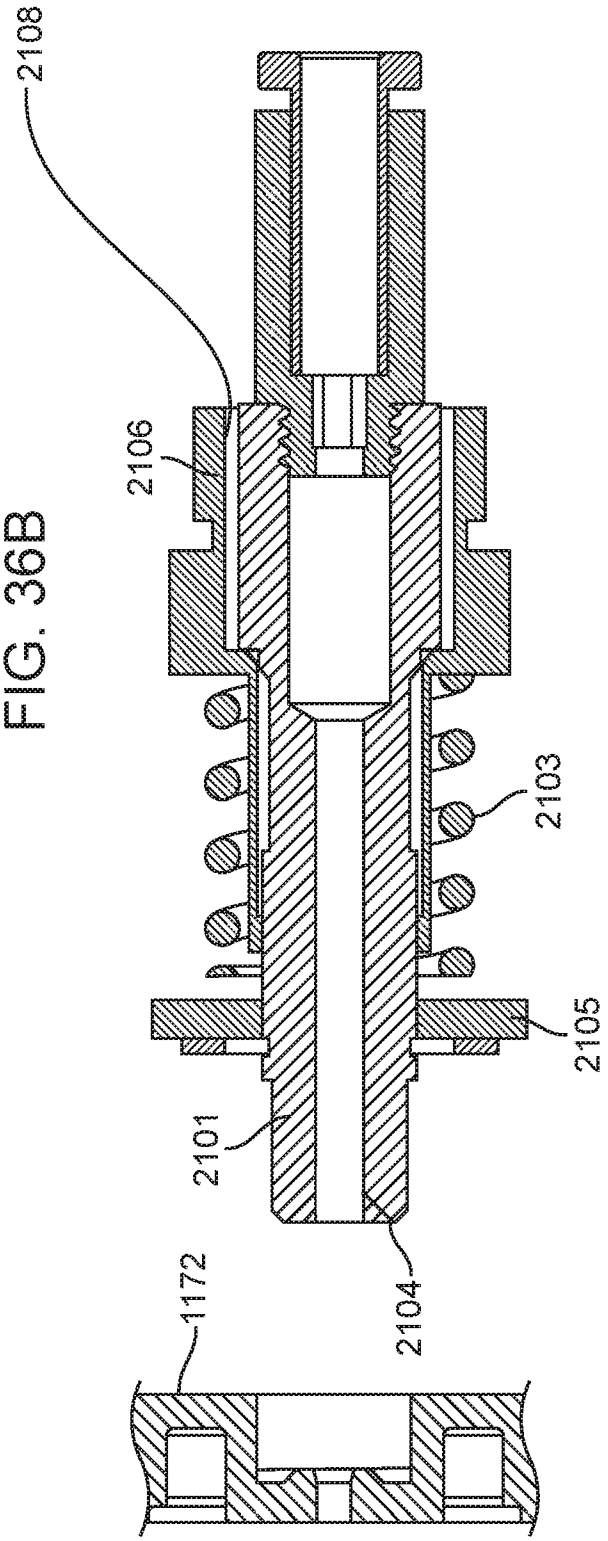


FIG. 36C

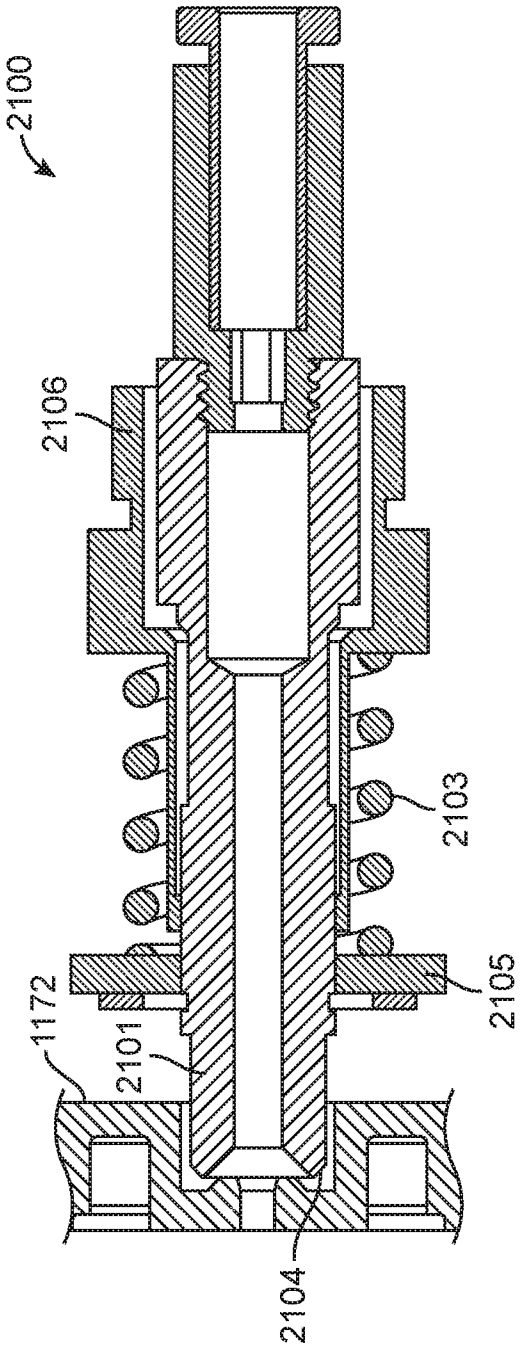


FIG. 37B

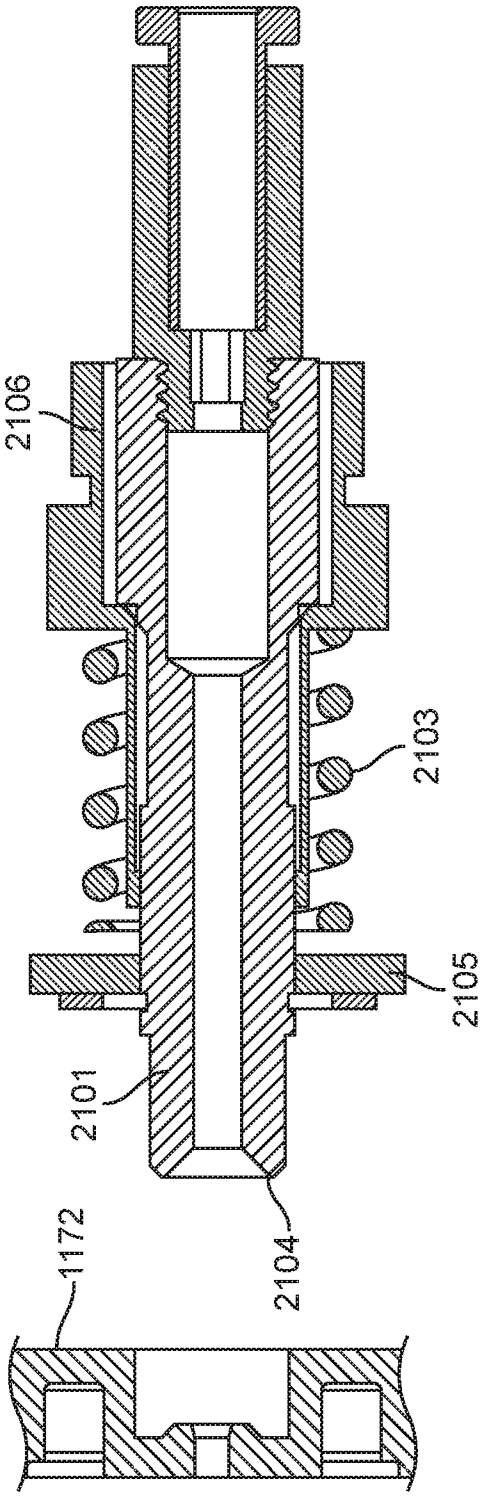


FIG. 37C

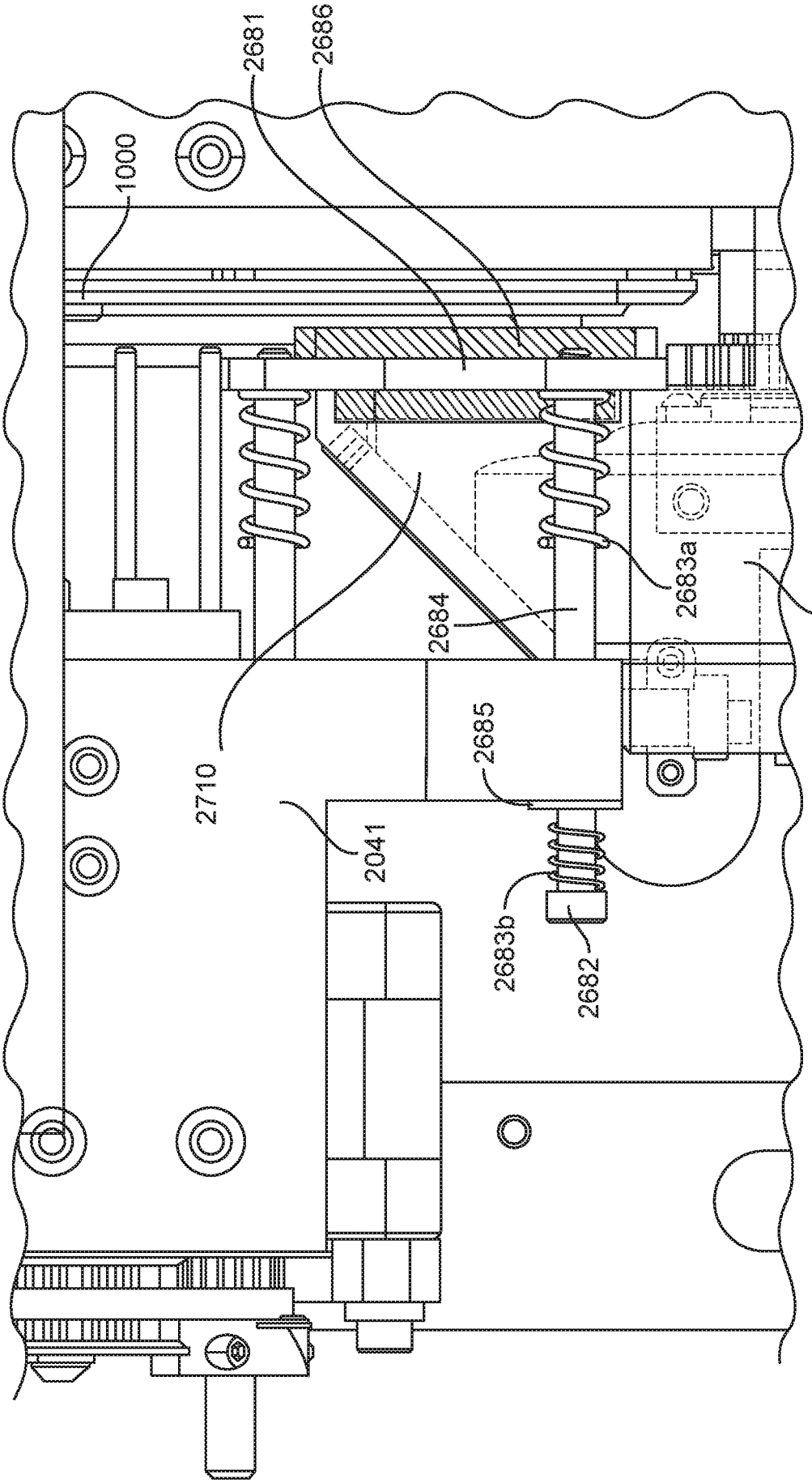


FIG. 38

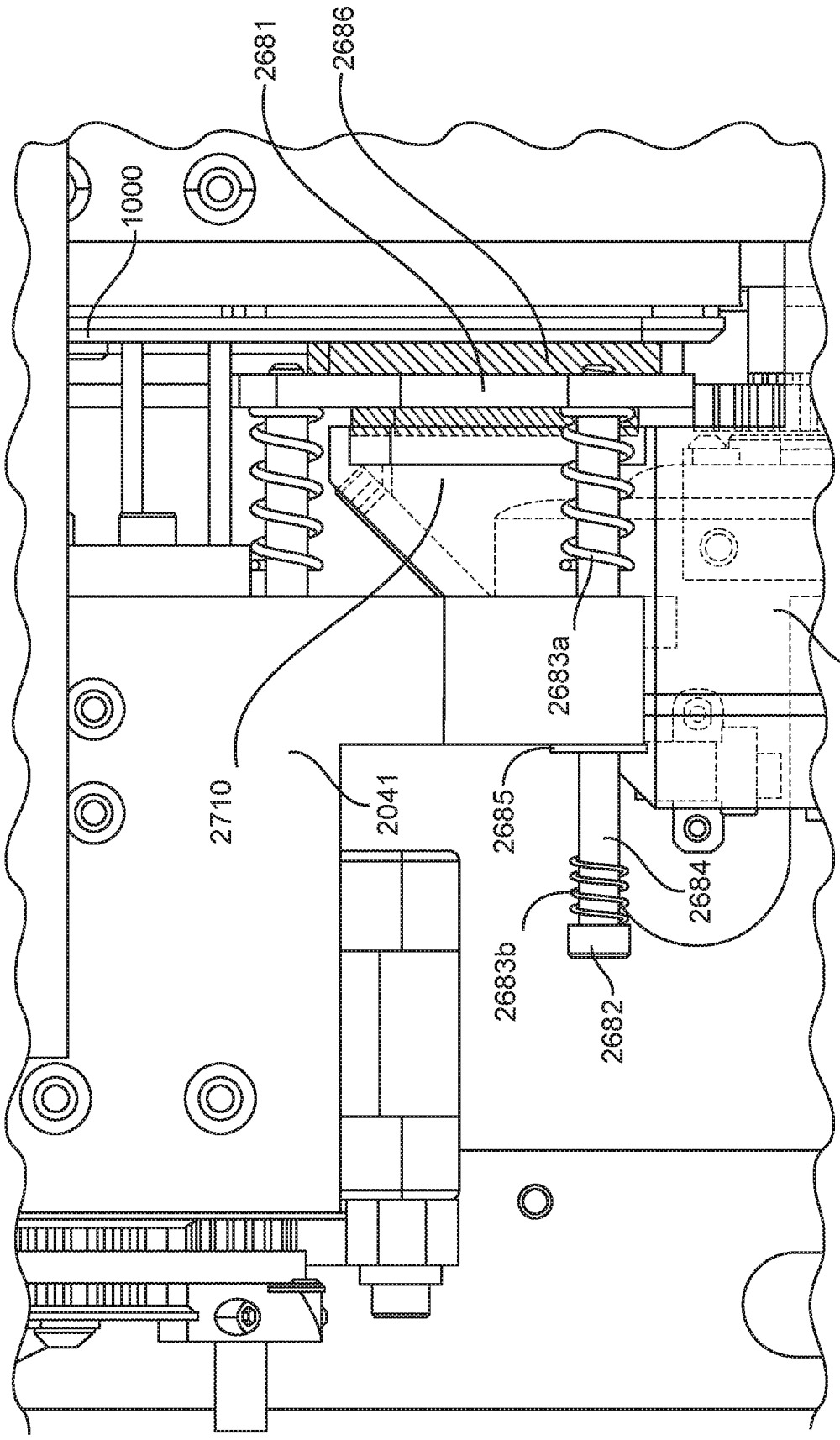


FIG. 39

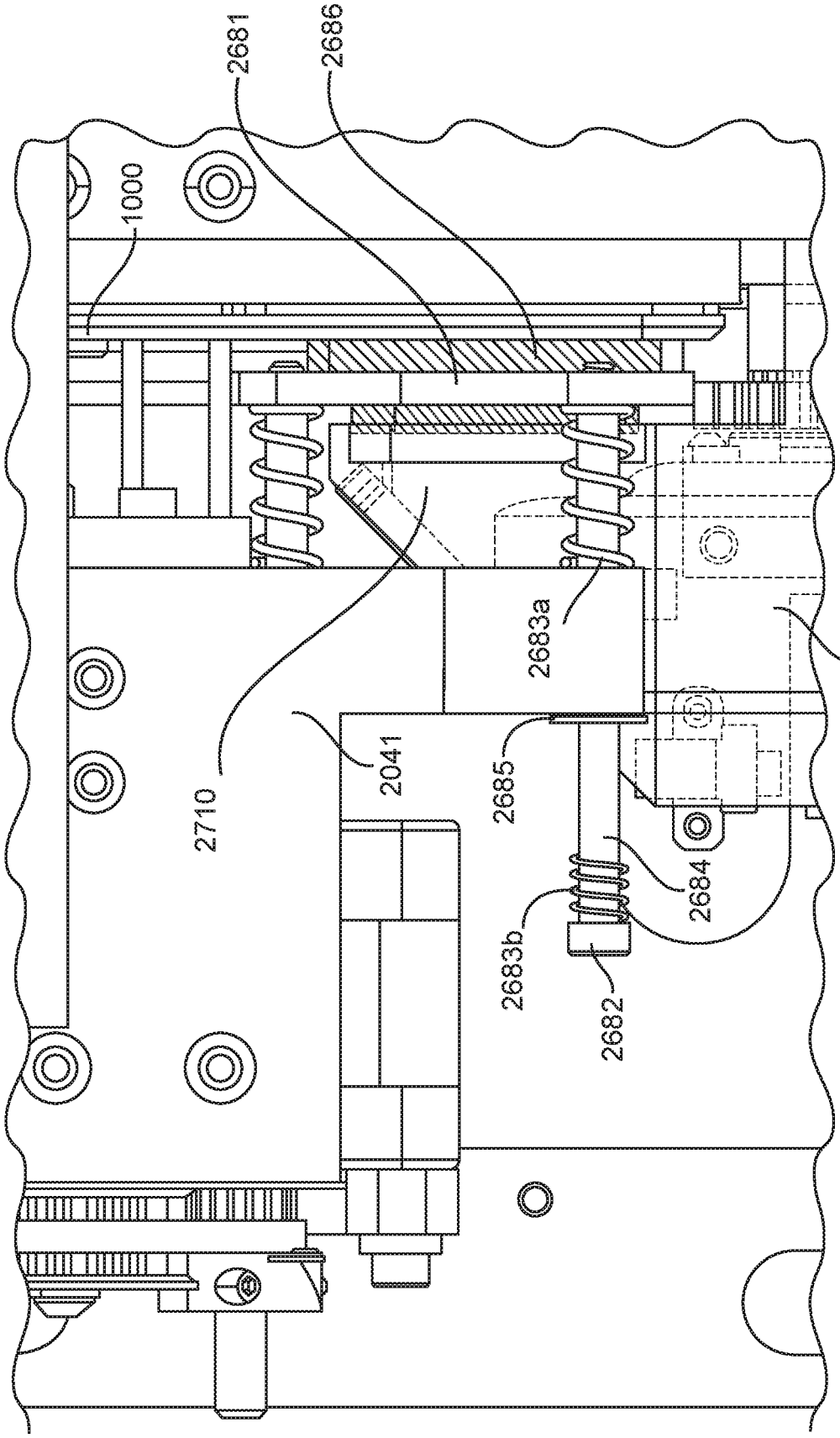


FIG. 40

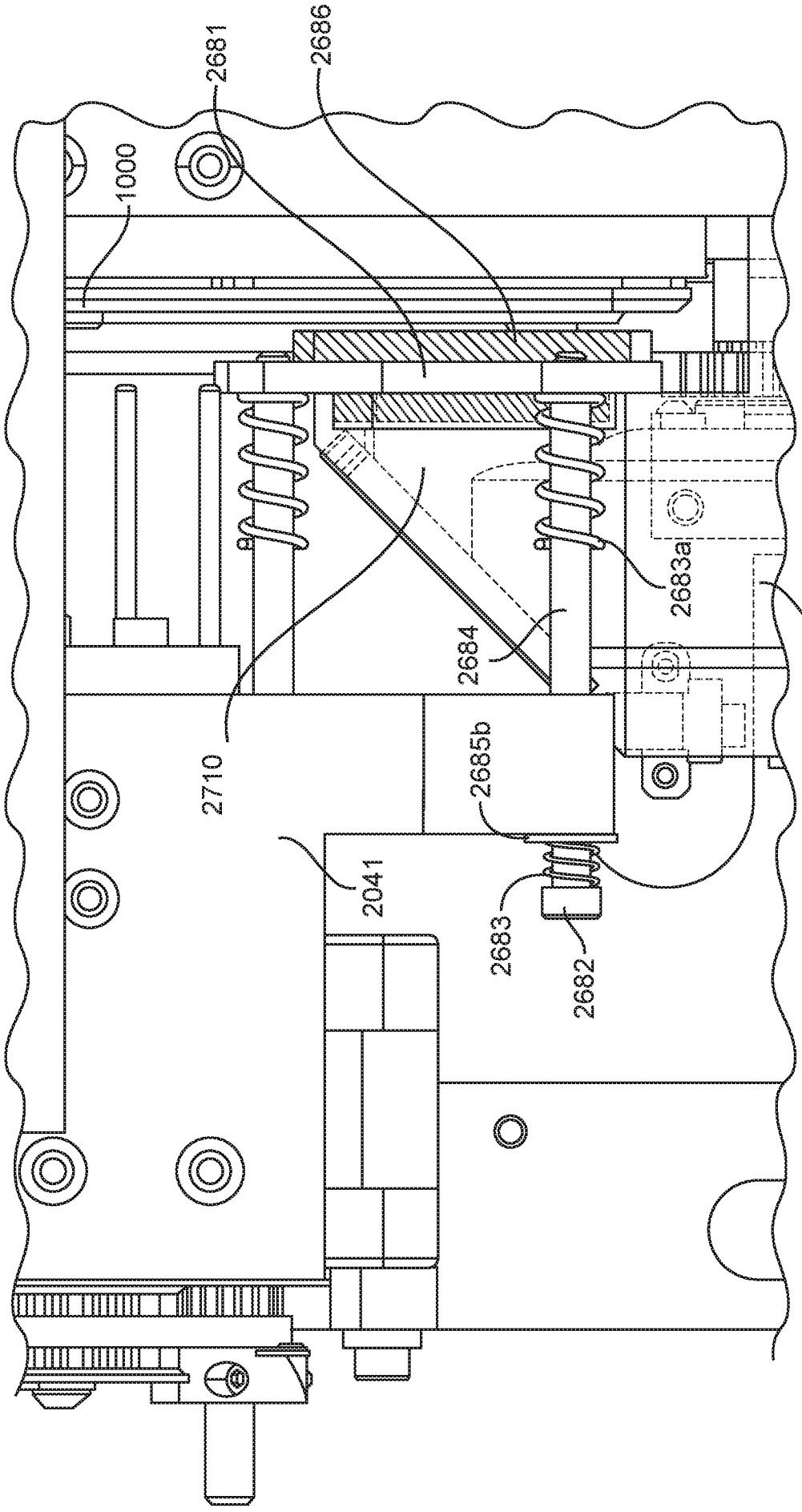


FIG. 41

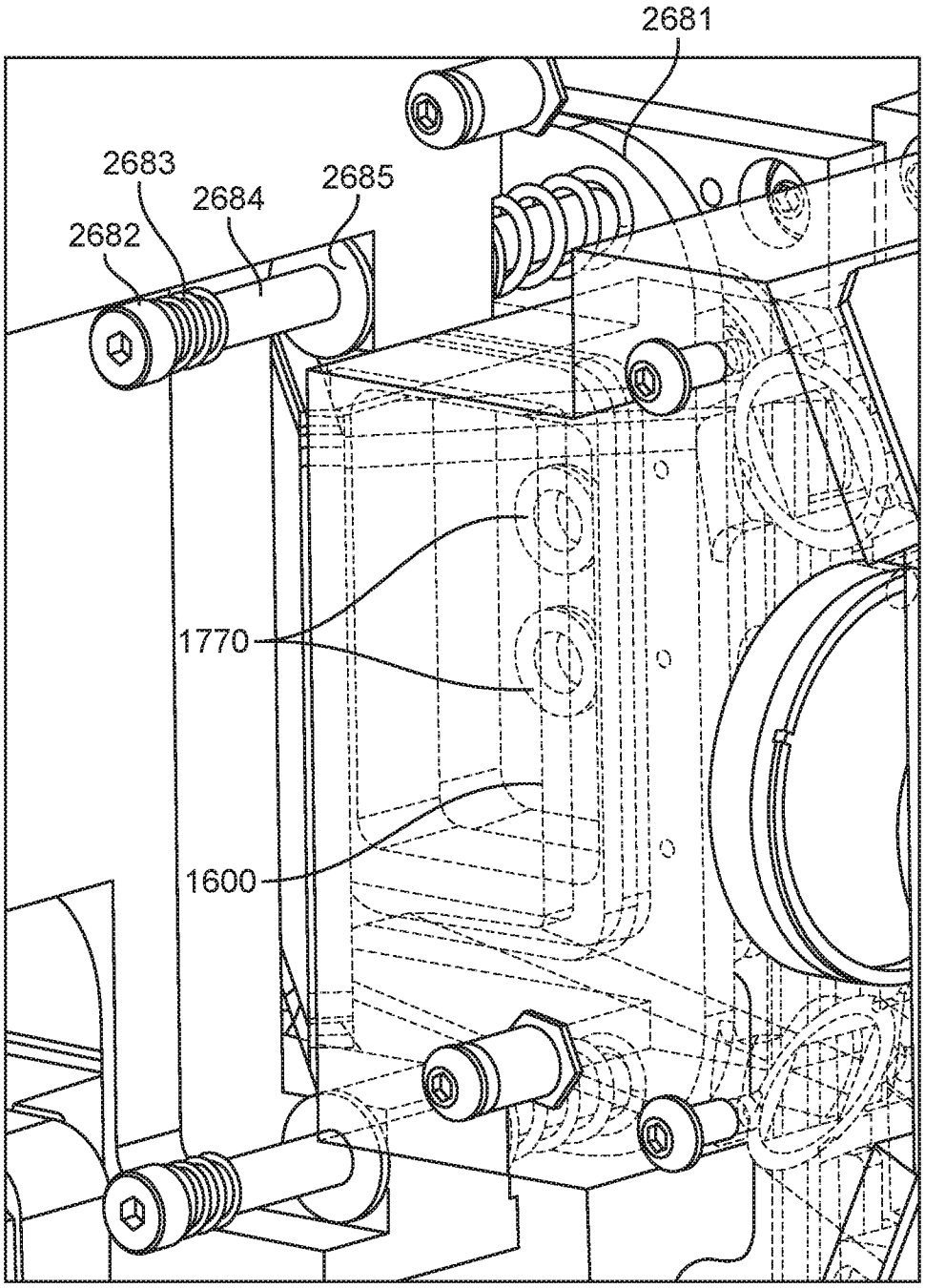
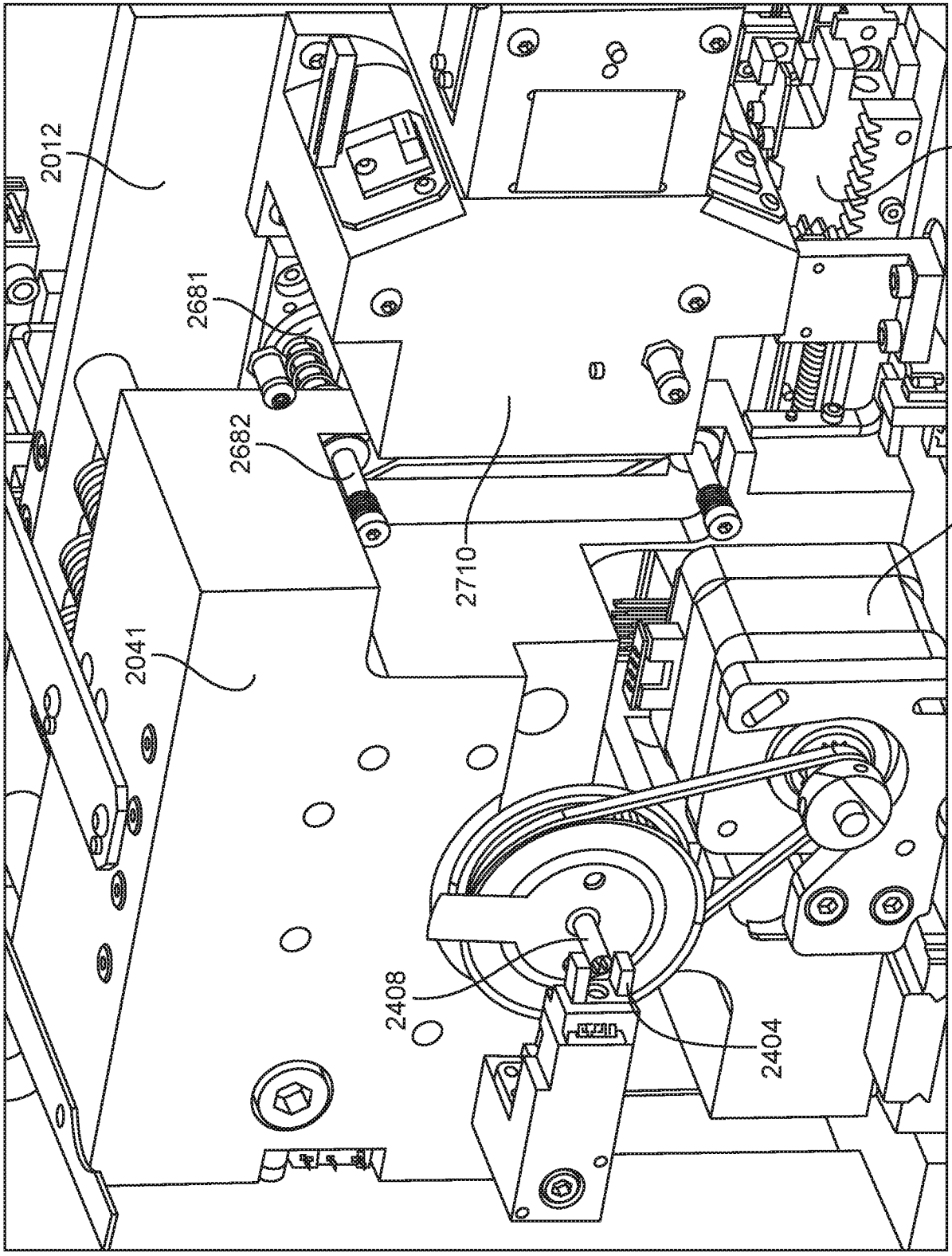


FIG. 42

FIG. 43



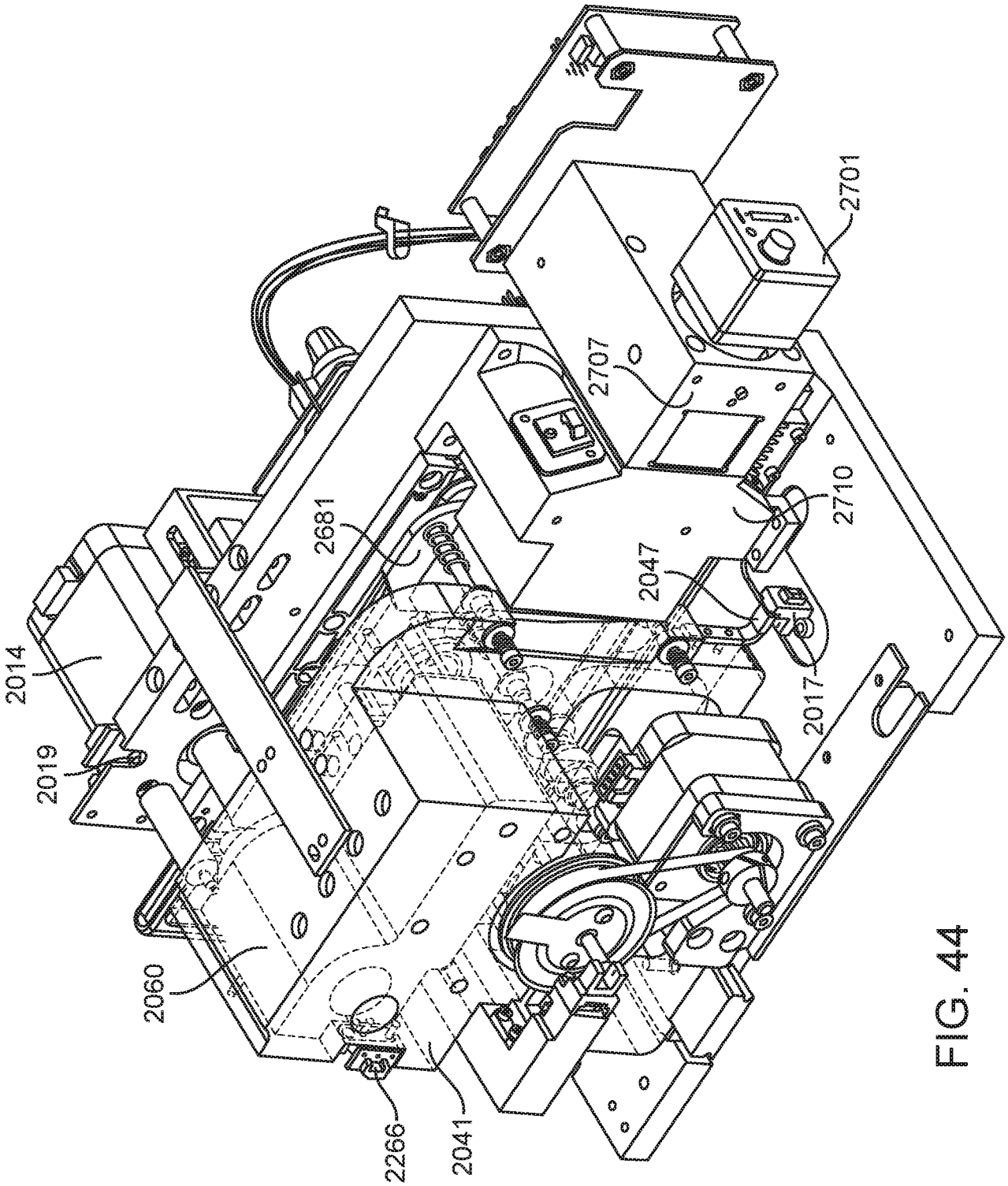


FIG. 44

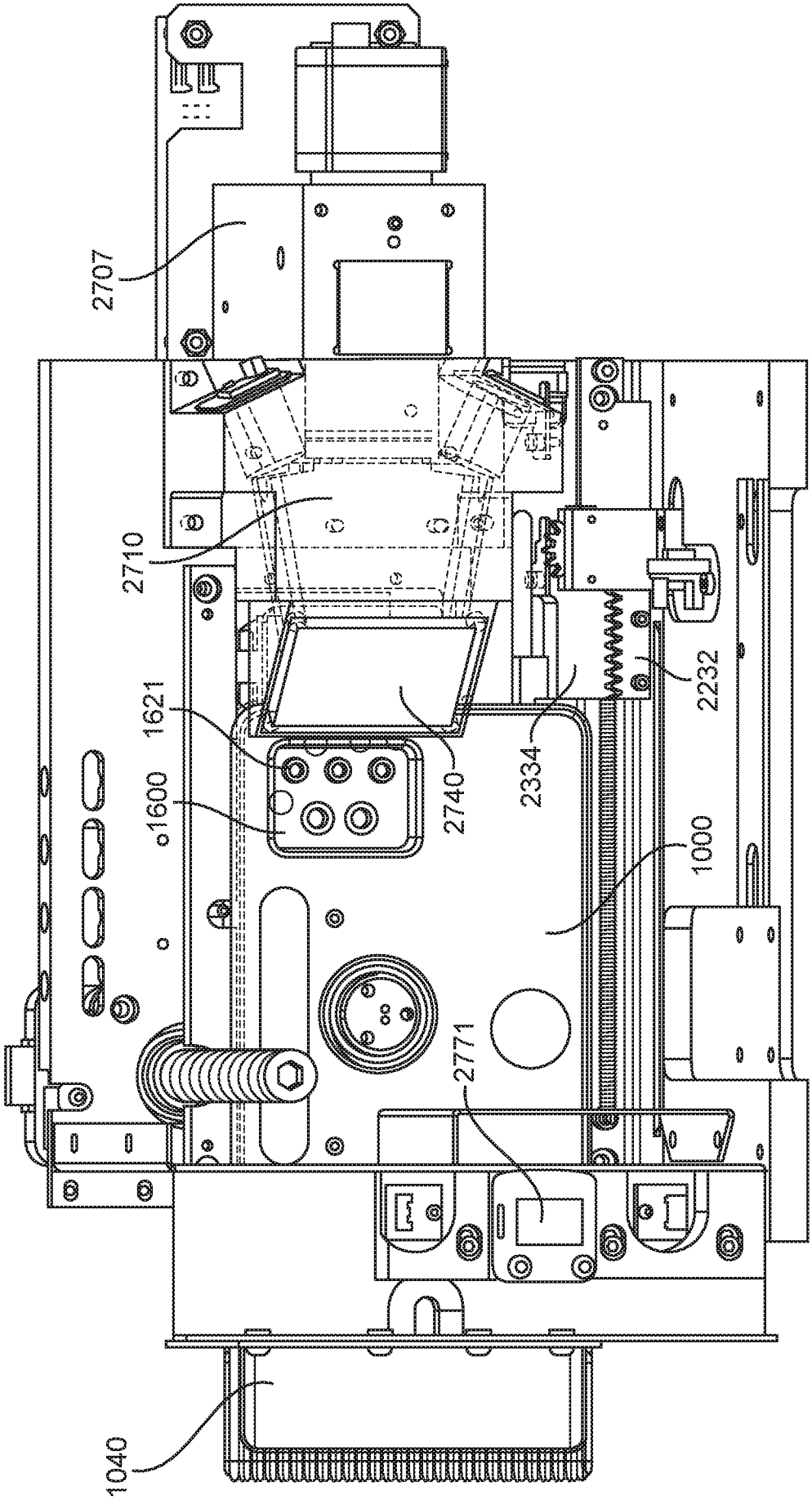


FIG. 45

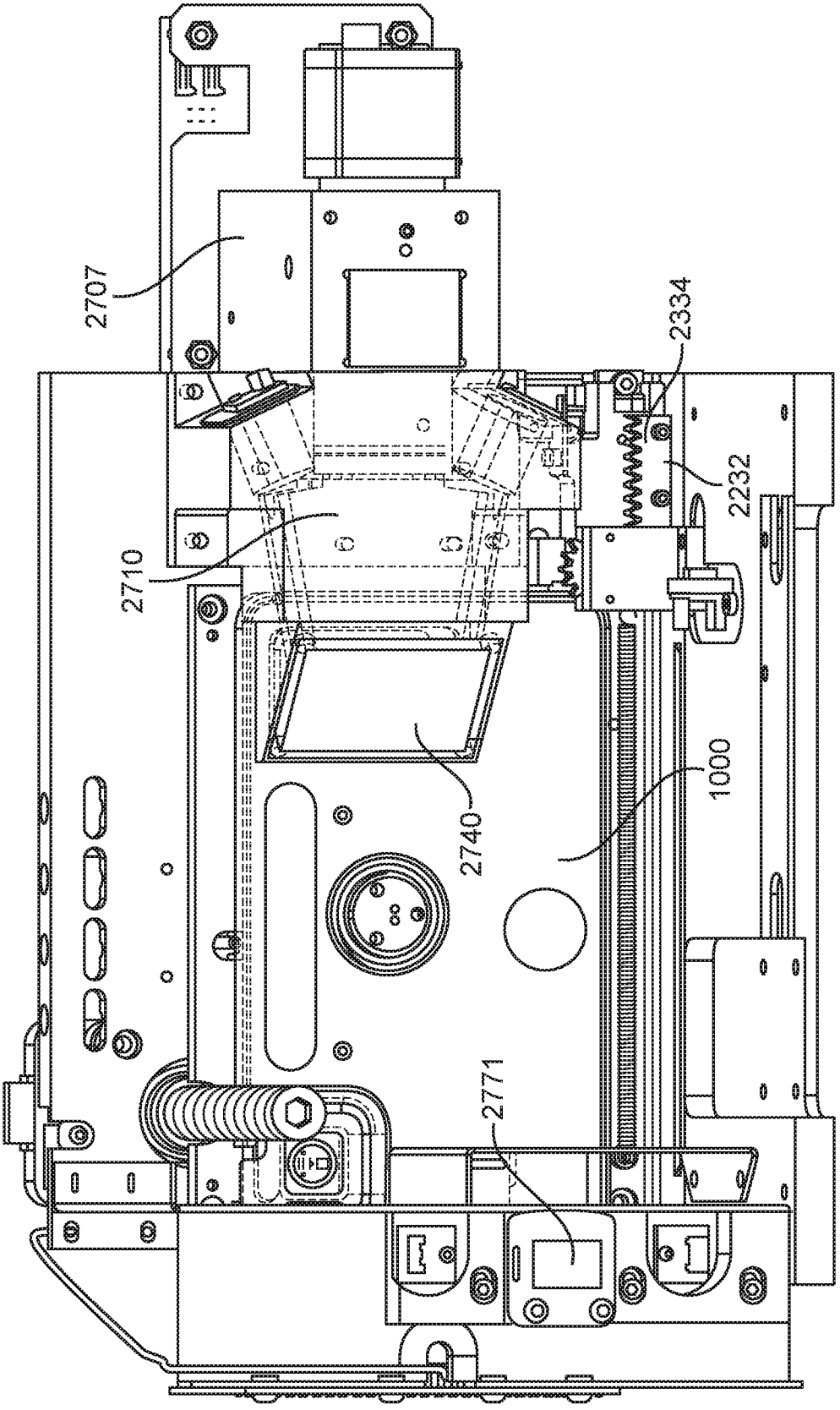


FIG. 46

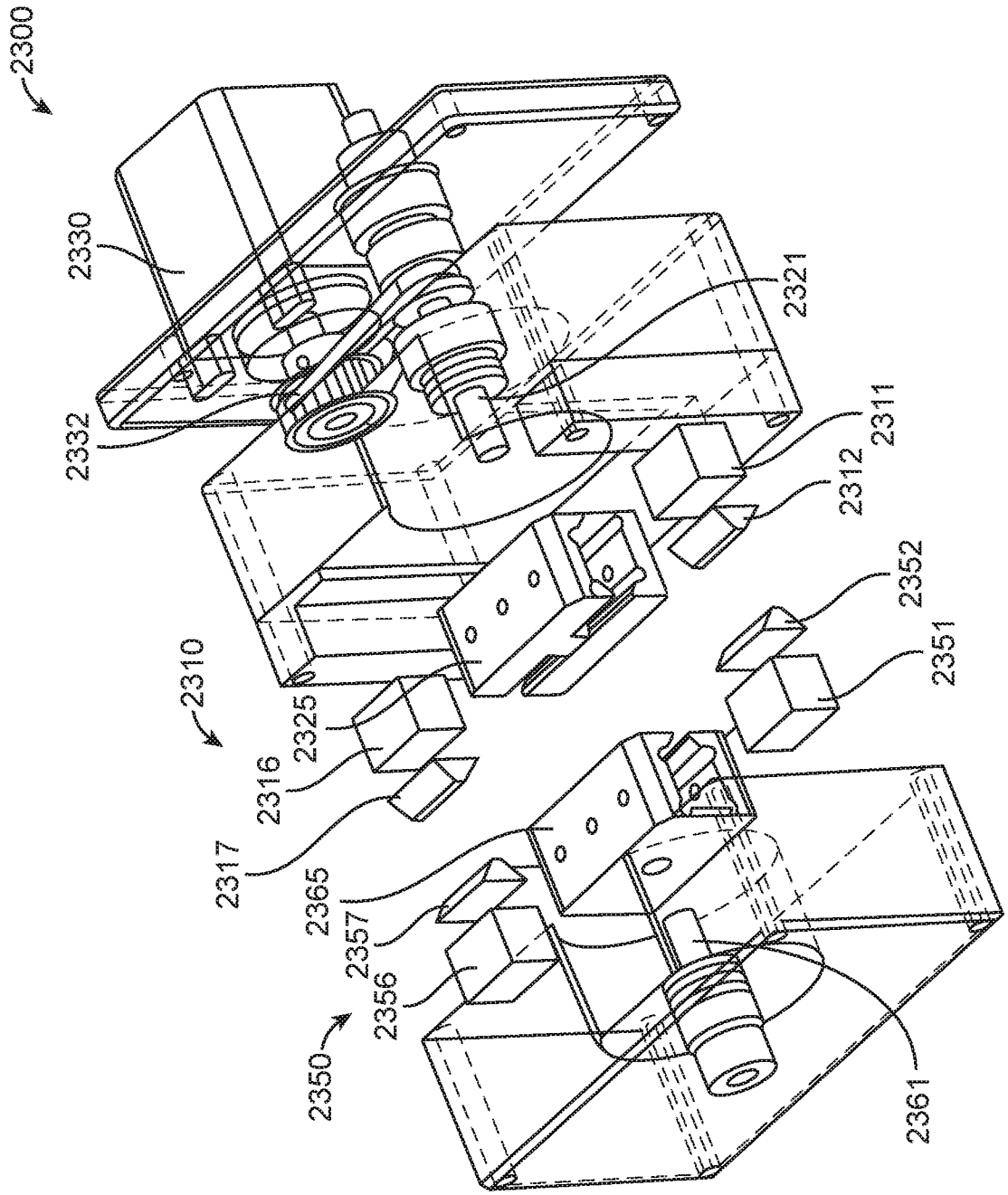

2300
2330
2332
2321
2311
2312
2352
2351
2310
2325
2316
2317
2365
2357
2356
2350
2361

FIG. 47A

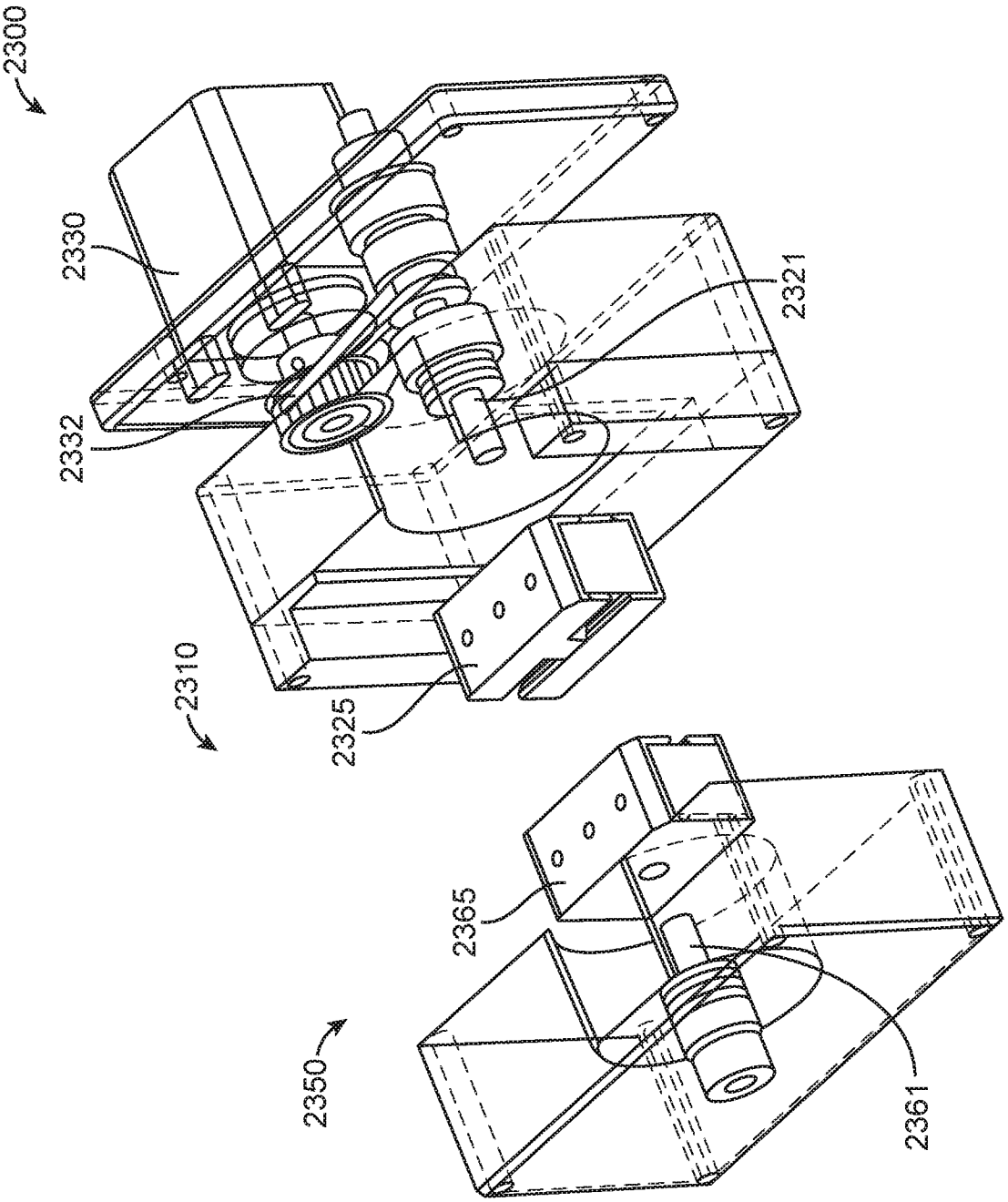


FIG. 47B

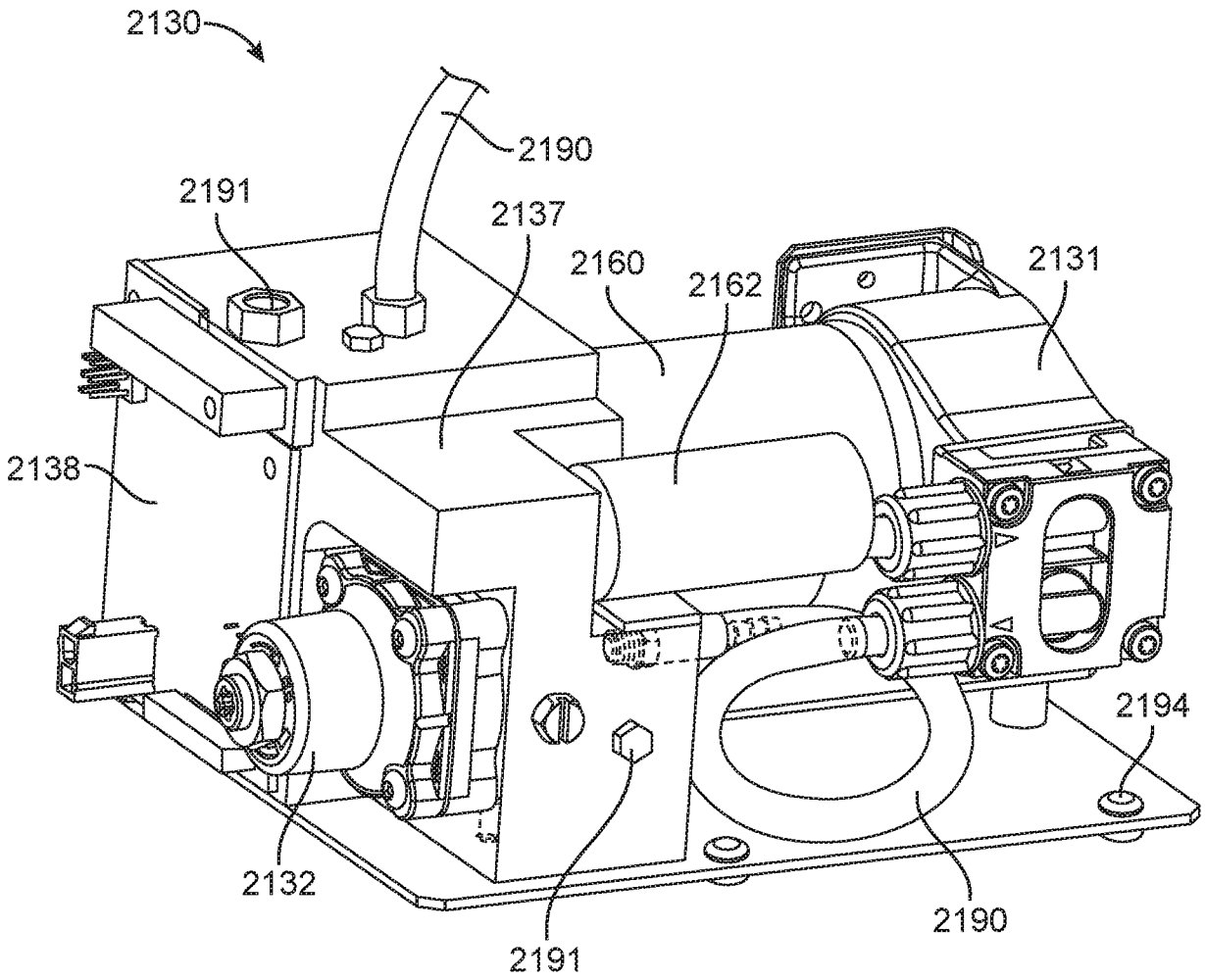


FIG. 48

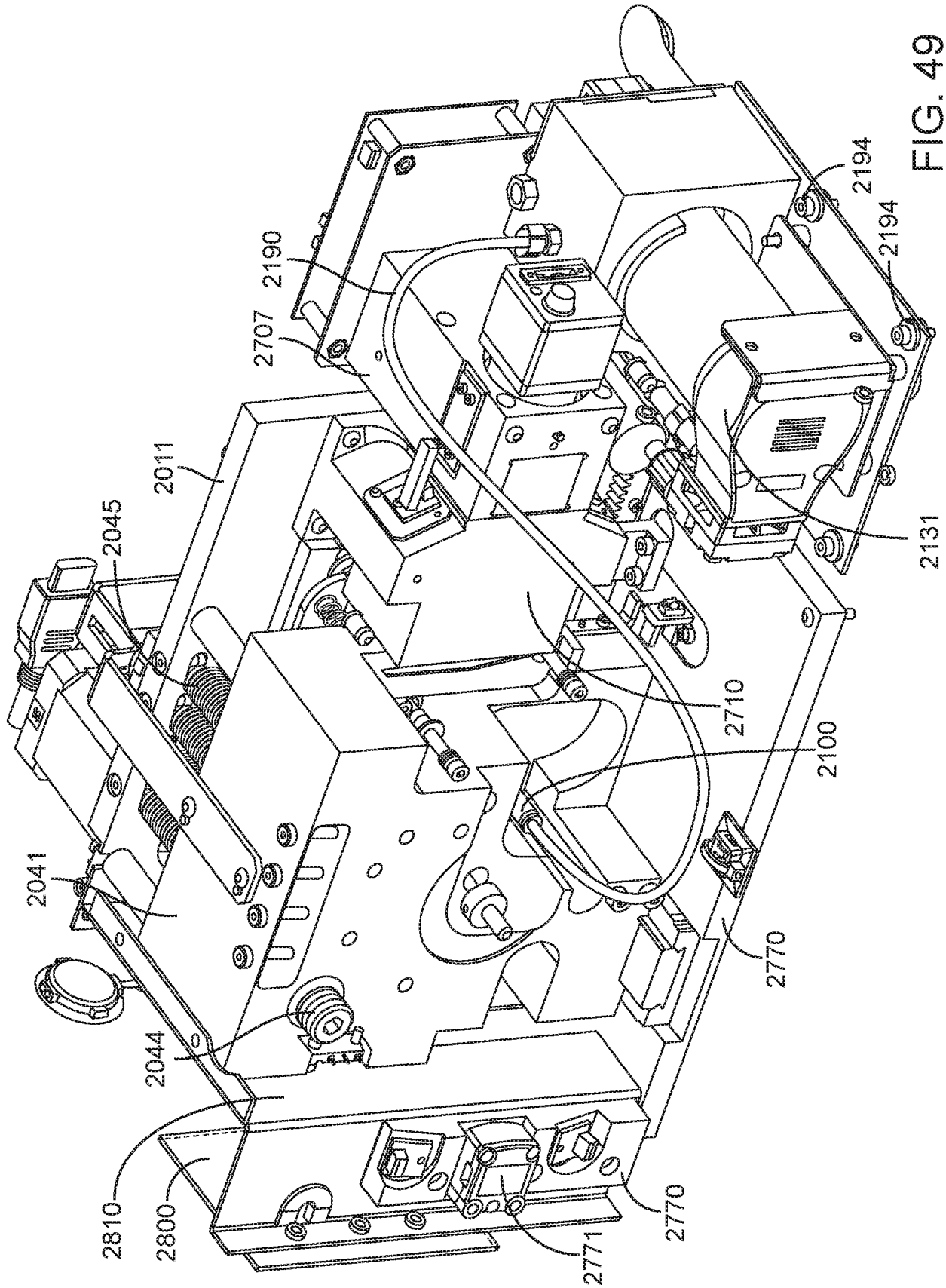

2810
2800
2044
2041
2045
2011
2707
2190
2194
2194
2131
2710
2100
2770
2771
2770

FIG. 49

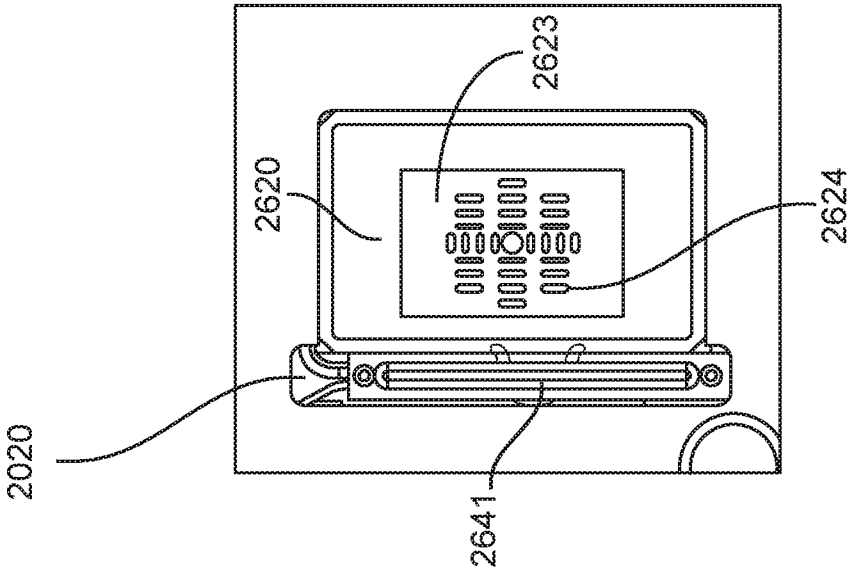


FIG. 51

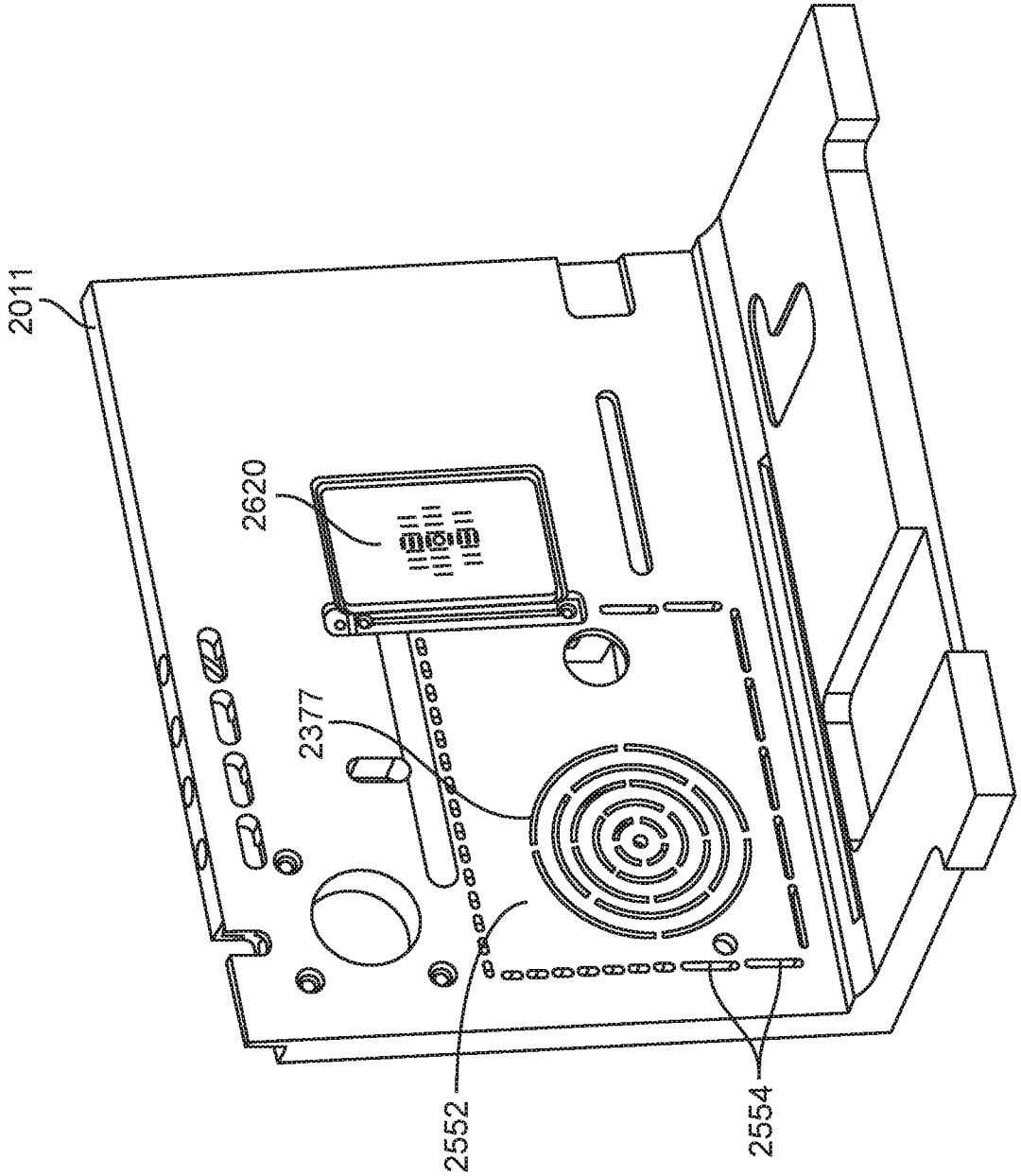


FIG. 50

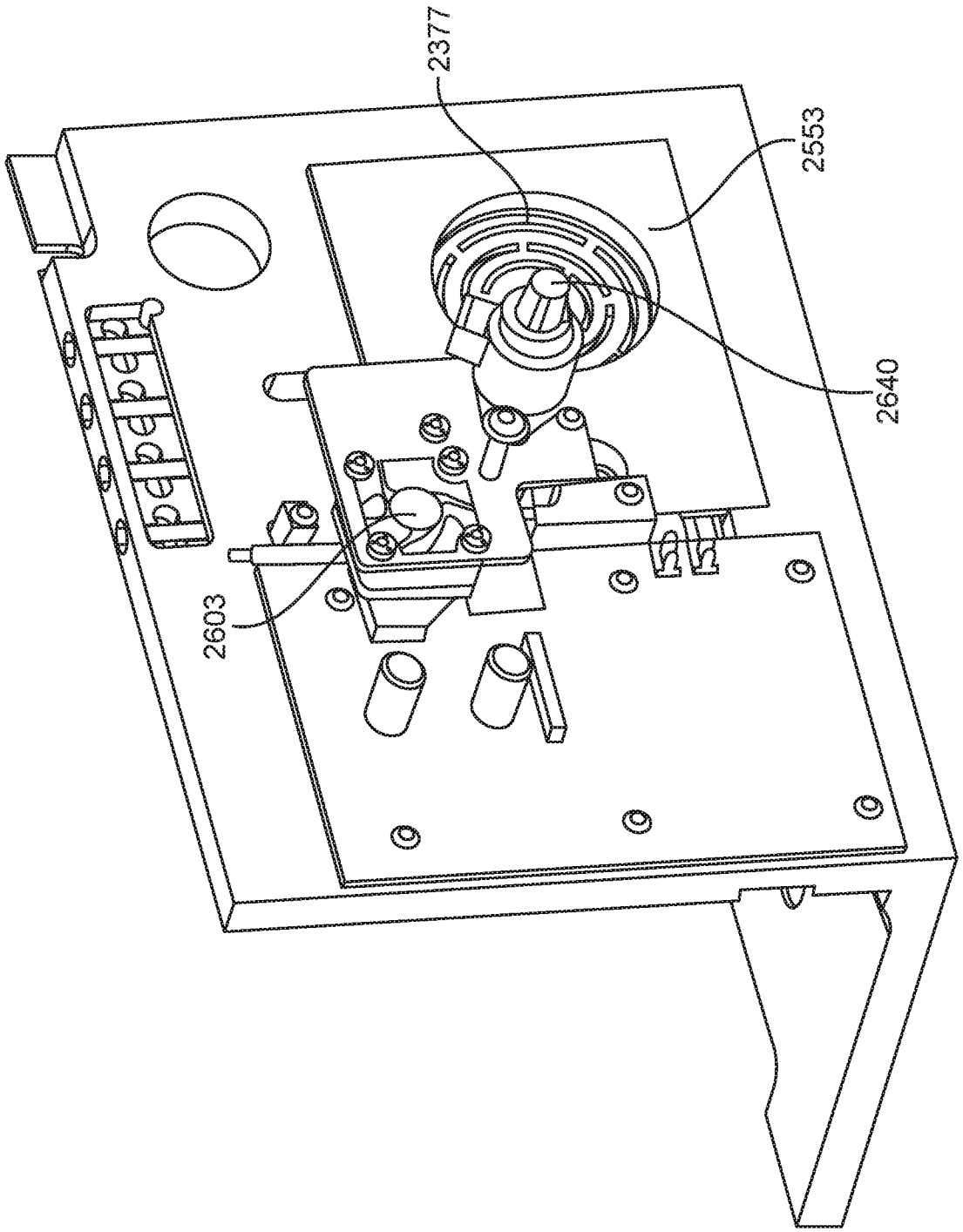


FIG. 52

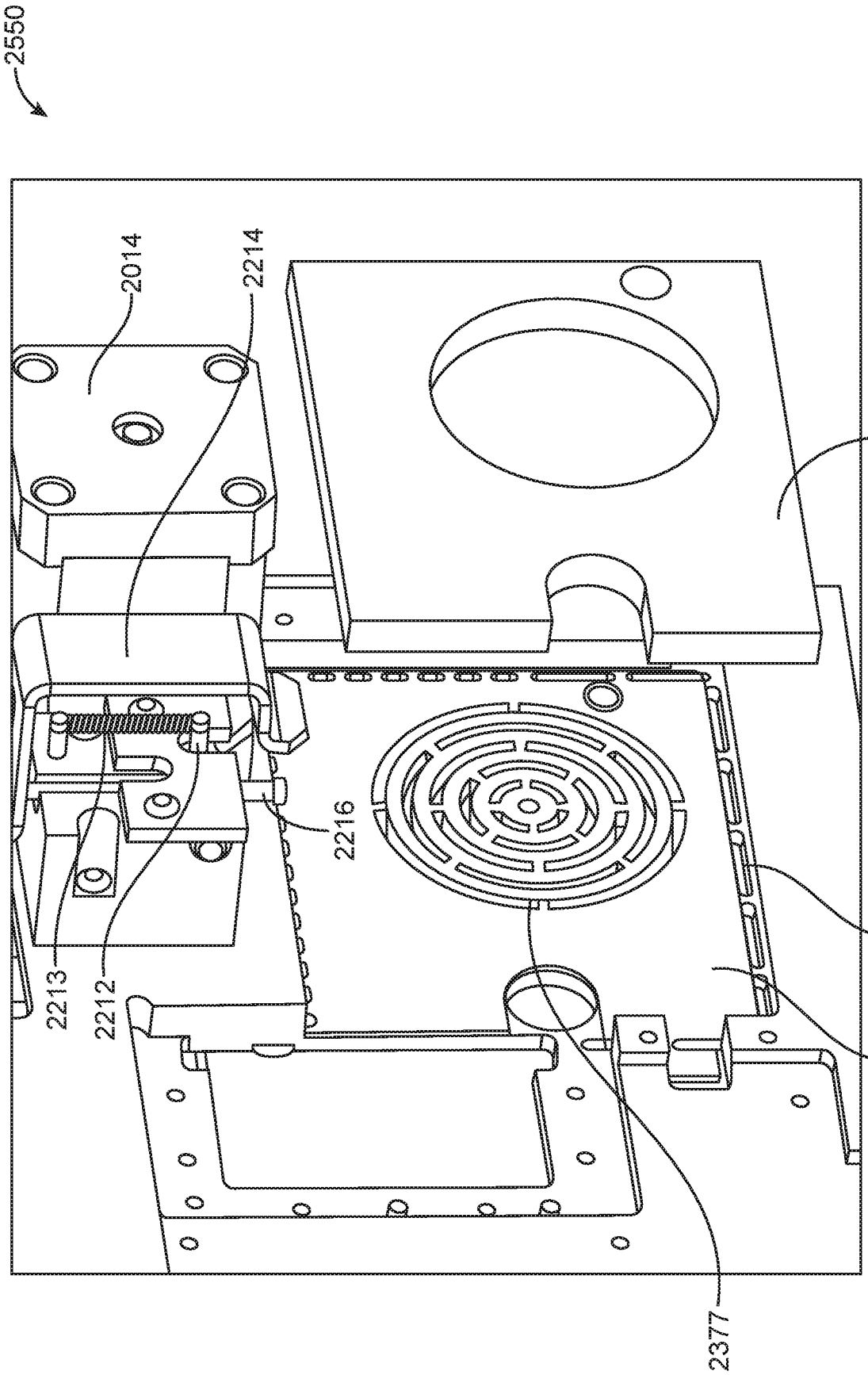


FIG. 53

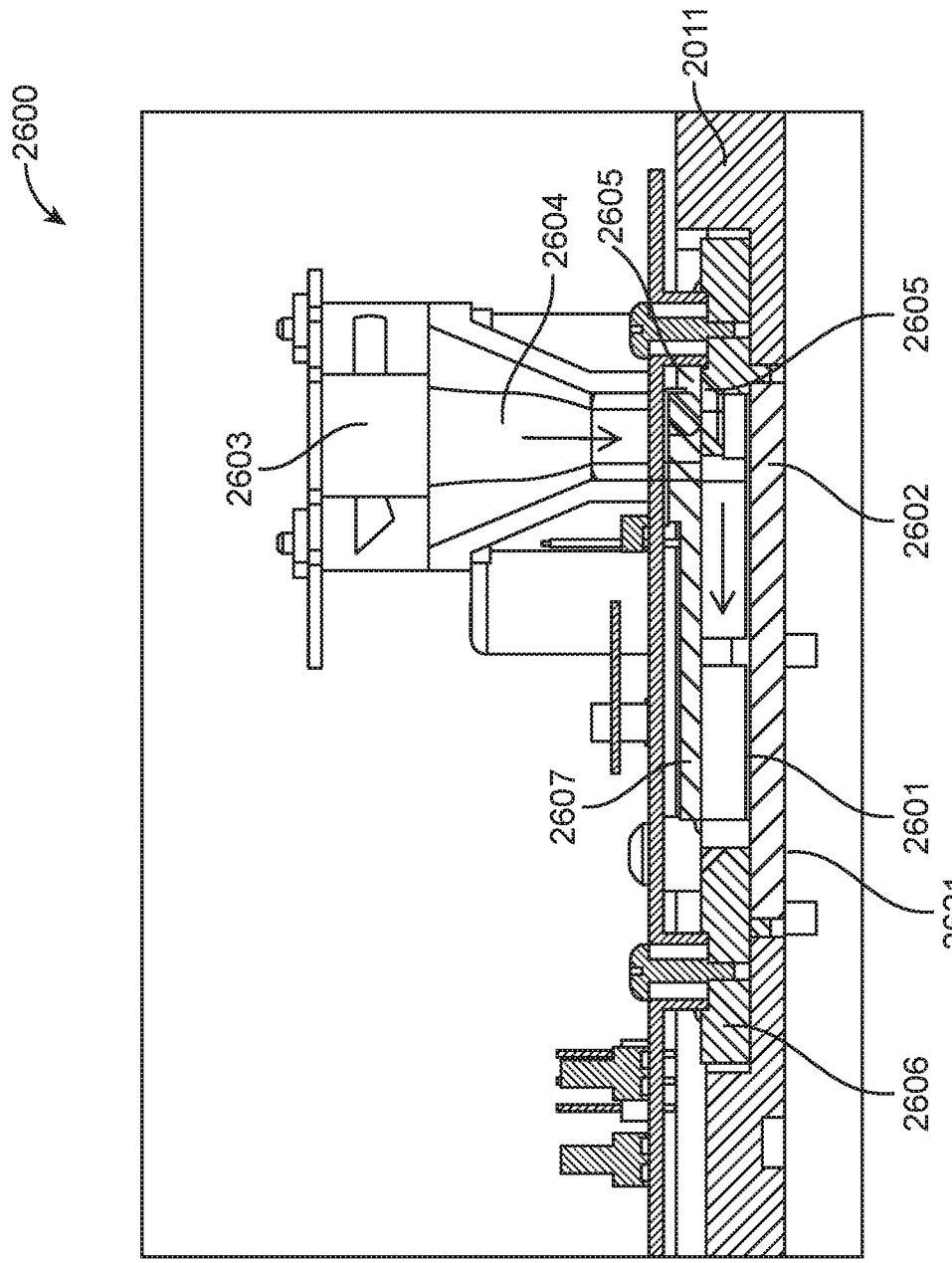


FIG. 54

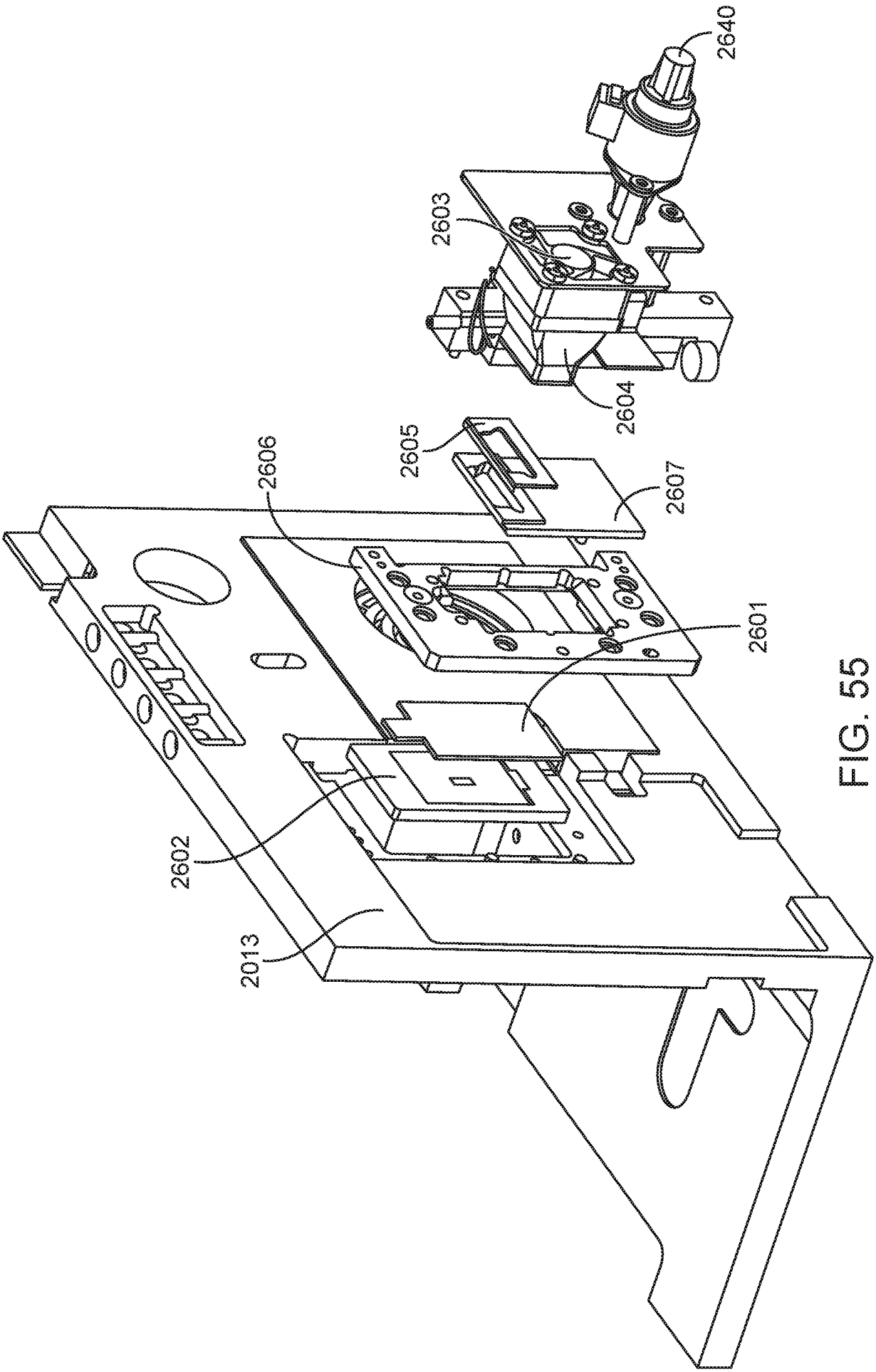


FIG. 55

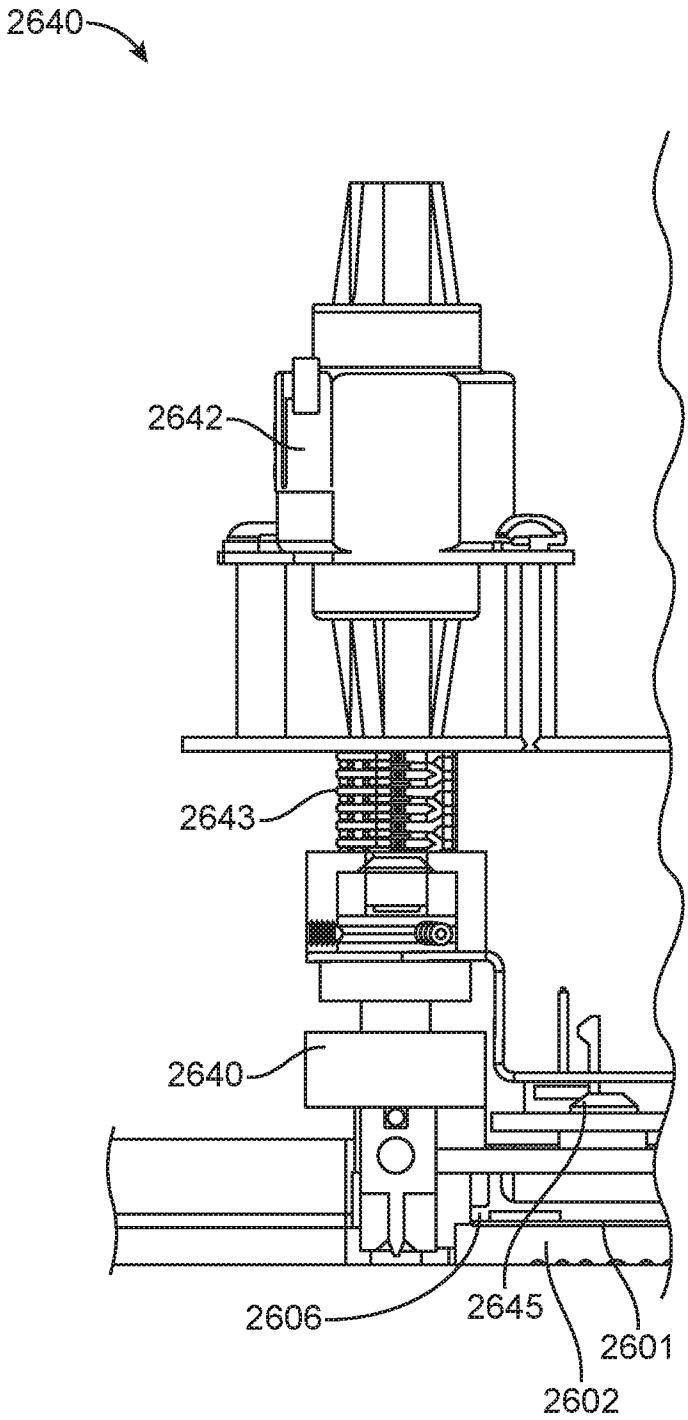


FIG. 56

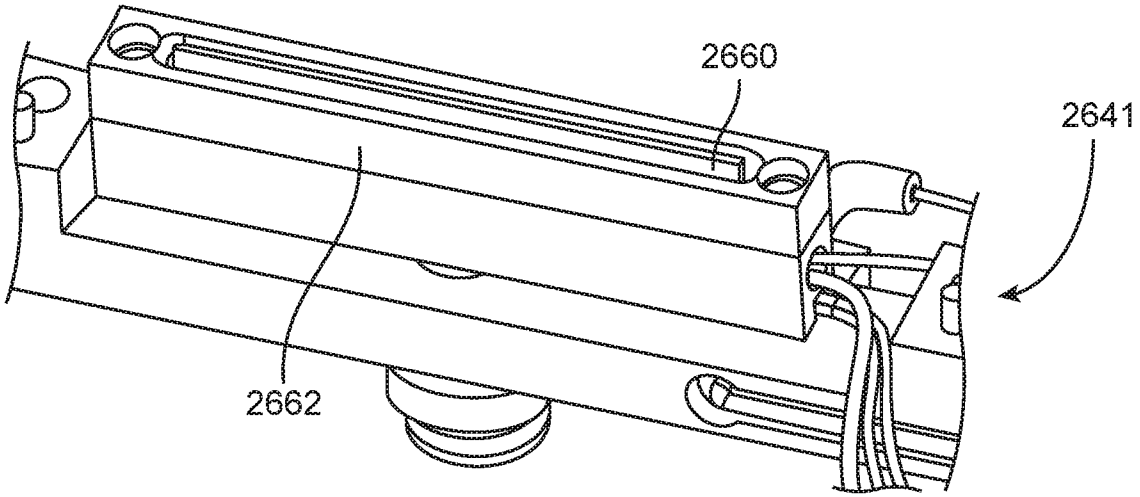


FIG. 57A

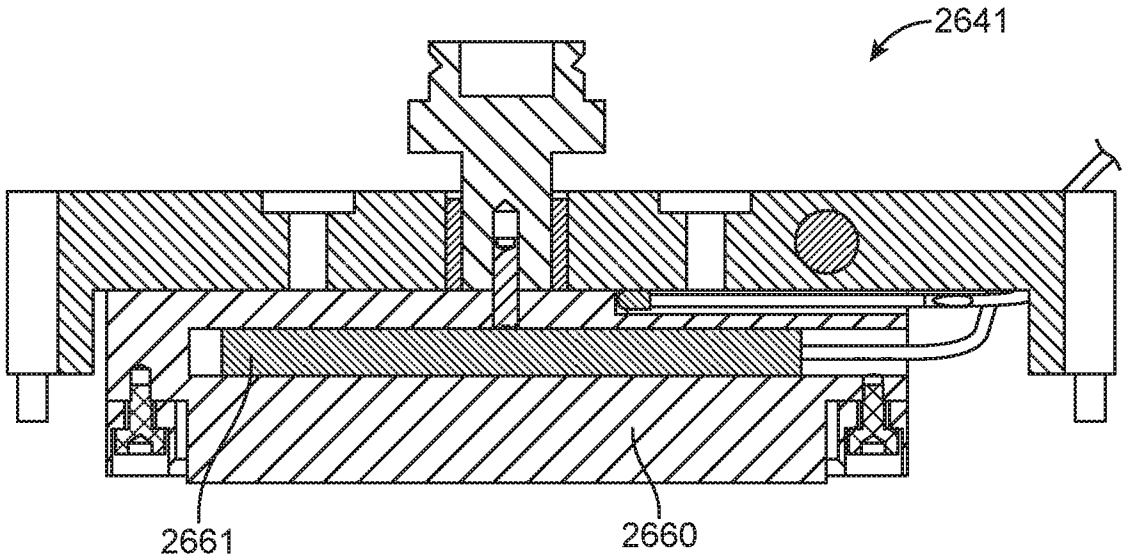


FIG. 57B

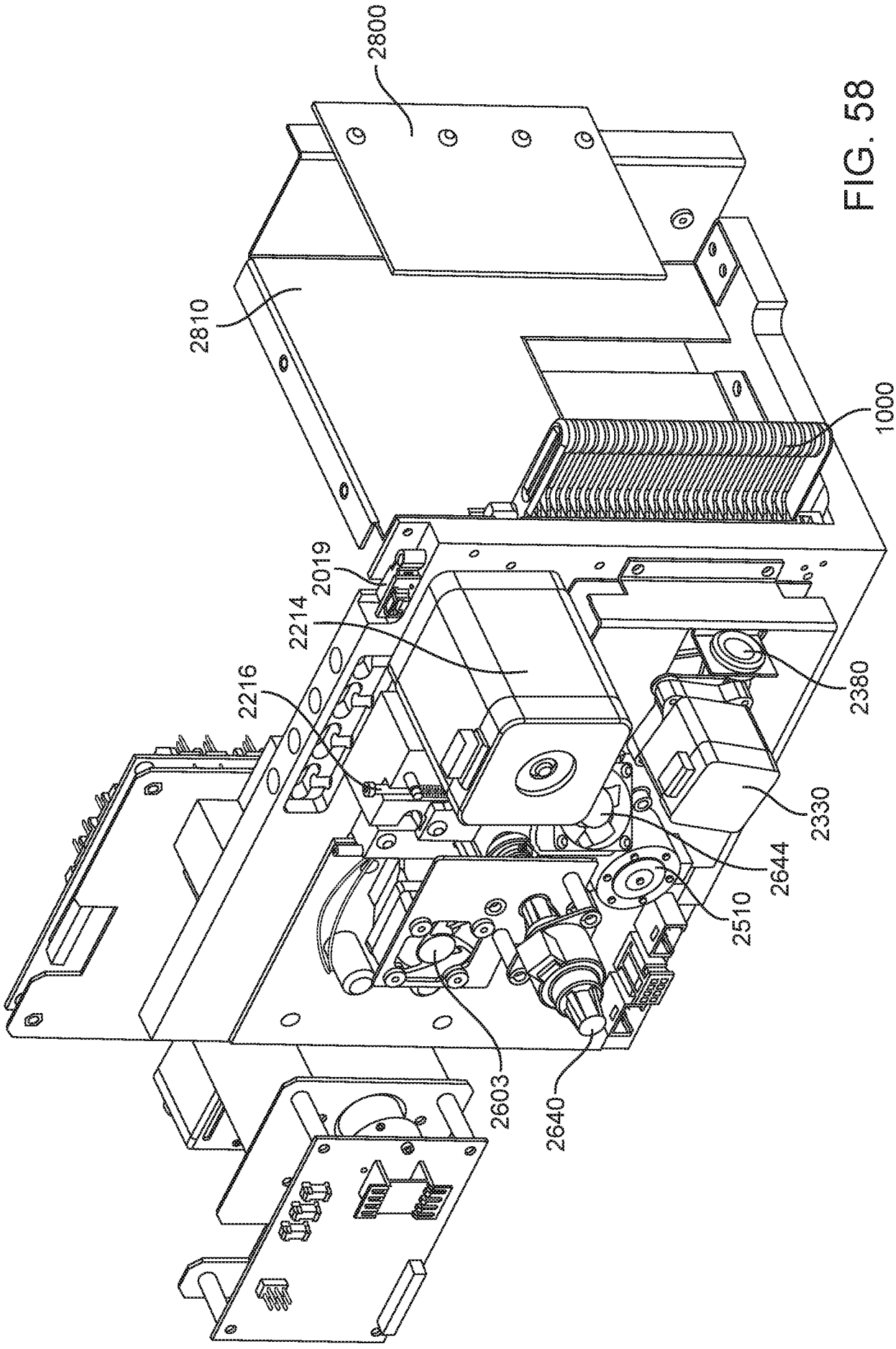


FIG. 58

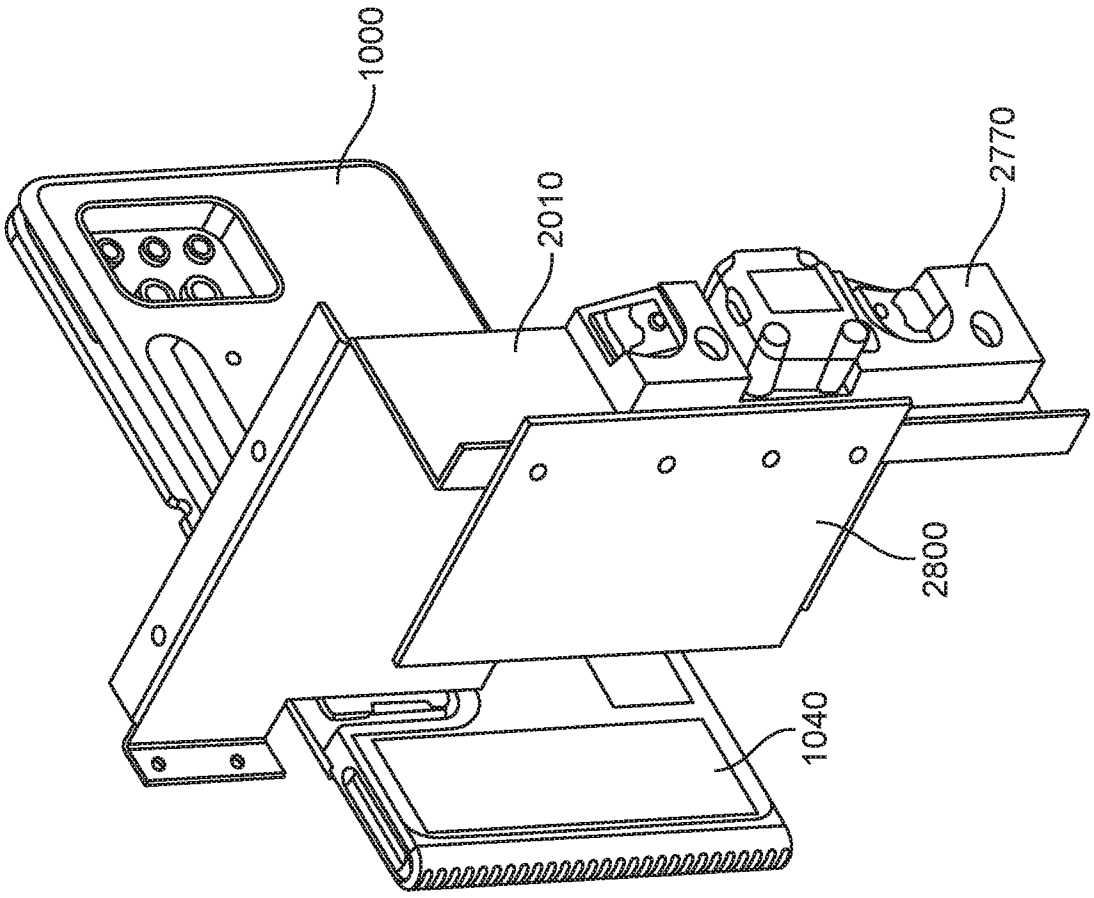


FIG. 59

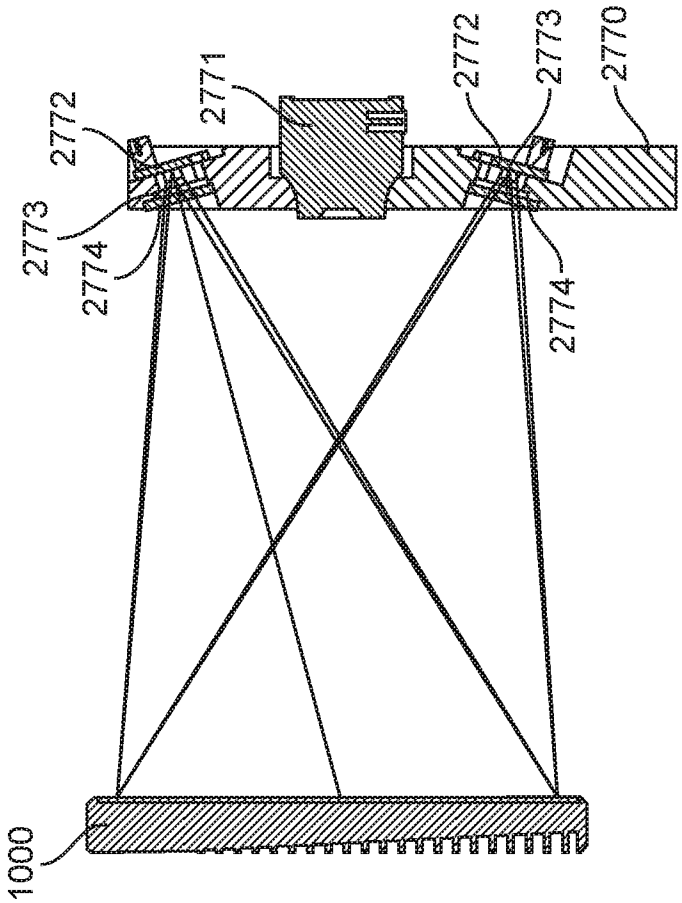

2772
2771
2773
2770
2772
2773
2774
2774
1000

FIG. 61

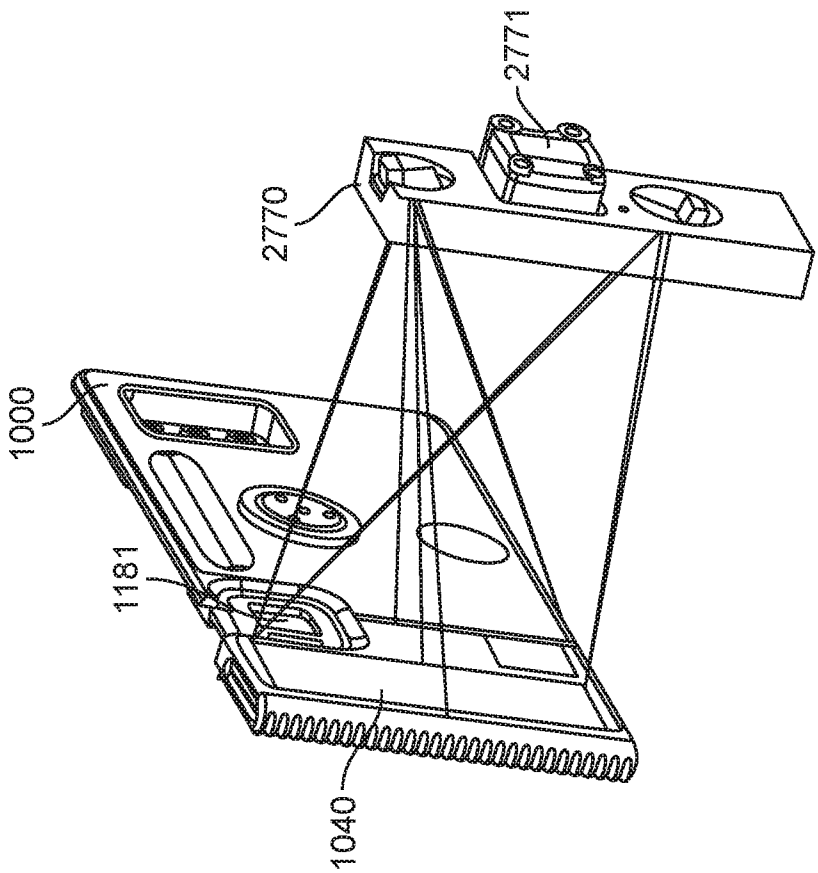

2771
2770
1000
1181
1040

FIG. 60

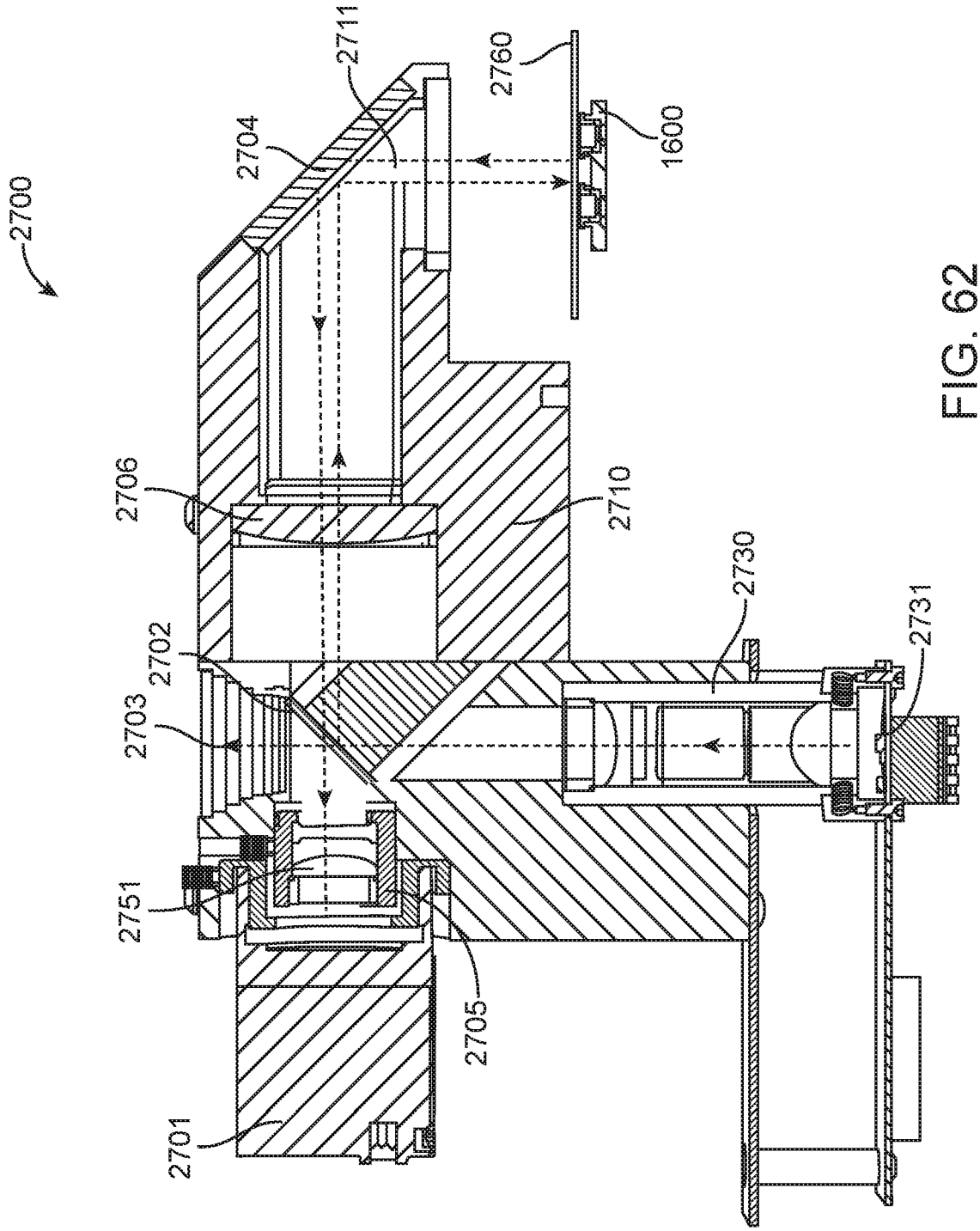

2700
2711
2760
2704
1600
2706
2710
2730
2731
2702
2703
2751
2705
2701

FIG. 62

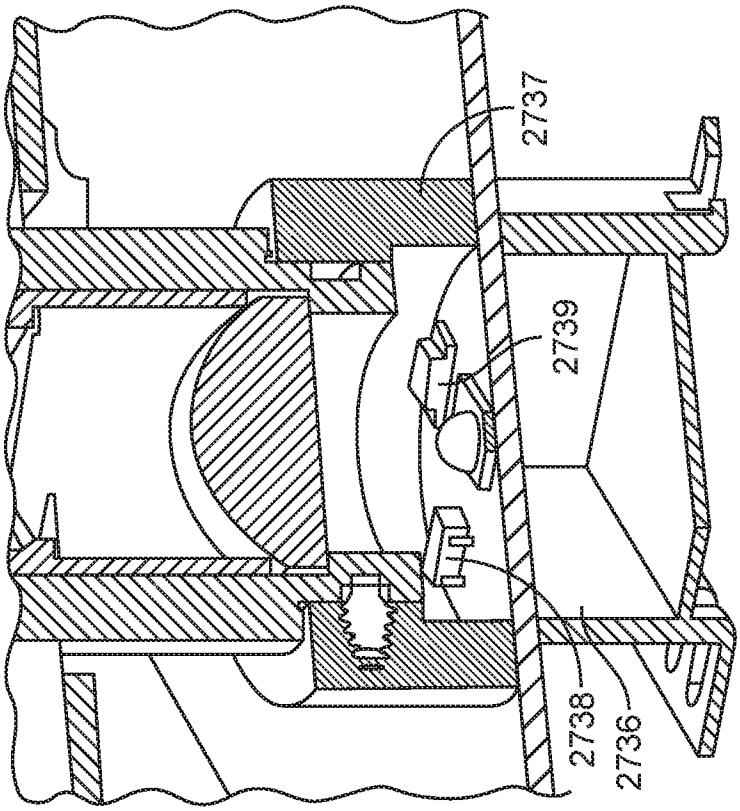

2737
2739
2738
2736

FIG. 64

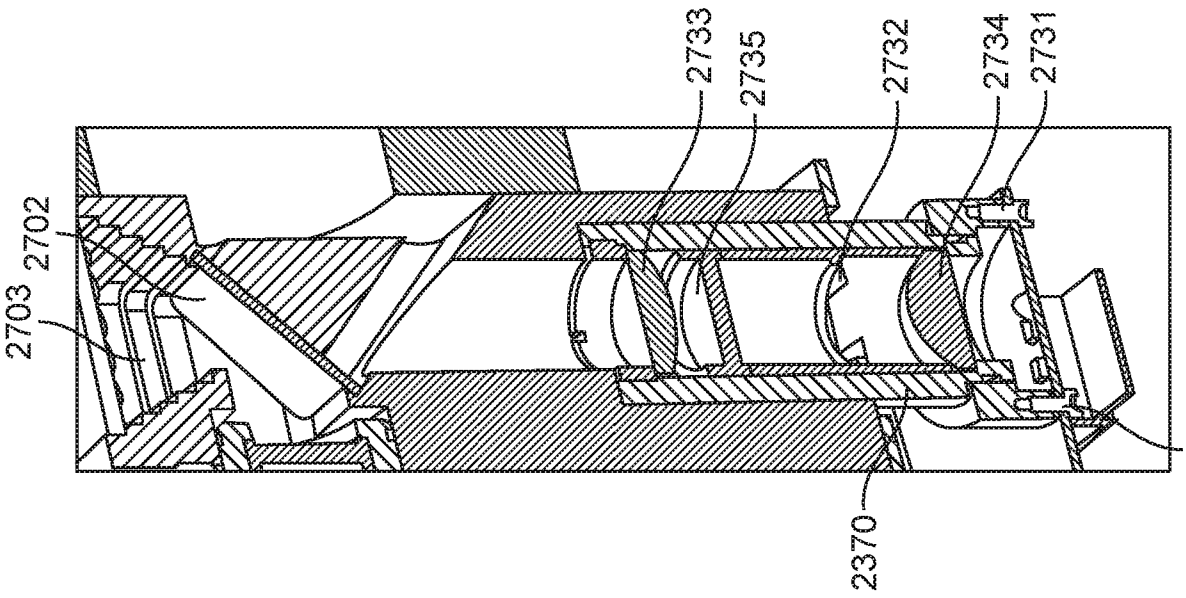

2733
2735
2732
2734
2731
2702
2703
2731
2370

FIG. 63

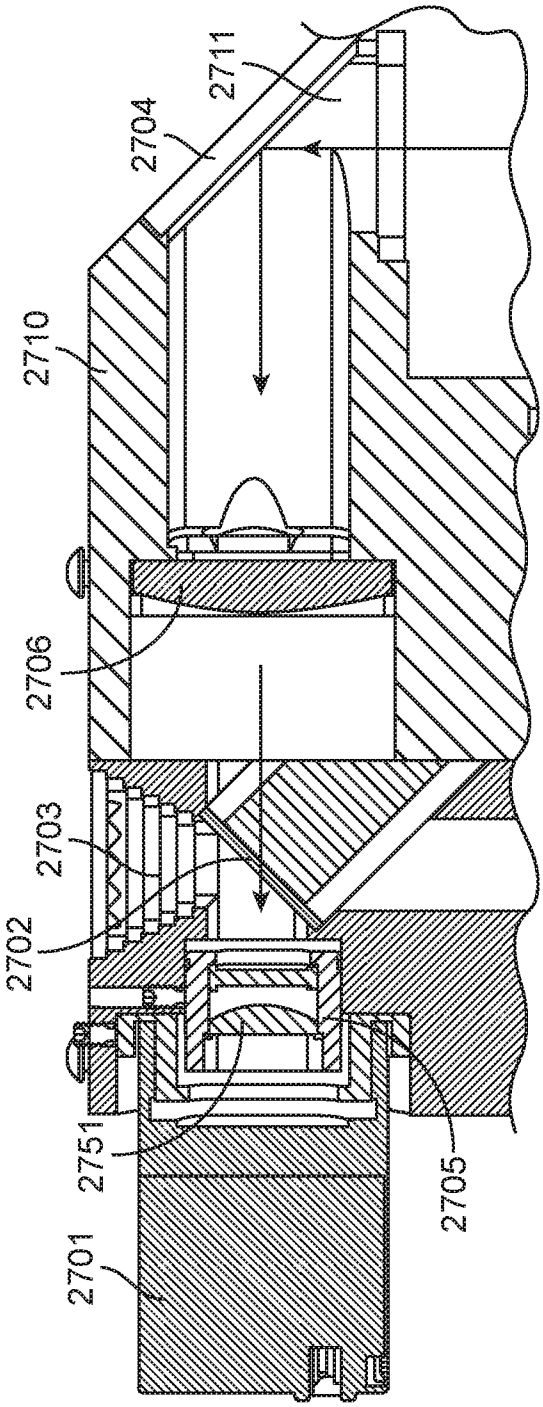


FIG. 65

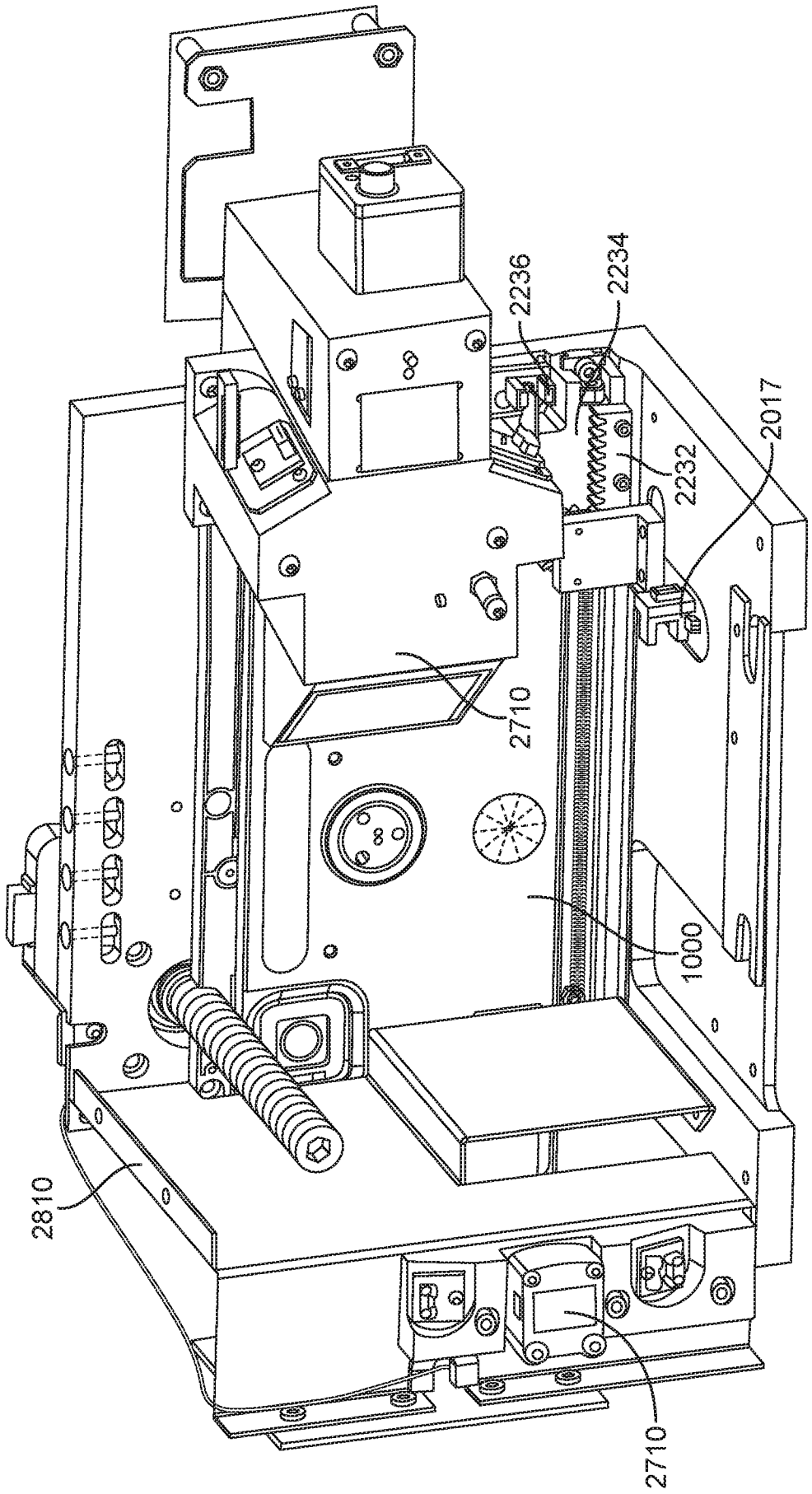


FIG. 66

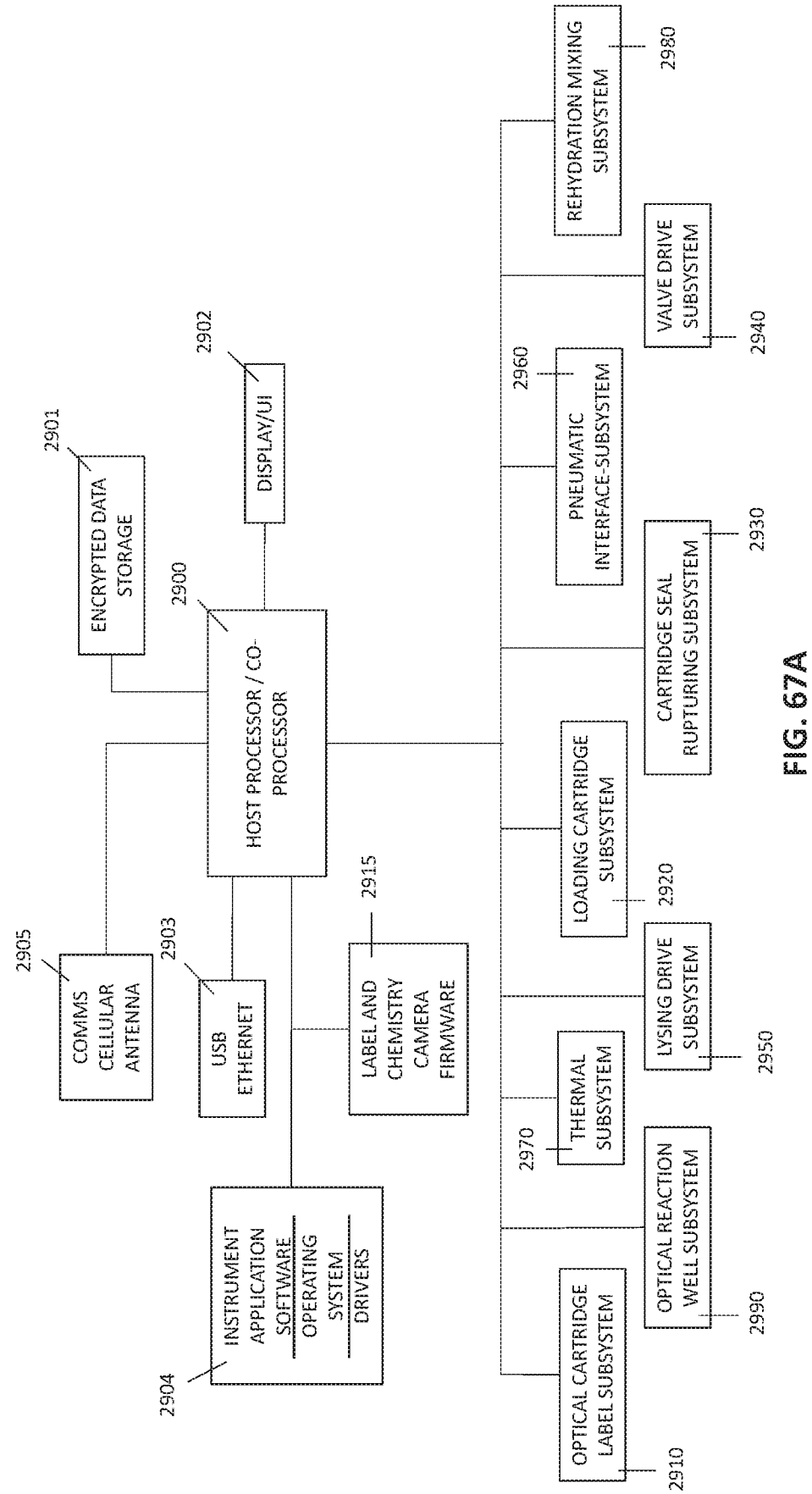

2901
ENCRYPTED DATA STORAGE
2902
DISPLAY/UI
2900
HOST PROCESSOR / CO-PROCESSOR
2905
COMMS CELLULAR ANTENNA
2903
USB ETHERNET
2915
LABEL AND CHEMISTRY CAMERA FIRMWARE
2904
INSTRUMENT APPLICATION SOFTWARE
OPERATING SYSTEM
DRIVERS
2960
PNEUMATIC INTERFACE-SUBSYSTEM
2980
REHYDRATION MIXING SUBSYSTEM
2940
VALVE DRIVE SUBSYSTEM
2930
CARTRIDGE SEAL RUPTURING SUBSYSTEM
2920
LOADING CARTRIDGE SUBSYSTEM
2950
LYSING DRIVE SUBSYSTEM
2970
THERMAL SUBSYSTEM
2990
OPTICAL REACTION WELL SUBSYSTEM
2910
OPTICAL CARTRIDGE LABEL SUBSYSTEM

FIG. 67A

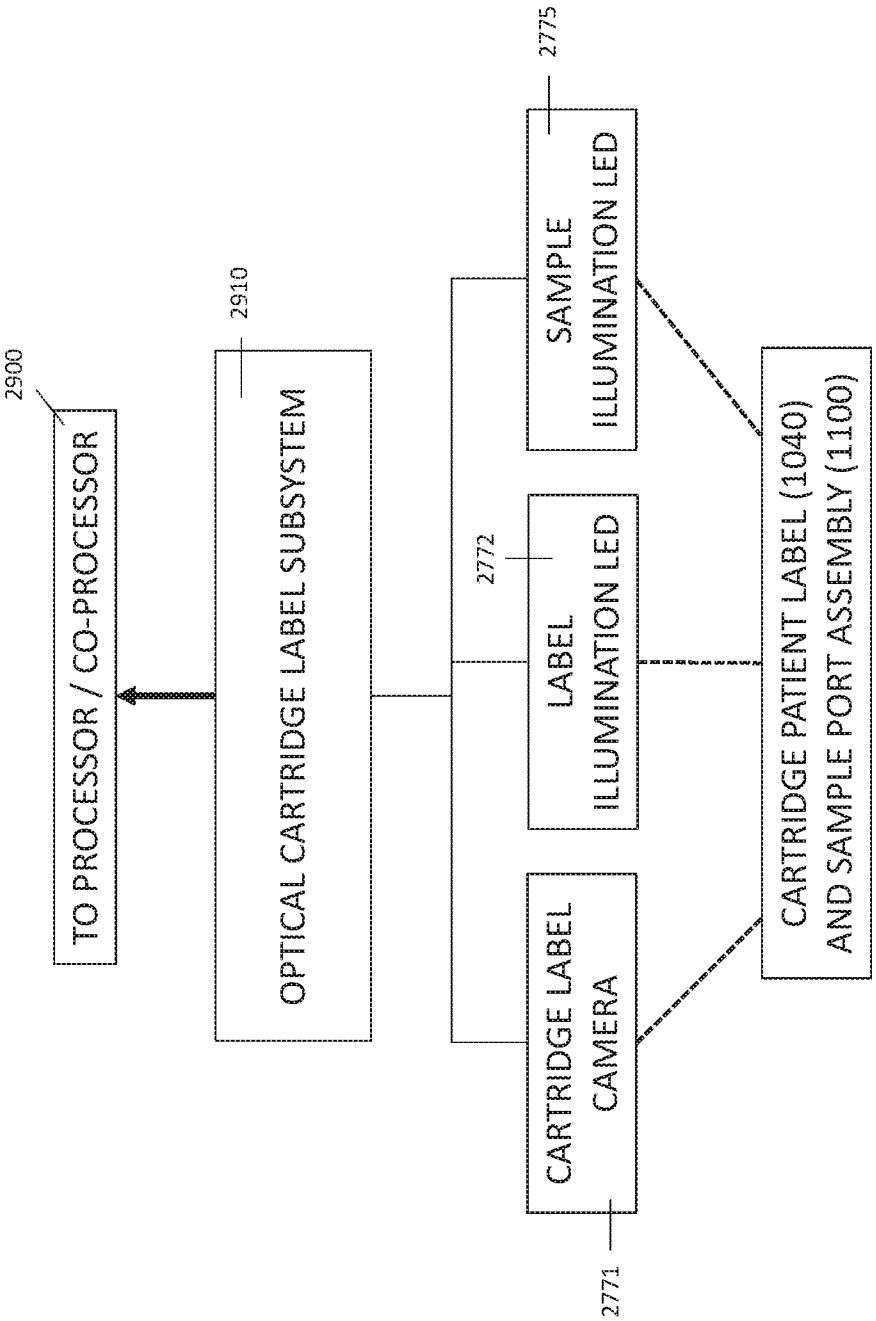

2900
TO PROCESSOR / CO-PROCESSOR
2910
OPTICAL CARTRIDGE LABEL SUBSYSTEM
2775
SAMPLE ILLUMINATION LED
2772
LABEL ILLUMINATION LED
2771
CARTRIDGE LABEL CAMERA
CARTRIDGE PATIENT LABEL (1040) AND SAMPLE PORT ASSEMBLY (1100)

FIG. 67B

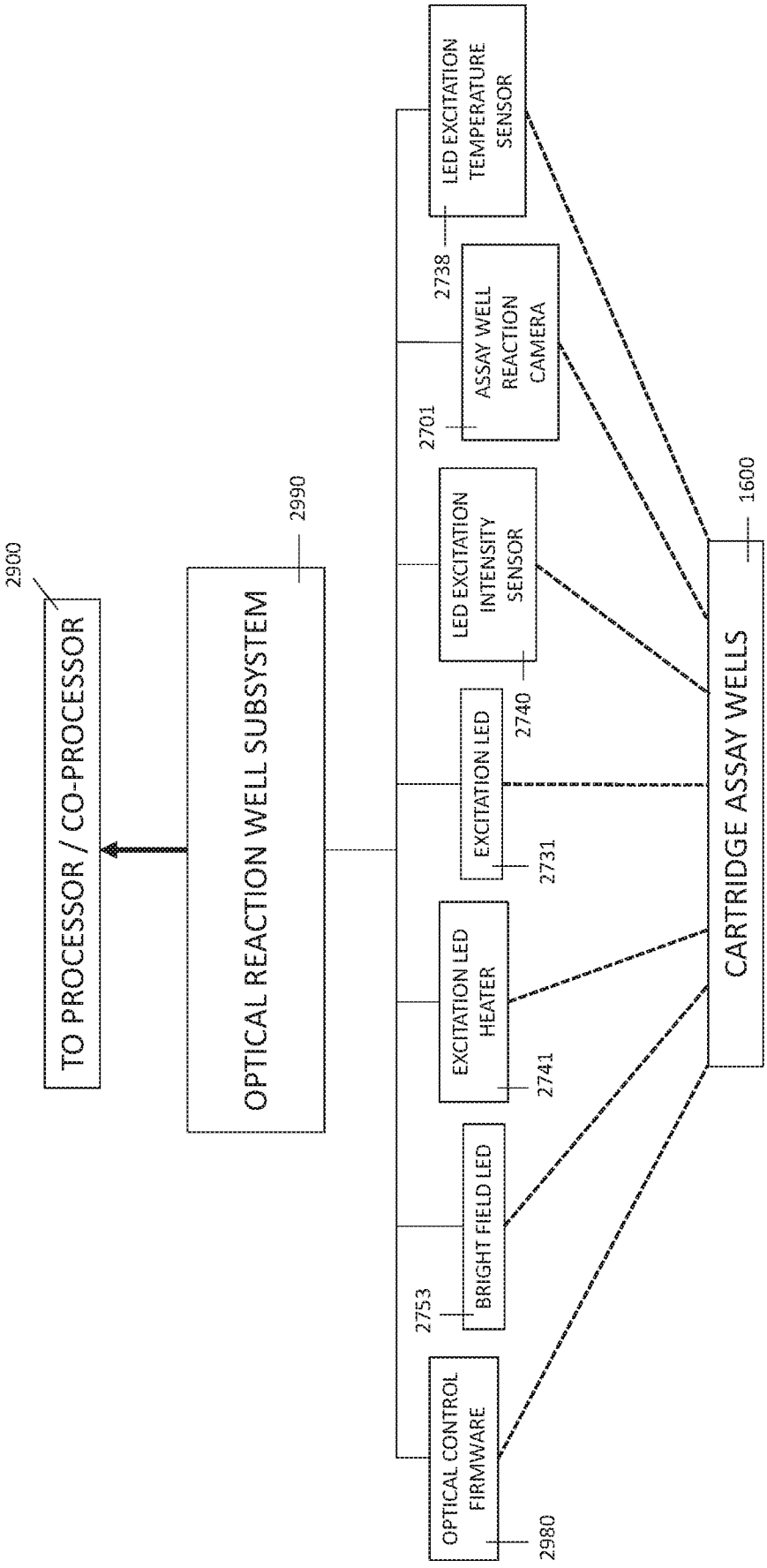

2900
TO PROCESSOR / CO-PROCESSOR
2990
OPTICAL REACTION WELL SUBSYSTEM
LED EXCITATION TEMPERATURE SENSOR
2738
ASSAY WELL REACTION CAMERA
2701
LED EXCITATION INTENSITY SENSOR
2740
EXCITATION LED
2731
EXCITATION LED HEATER
2741
BRIGHT FIELD LED
2753
OPTICAL CONTROL FIRMWARE
2980
1600
CARTRIDGE ASSAY WELLS

FIG. 67C

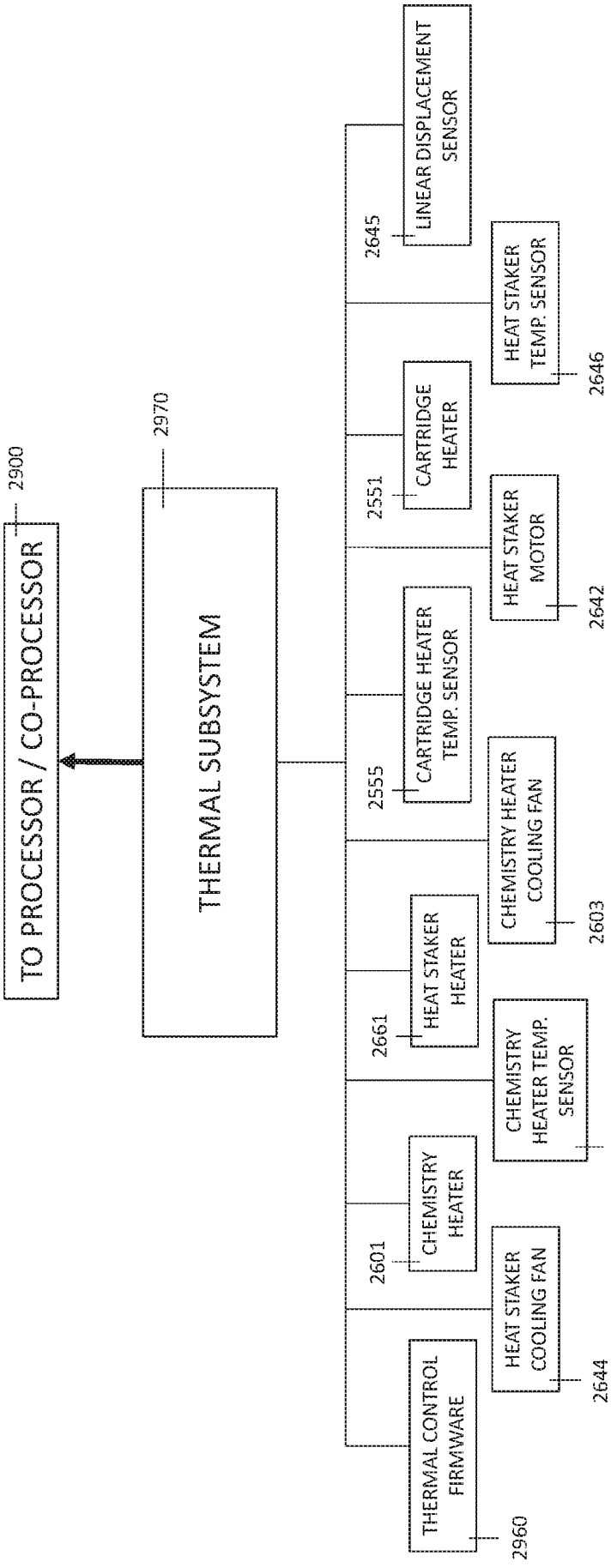

TO PROCESSOR / CO-PROCESSOR
2900
THERMAL SUBSYSTEM
2970
THERMAL CONTROL FIRMWARE
2960
2601
CHEMISTRY HEATER
2661
HEAT STAKER HEATER
2555
CARTRIDGE HEATER TEMP. SENSOR
2551
CARTRIDGE HEATER
2645
LINEAR DISPLACEMENT SENSOR
HEAT STAKER COOLING FAN
2644
CHEMISTRY HEATER TEMP. SENSOR
2608
CHEMISTRY HEATER COOLING FAN
2603
HEAT STAKER MOTOR
2642
HEAT STAKER TEMP. SENSOR
2646

FIG. 67D

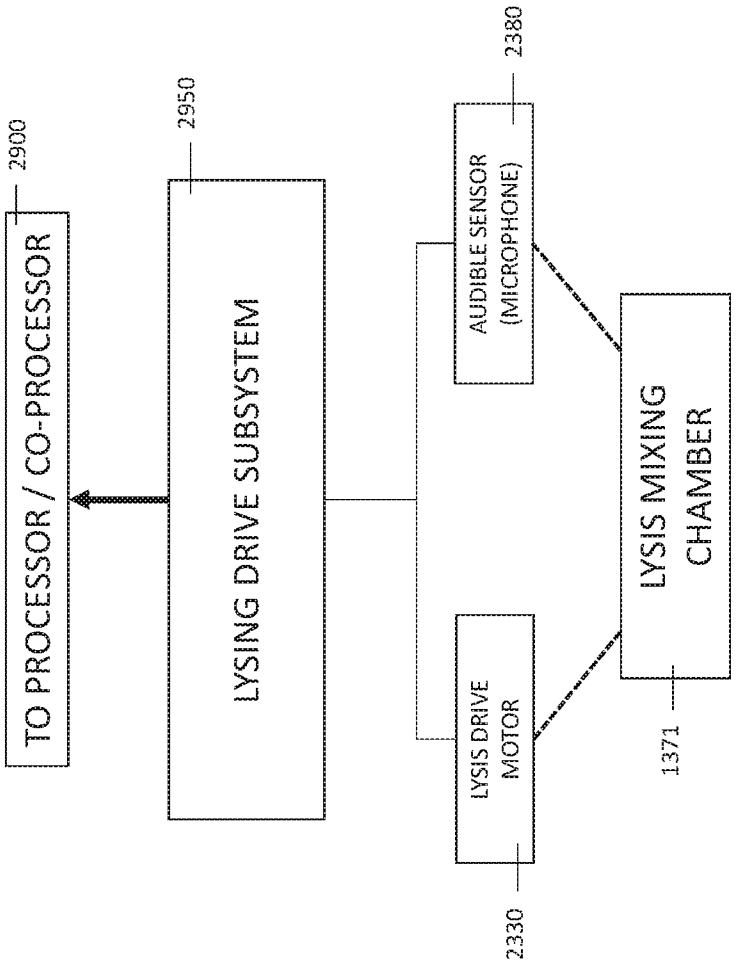

2900
TO PROCESSOR / CO-PROCESSOR
2950
LYSING DRIVE SUBSYSTEM
2380
AUDIBLE SENSOR (MICROPHONE)
LYSIS DRIVE MOTOR
2330
LYSIS MIXING CHAMBER
1371

FIG. 67E

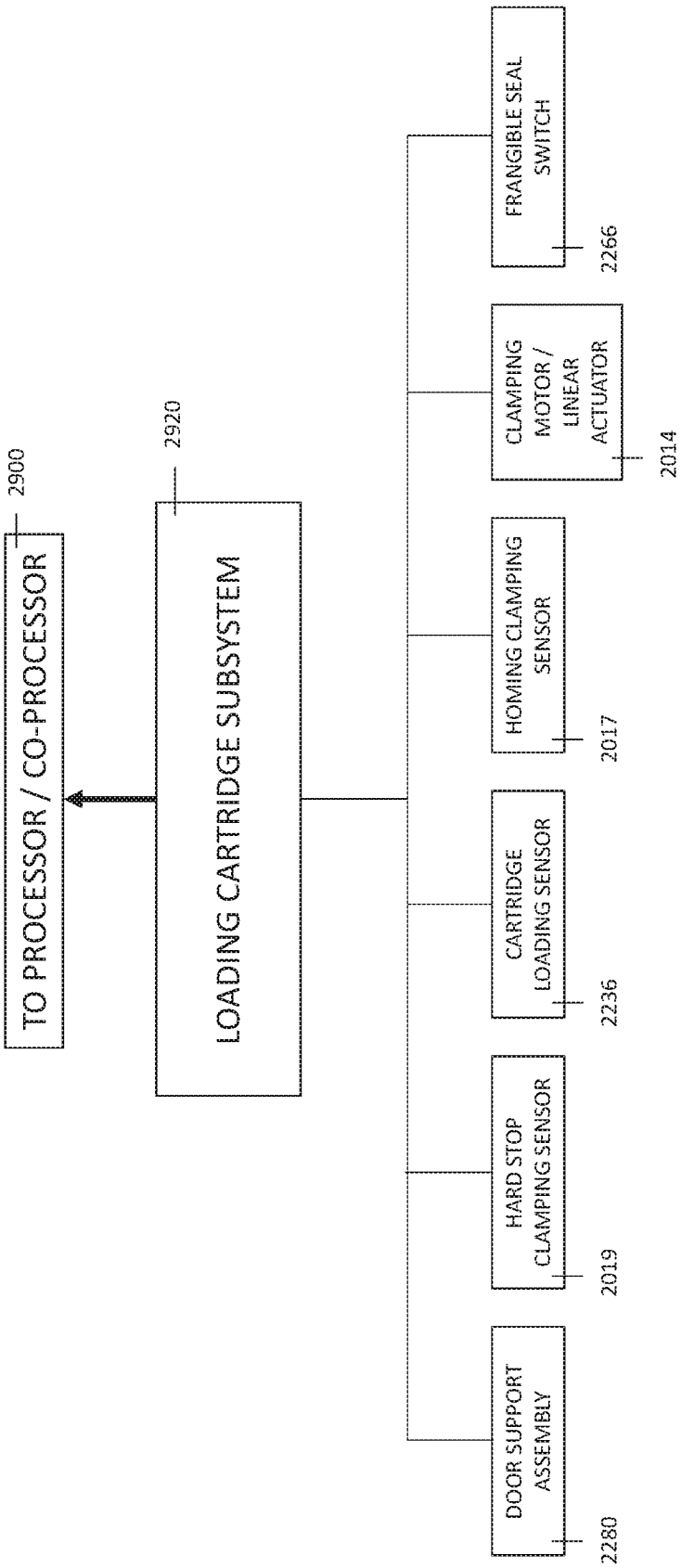

TO PROCESSOR / CO-PROCESSOR
2900
LOADING CARTRIDGE SUBSYSTEM
2920
DOOR SUPPORT ASSEMBLY
2280
HARD STOP CLAMPING SENSOR
2019
CARTRIDGE LOADING SENSOR
2236
HOMING CLAMPING SENSOR
2017
CLAMPING MOTOR / LINEAR ACTUATOR
2014
FRANGIBLE SEAL SWITCH
2266

FIG. 67F

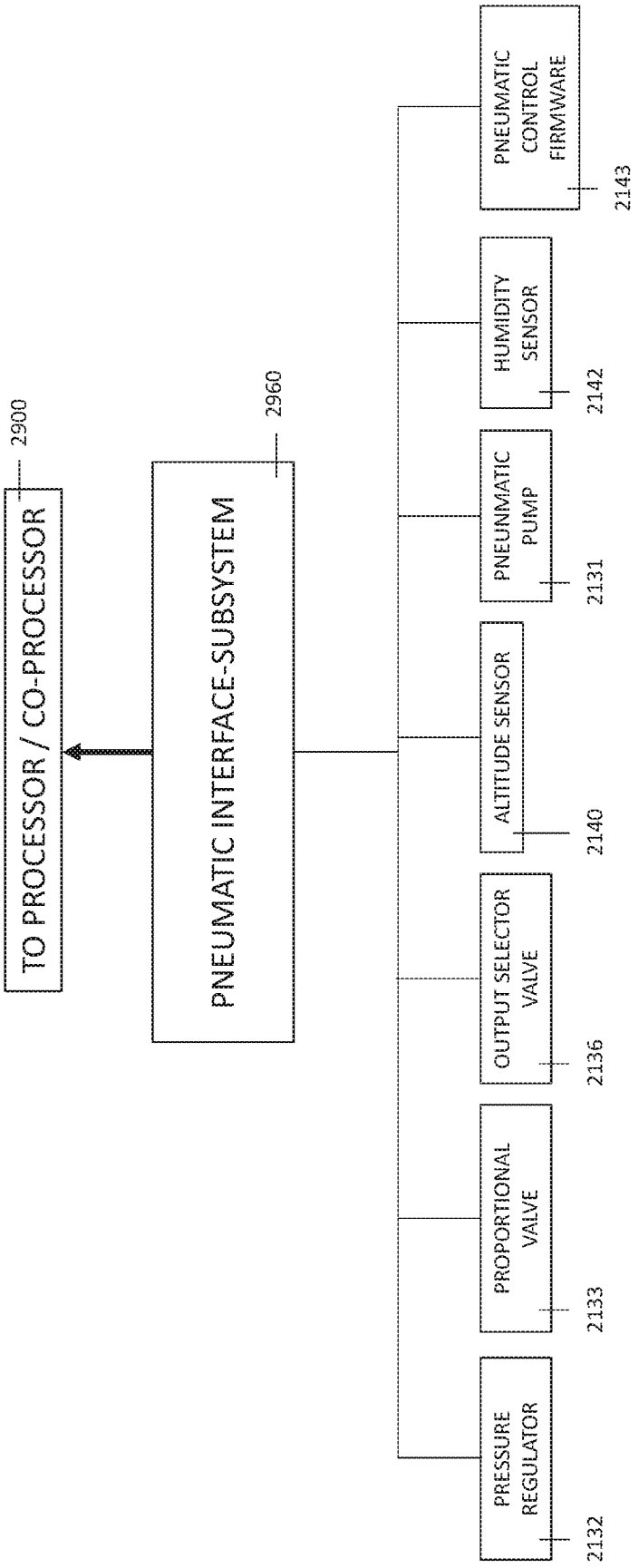

TO PROCESSOR / CO-PROCESSOR
2900
PNEUMATIC INTERFACE-SUBSYSTEM
2960
PRESSURE REGULATOR
2132
PROPORTIONAL VALVE
2133
OUTPUT SELECTOR VALVE
2136
ALTITUDE SENSOR
2140
PNEUNMATIC PUMP
2131
HUMIDITY SENSOR
2142
PNEUMATIC CONTROL FIRMWARE
2143

FIG. 67G

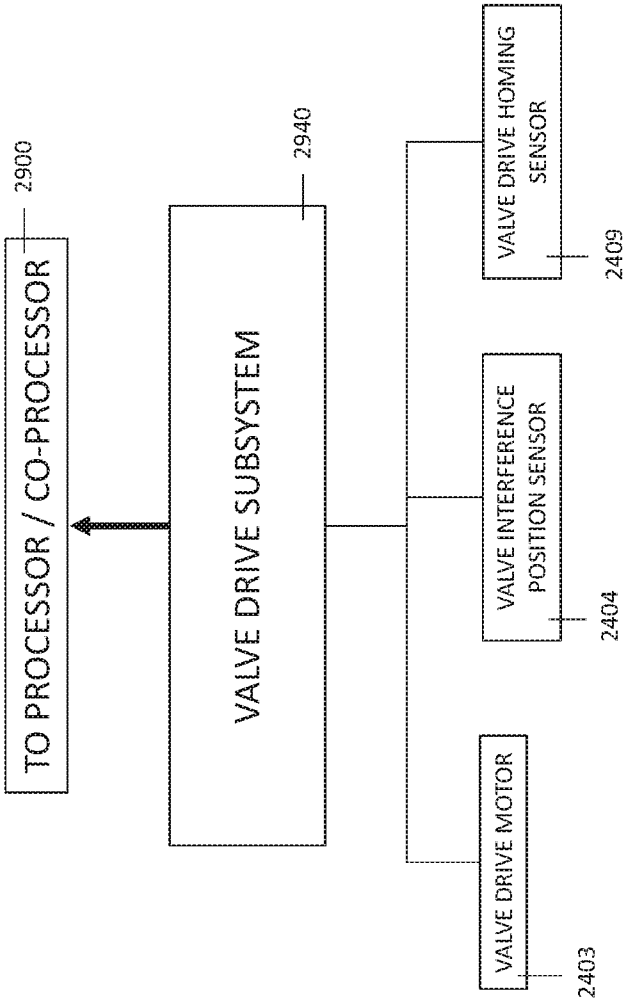

TO PROCESSOR / CO-PROCESSOR
2900
VALVE DRIVE SUBSYSTEM
2940
VALVE DRIVE MOTOR
2403
VALVE INTERFERENCE POSITION SENSOR
2404
VALVE DRIVE HOMING SENSOR
2409

FIG. 67H

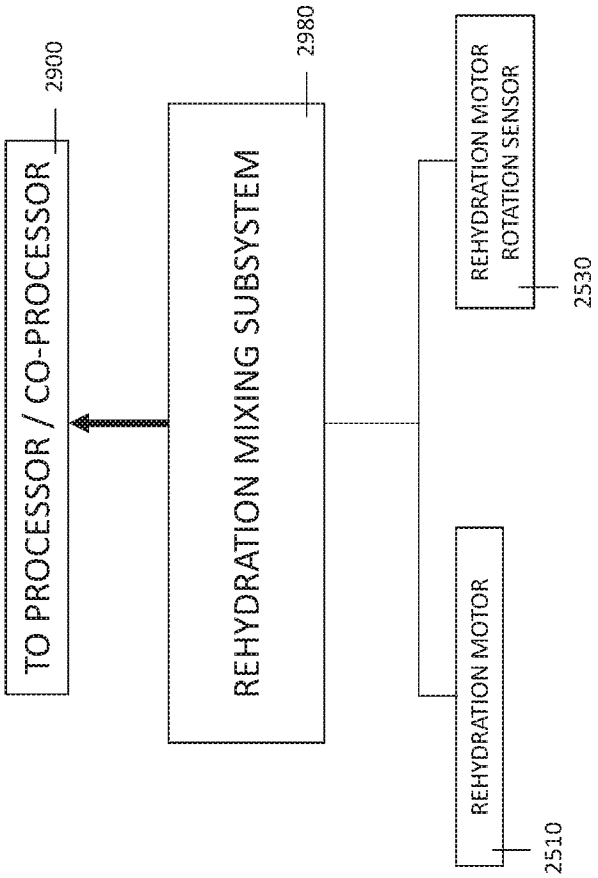

TO PROCESSOR / CO-PROCESSOR
2900
REHYDRATION MIXING SUBSYSTEM
2980
REHYDRATION MOTOR
2510
REHYDRATION MOTOR ROTATION SENSOR
2530

FIG. 67I

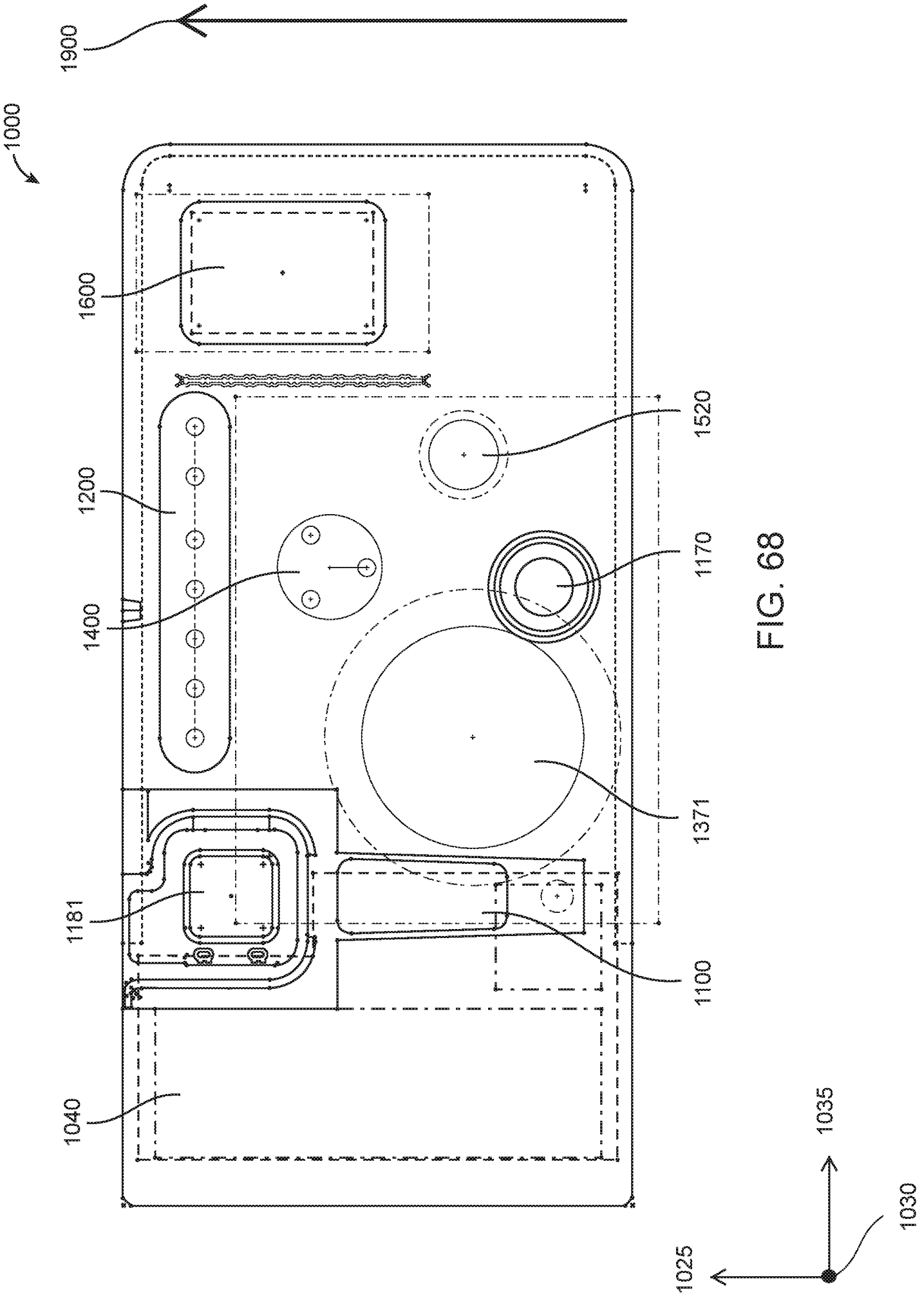

1900
1000
1600
1200
1400
1520
1170
1371
1181
1100
1040
1035
1030
1025

FIG. 68

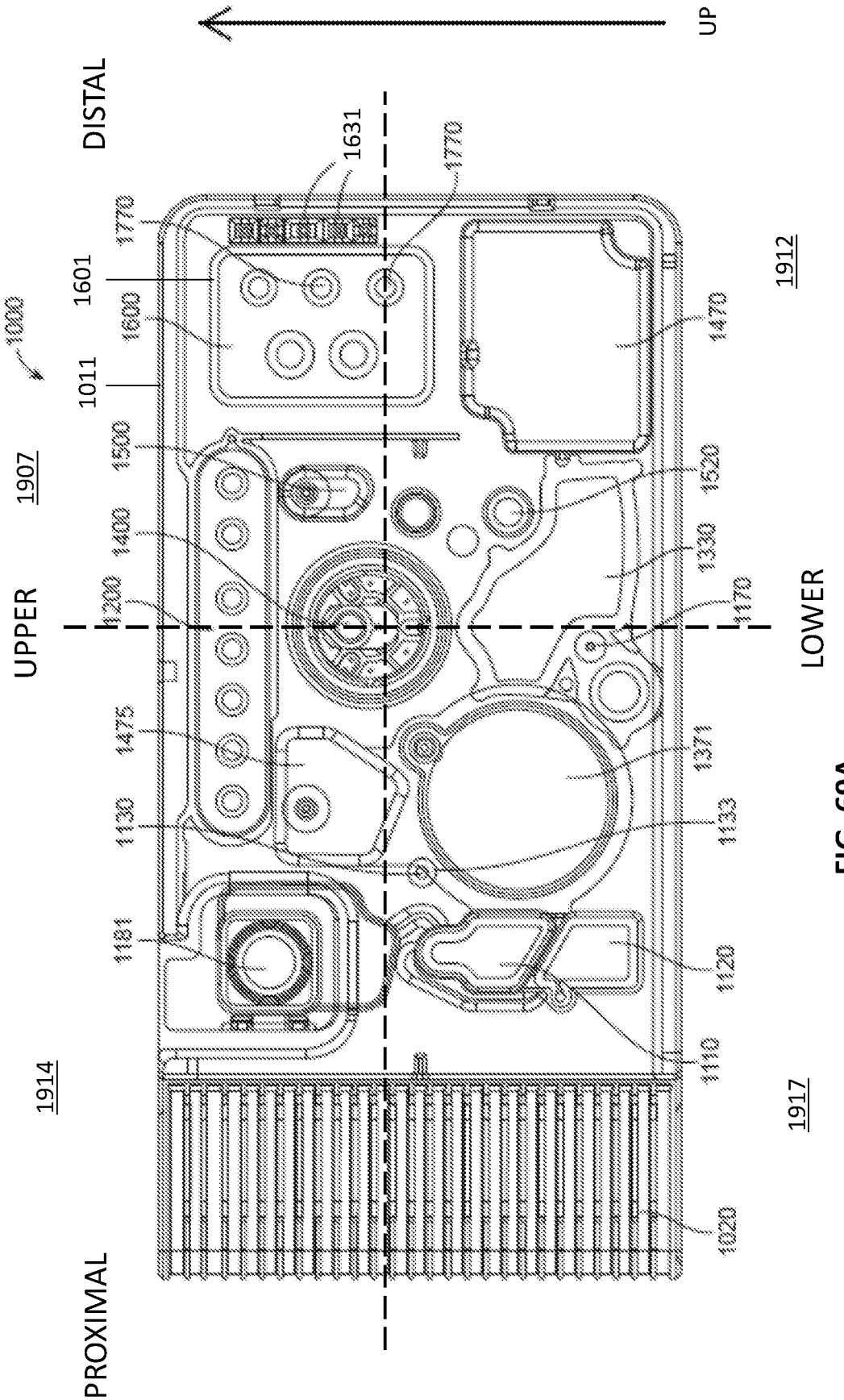

UP
DISTAL
PROXIMAL
UPPER
LOWER
1000
1907
1912
1914
1917
1631
1770
1601
1600
1011
1500
1400
1200
1475
1130
1181
1470
1620
1330
1170
1371
1133
1120
1110
1020

FIG. 69A

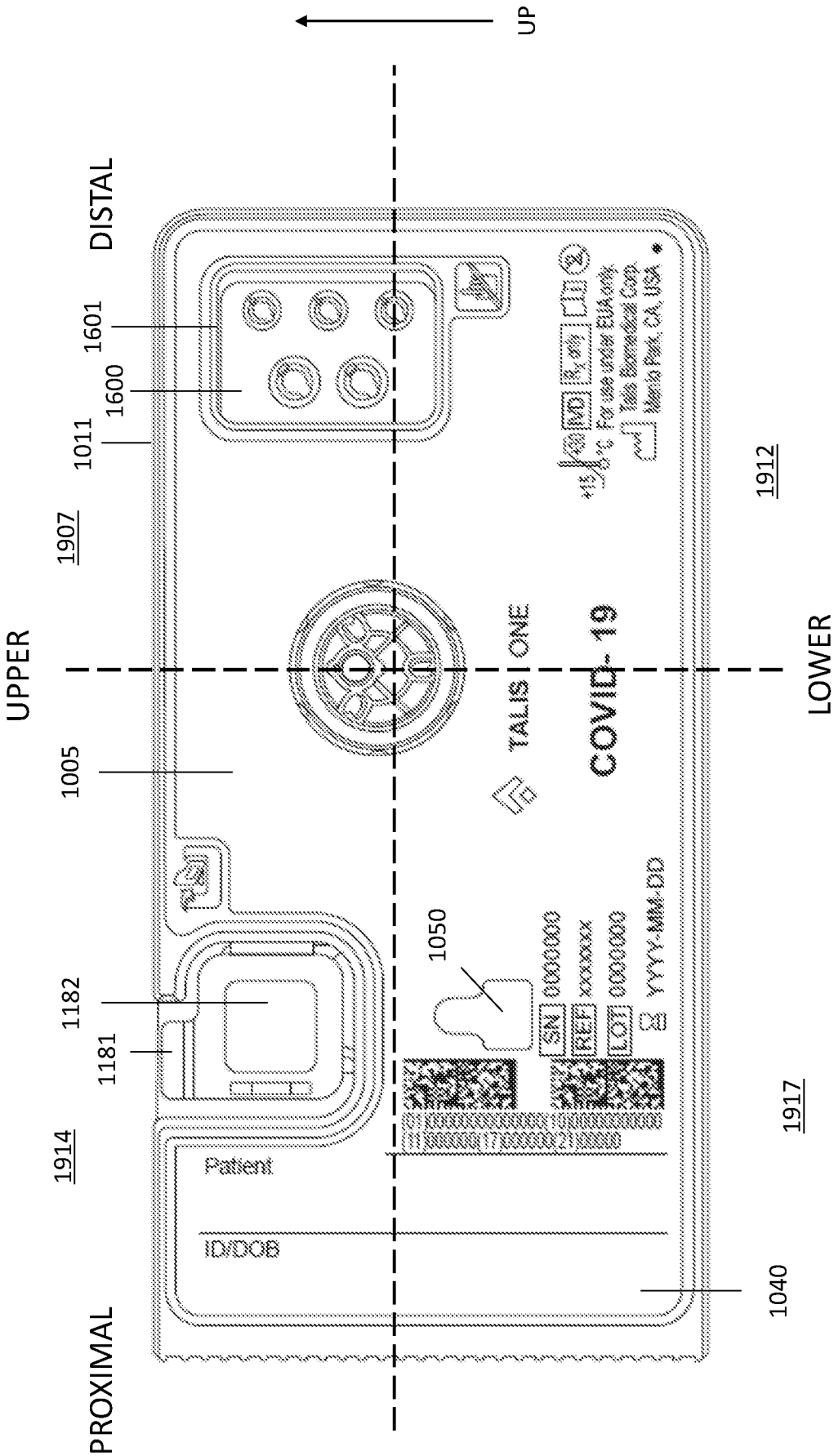

UP
DISTAL
PROXIMAL
UPPER
LOWER
1601
1600
1011
1907
1912
1005
1182
1181
1914
1917
1040
1050
TALIS ONE
COVID-19
Rx only
For use under EUA only
Talis Biomedical Corp.
Menlo Park, CA, USA
SN 000000
REF xxxxxx
LOT 000000
YYYY-MM-DD
Patient
ID/DOB

FIG. 69B

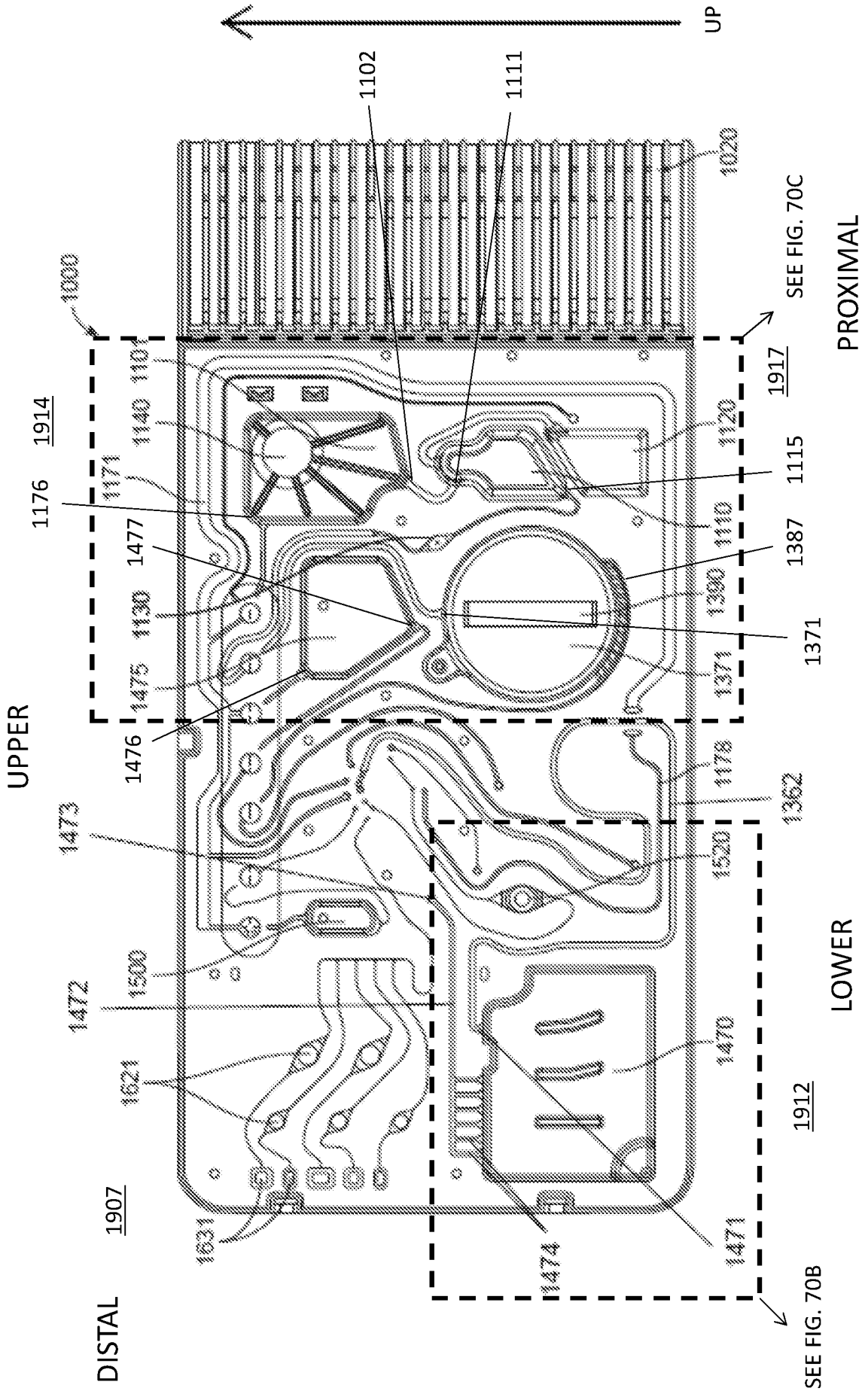

UP
1102
1111
1020
1000
1914
1104
1140
1171
1176
1477
1130
1475
1476
1917
SEE FIG. 70C
PROXIMAL
1120
1115
1110
1387
1390
1371
1371
UPPER
1178
1362
1473
1520
1500
1472
1621
1470
1912
LOWER
1907
DISTAL
1631
1474
1471
SEE FIG. 70B

FIG. 70A

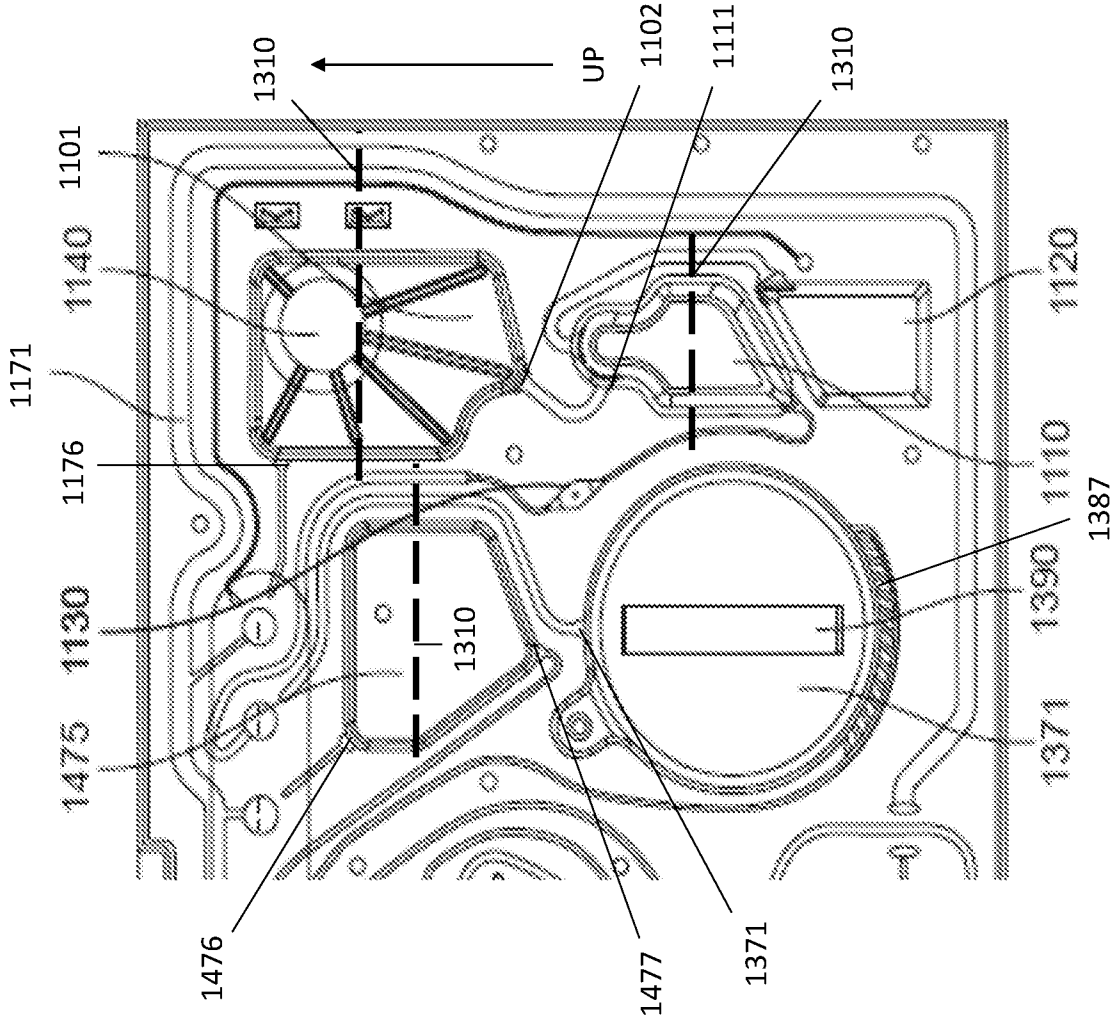


FIG. 70C

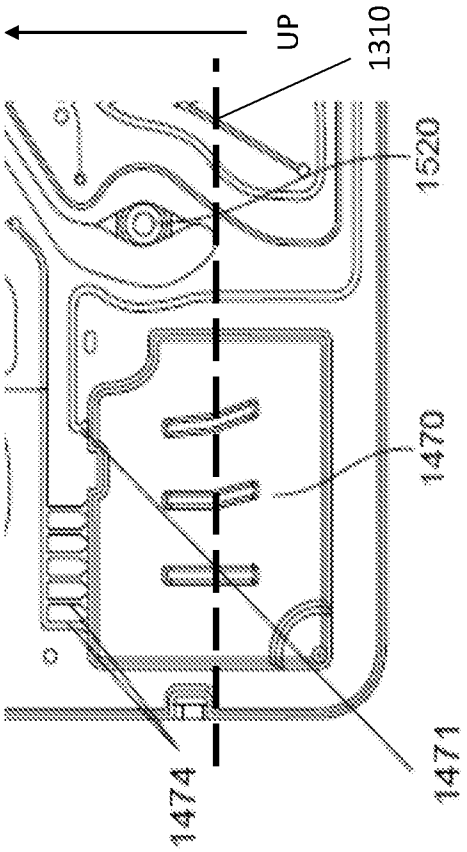


FIG. 70B

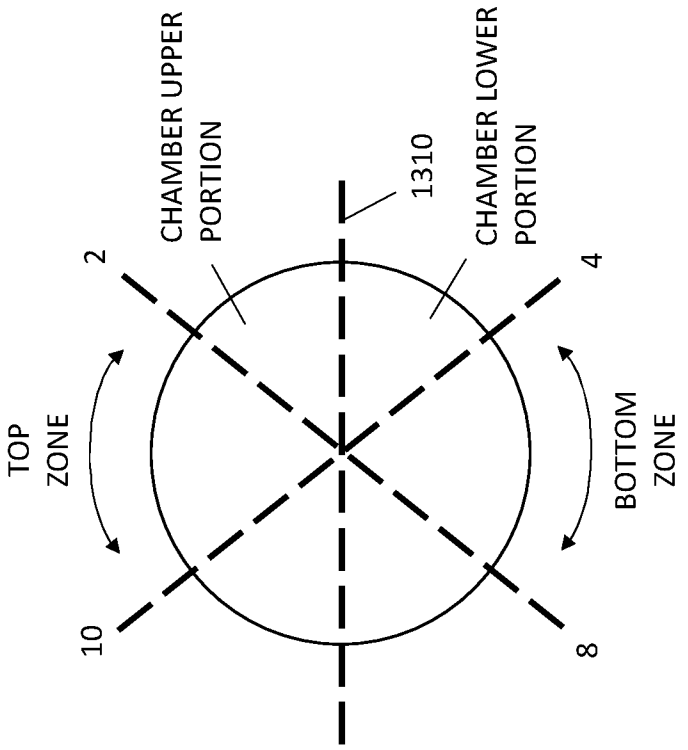


FIG. 70F

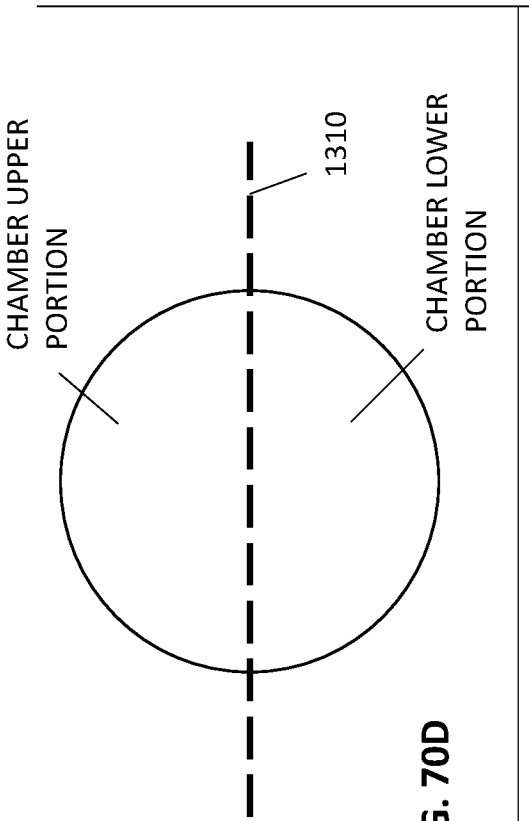


FIG. 70D

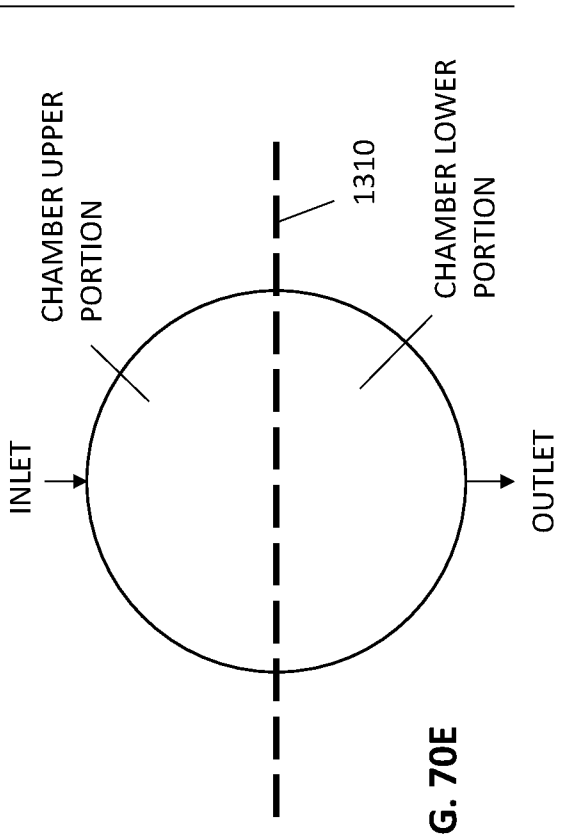


FIG. 70E

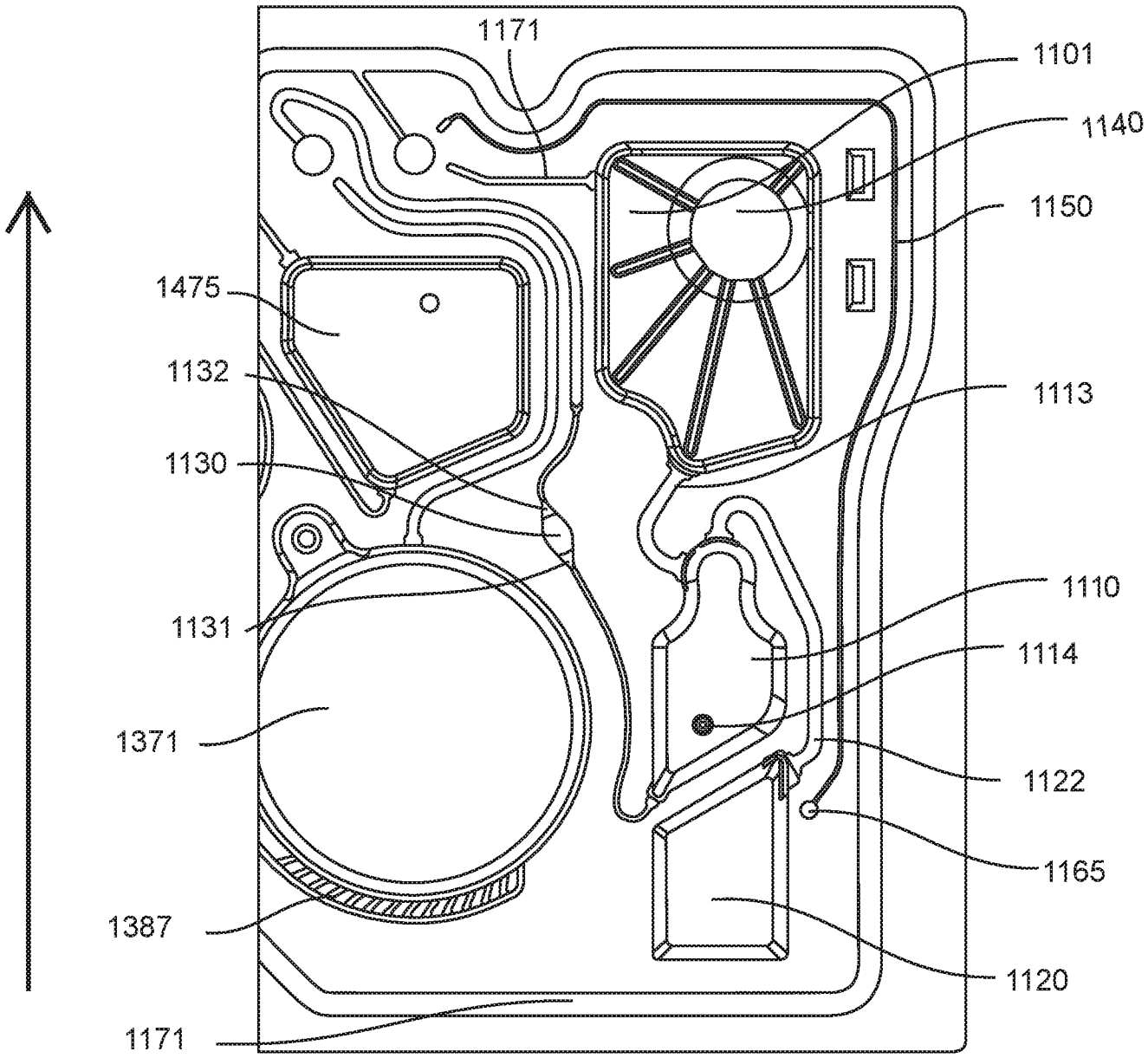

1171
1101
1140
1150
1475
1132
1113
1130
1110
1131
1114
1371
1122
1165
1387
1120
1171

FIG. 71

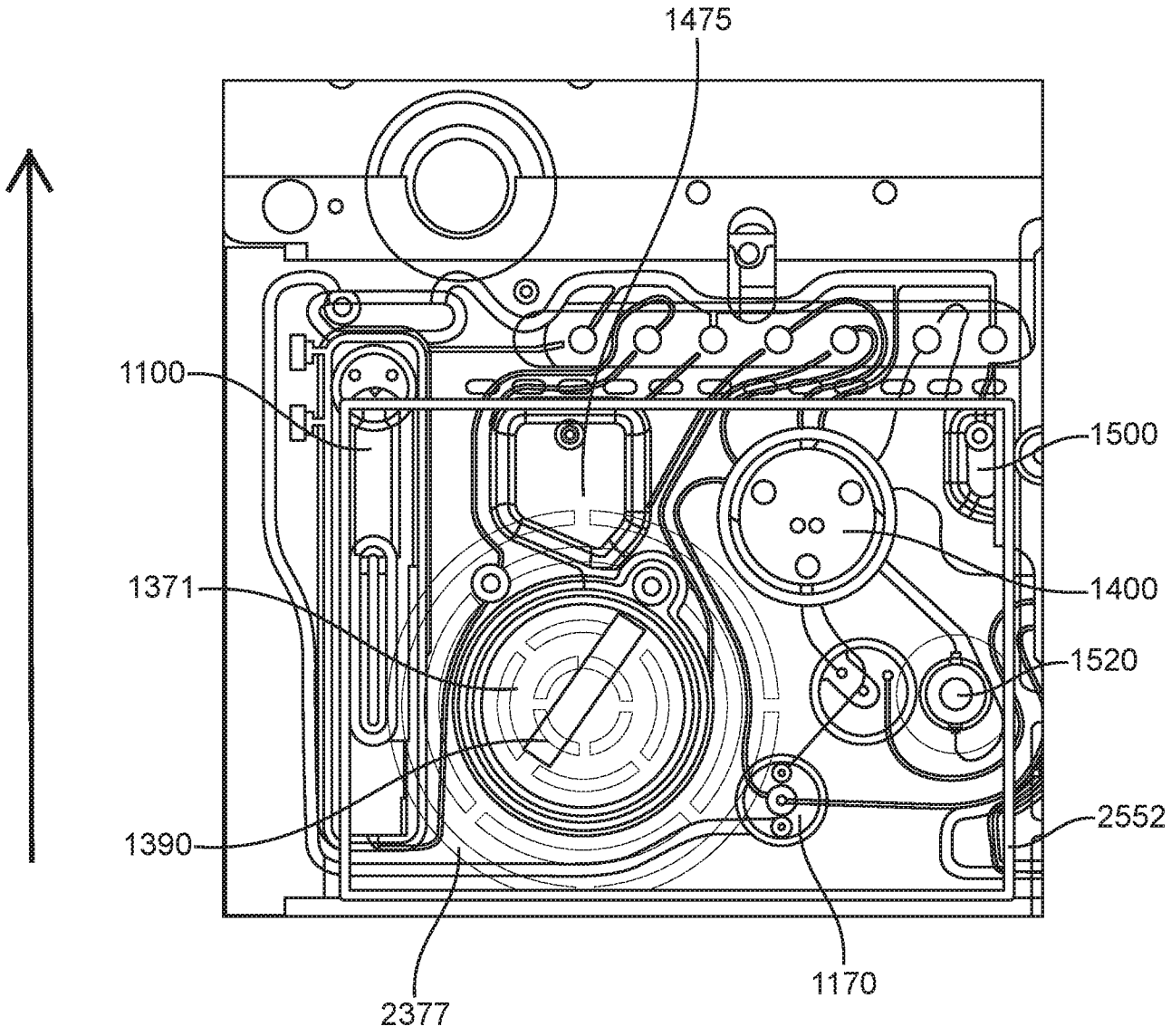


FIG. 72

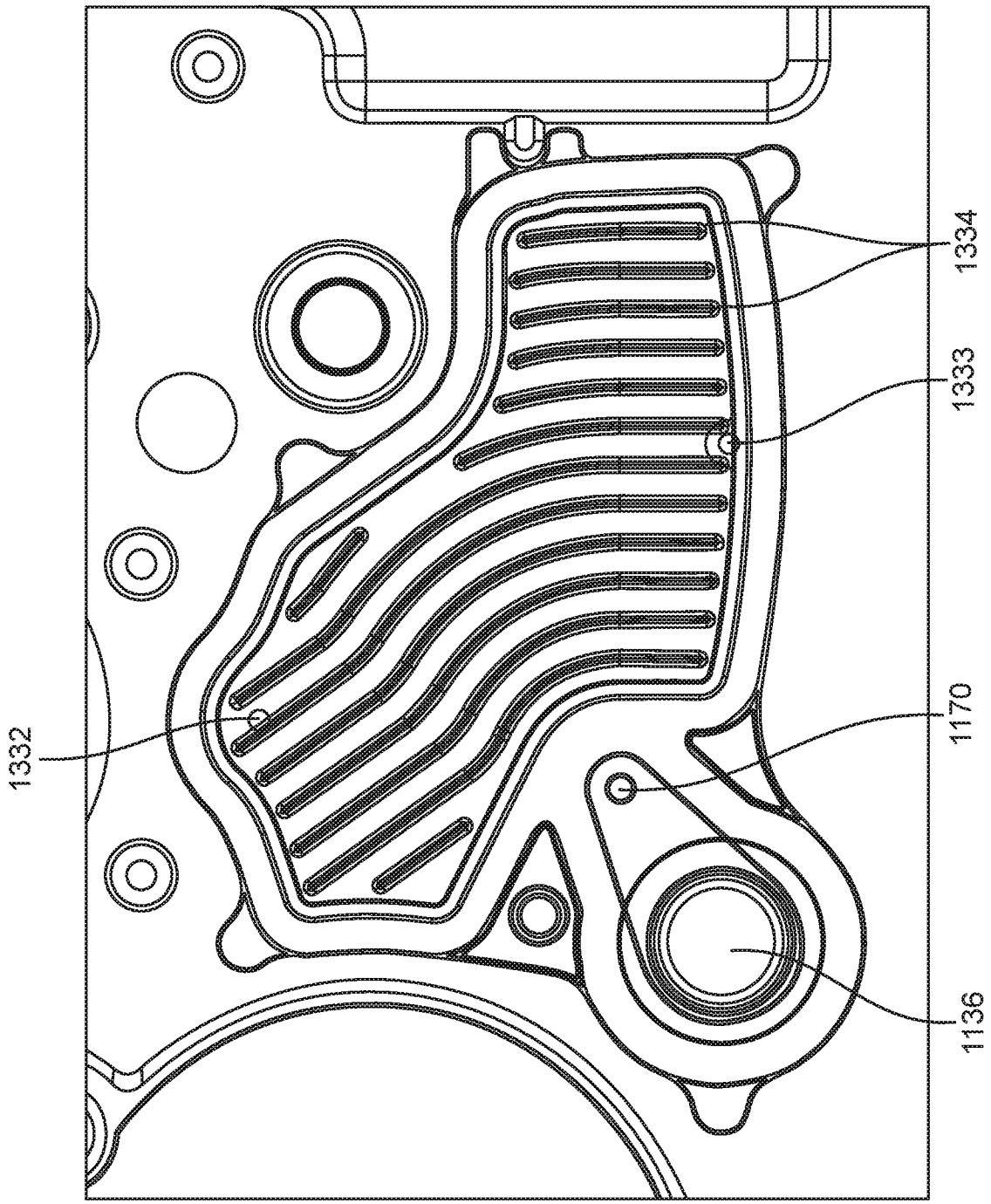


FIG. 73

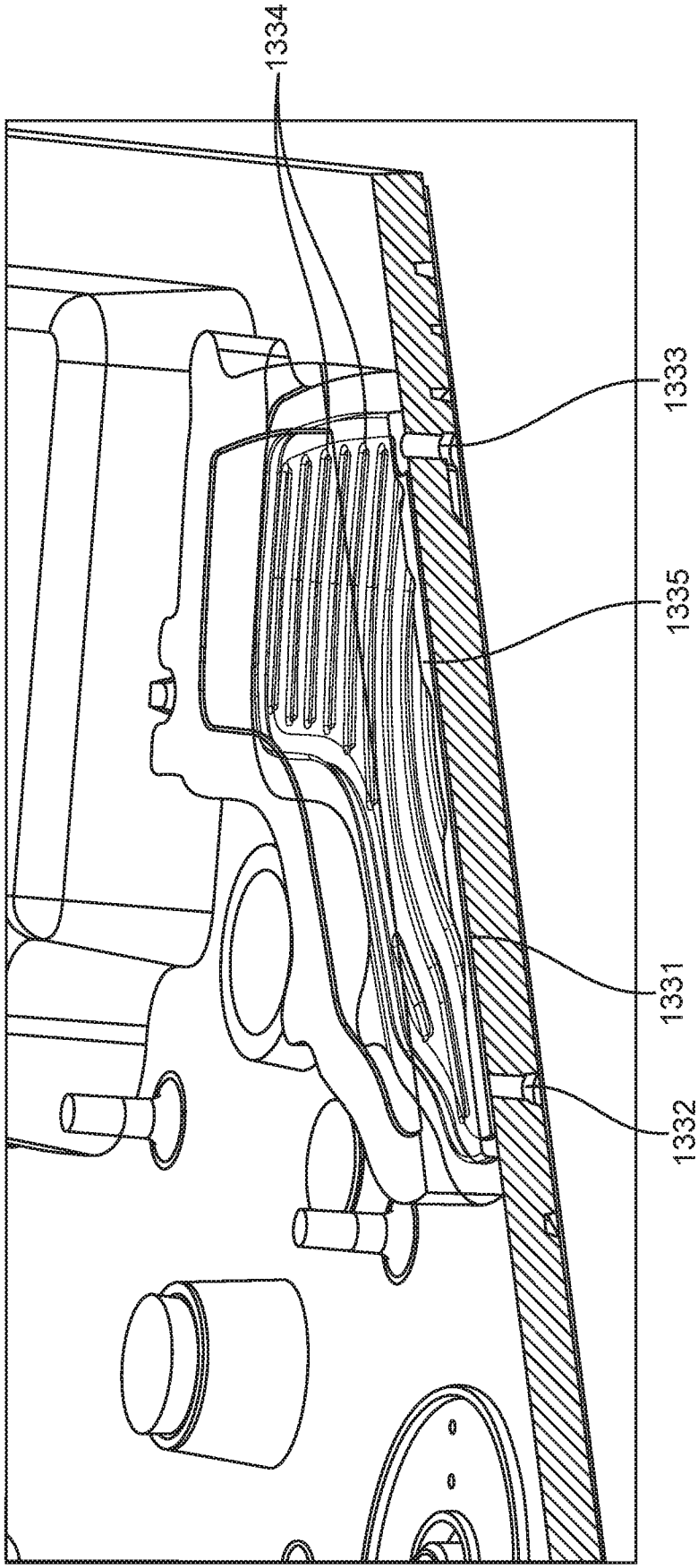


FIG. 74

FIG. 75A

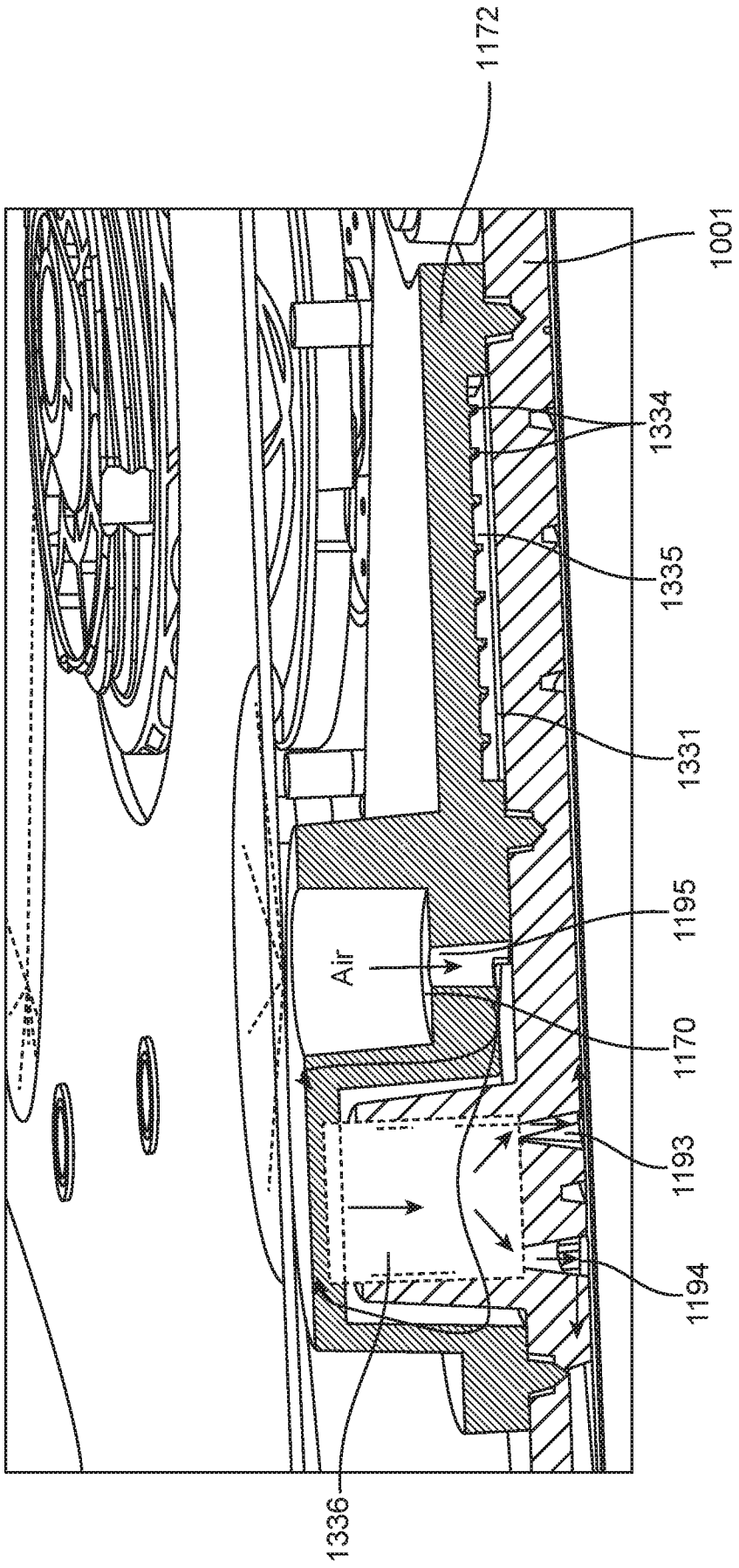


FIG. 75B

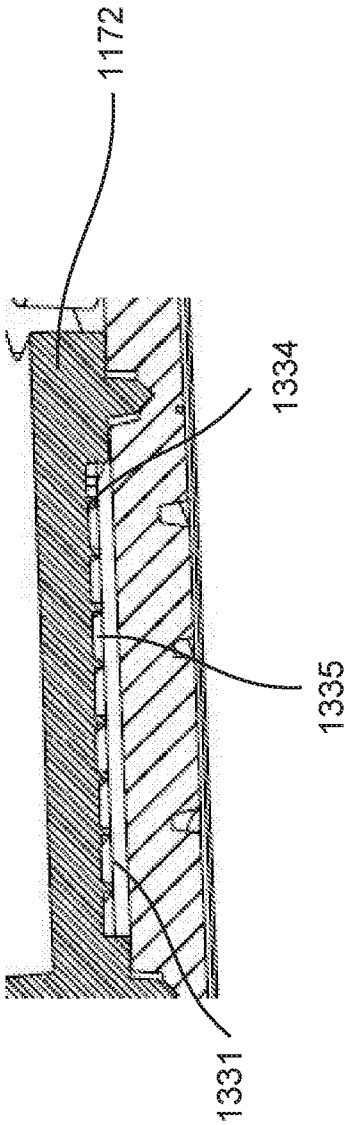


FIG. 76A

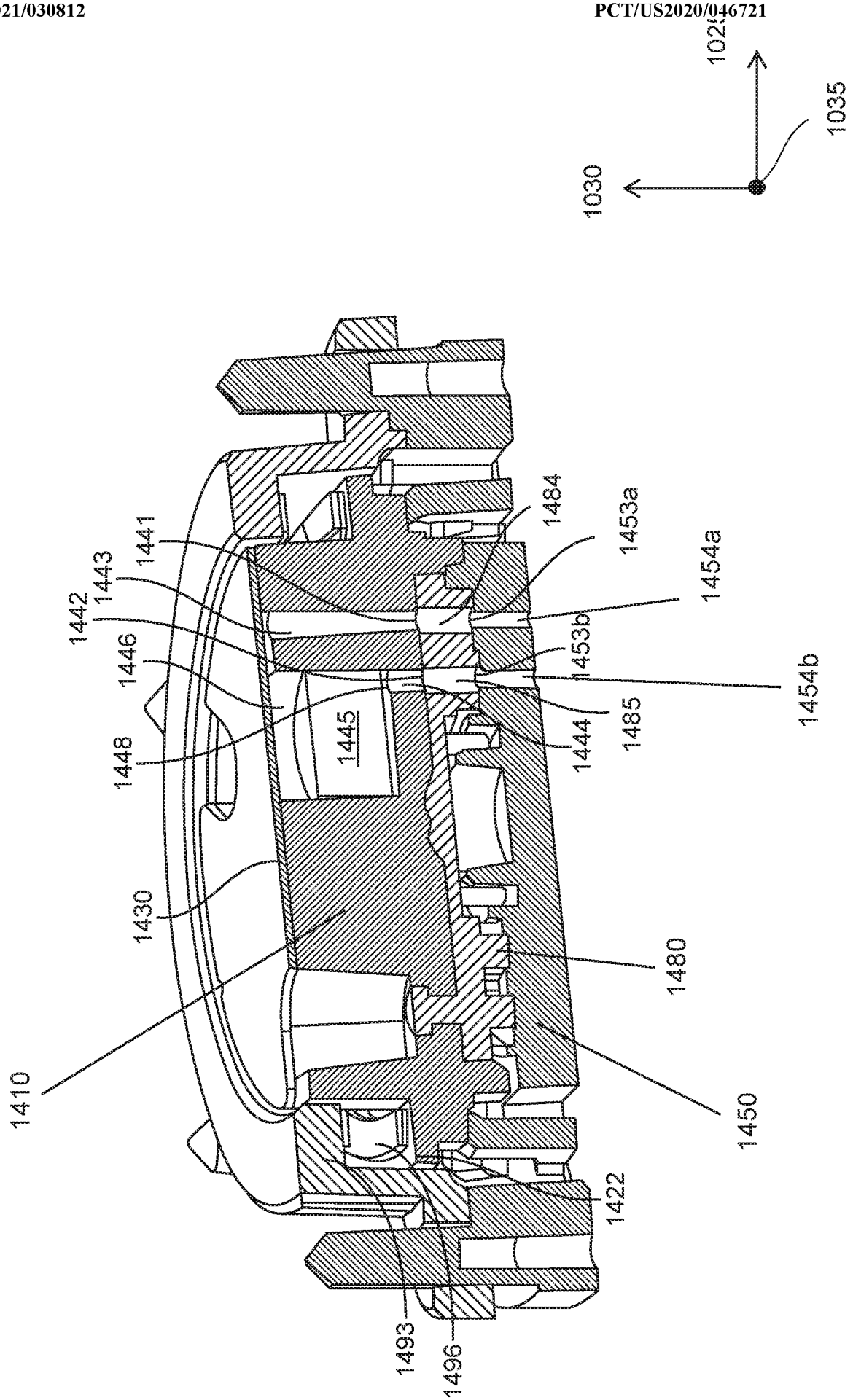

1021
1035
1030
1484
1453a
1454a
1441
1443
1442
1446
1453b
1454b
1448
1445
1485
1444
1430
1480
1410
1450
1422
1493
1496

FIG. 76B

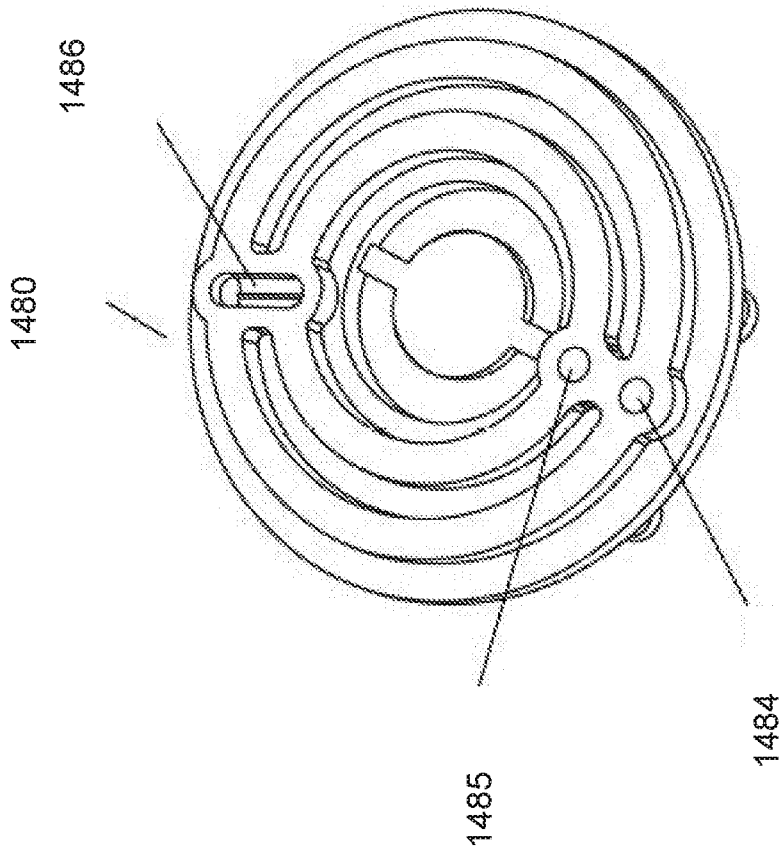
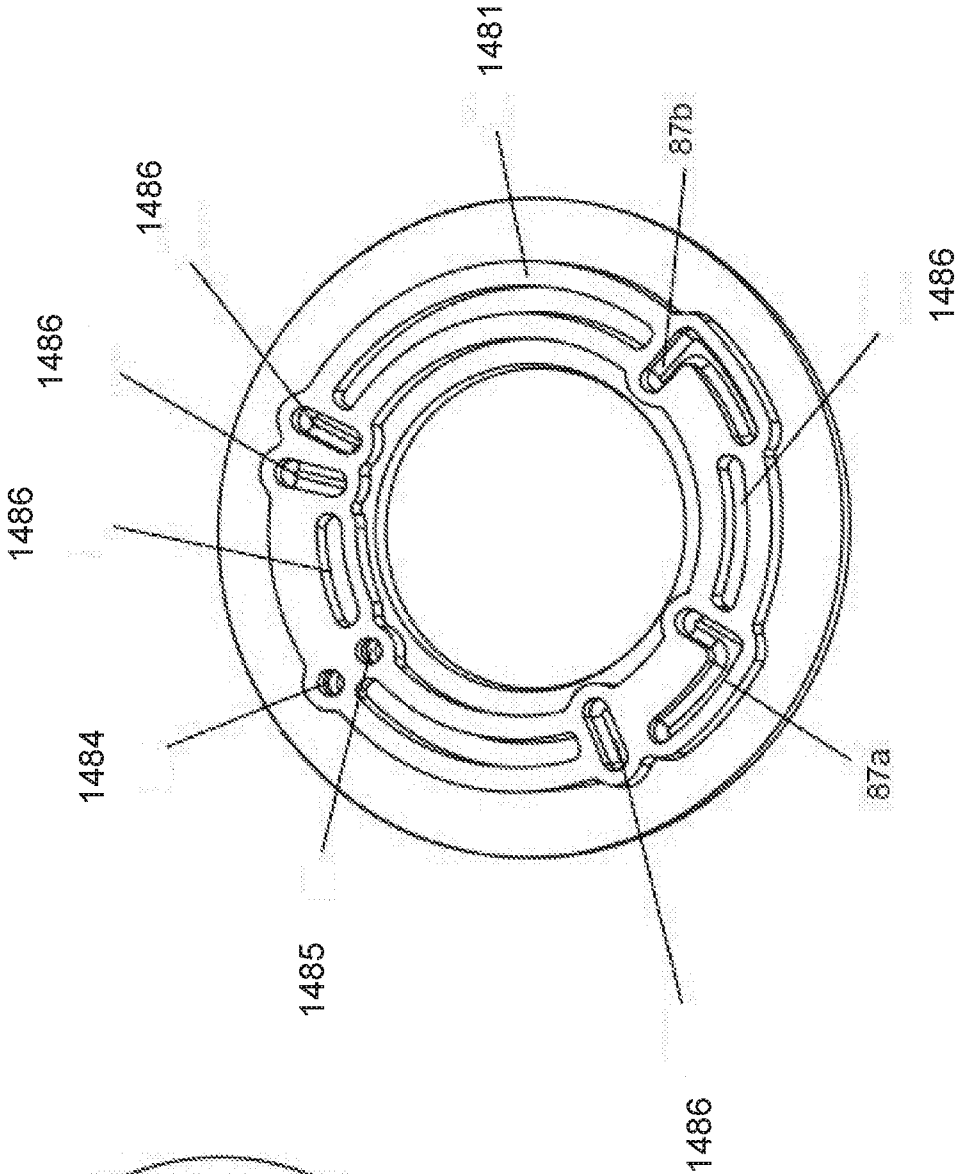


FIG. 76C



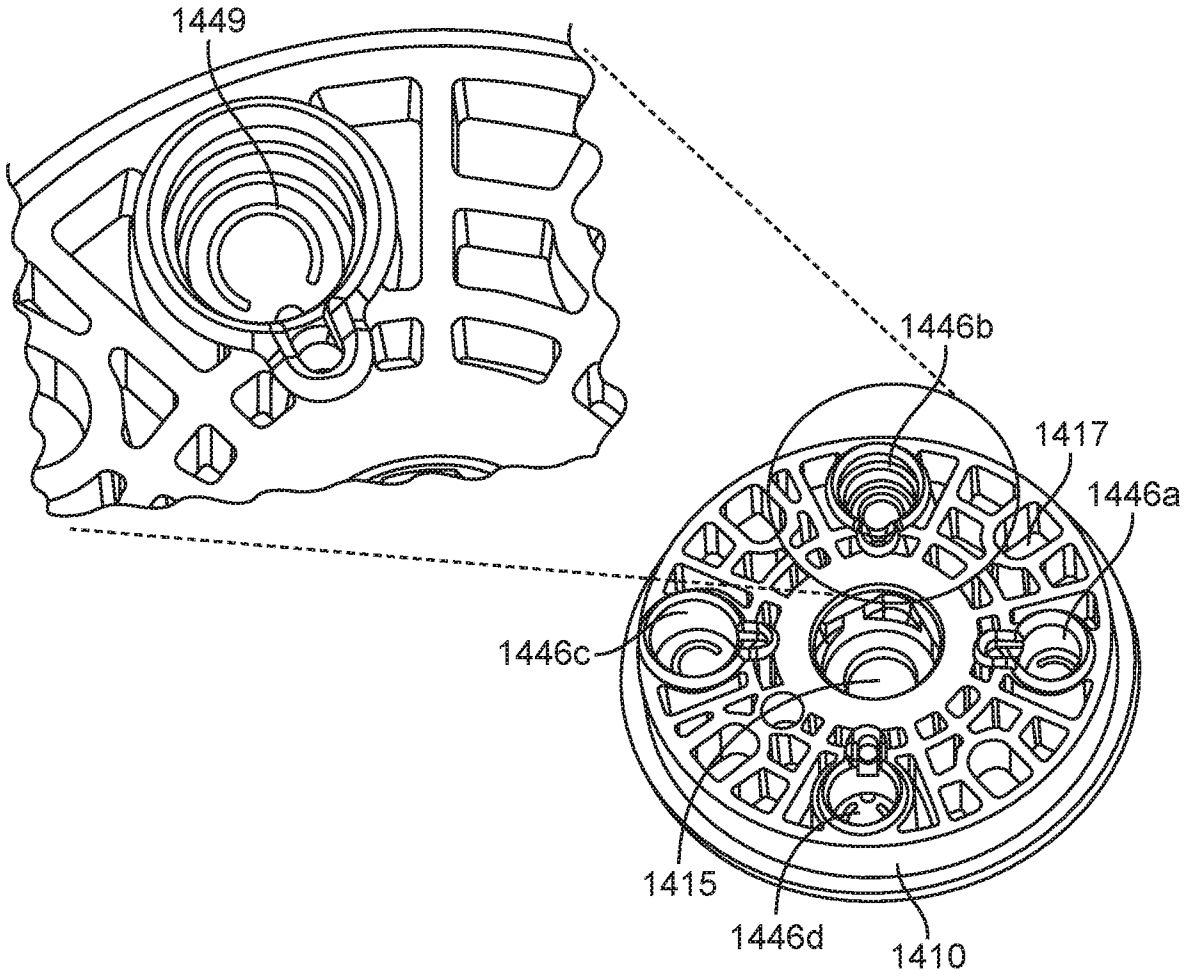


FIG. 77

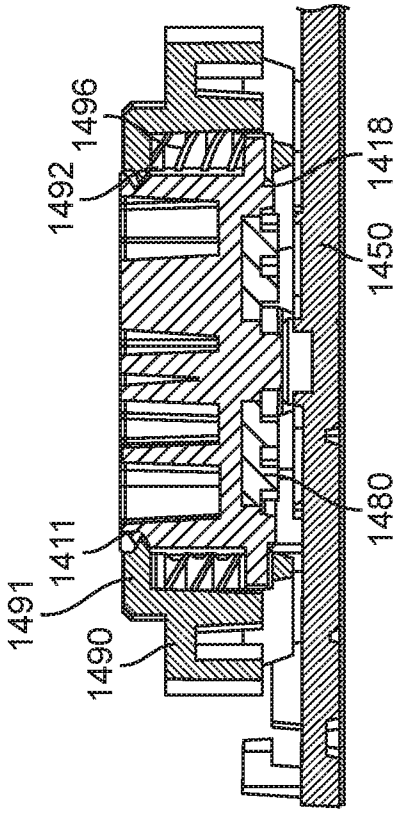

1496
1492
1418
1450
1480
1411
1491
1490

FIG. 79

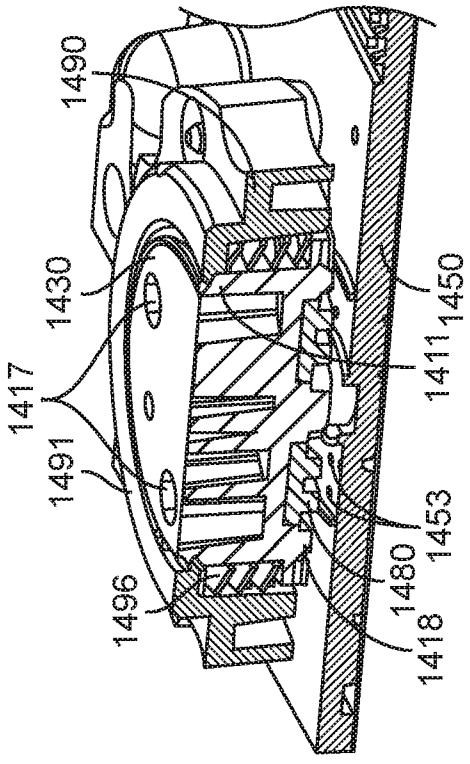

1490
1430
1450
1417
1411
1491
1453
1480
1496
1418

FIG. 78

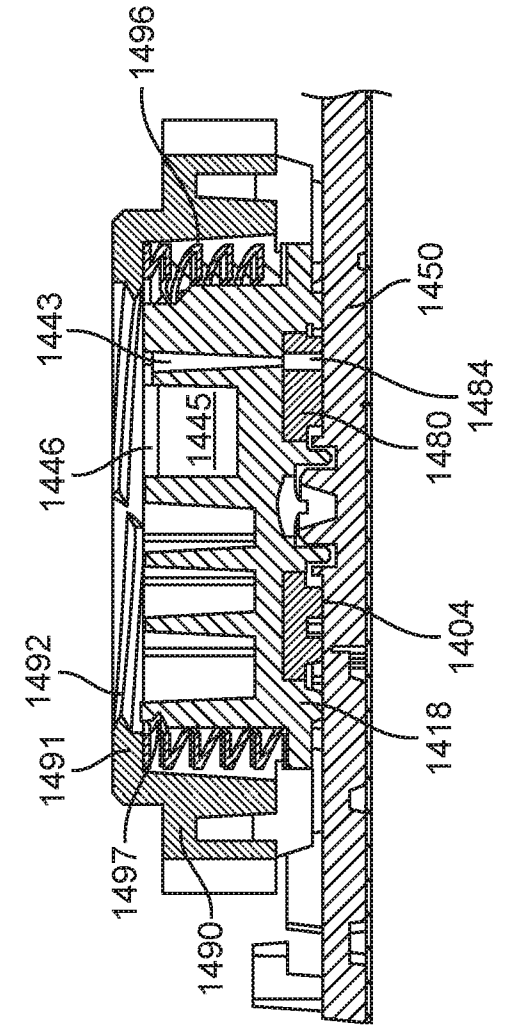

1496
1450
1443
1484
1445
1480
1446
1404
1492
1418
1491
1497
1490

FIG. 81

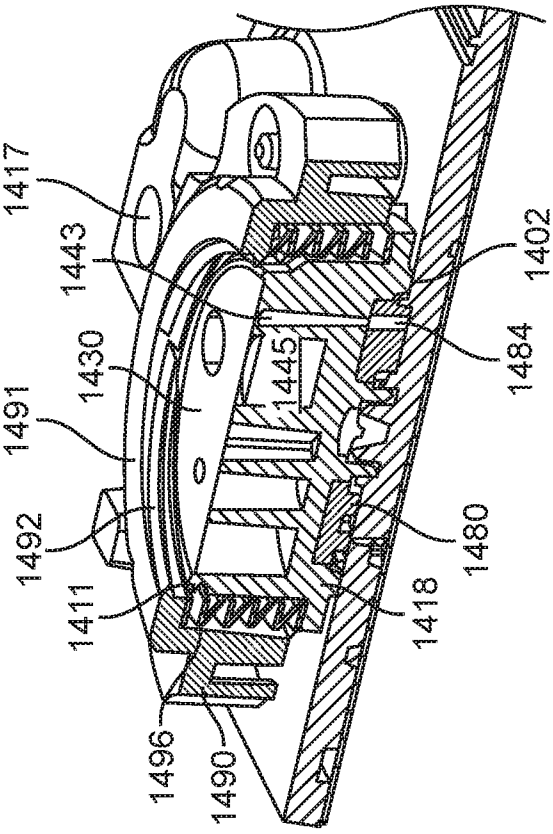

1417
1443
1402
1484
1445
1430
1491
1480
1492
1418
1411
1496
1490

FIG. 80

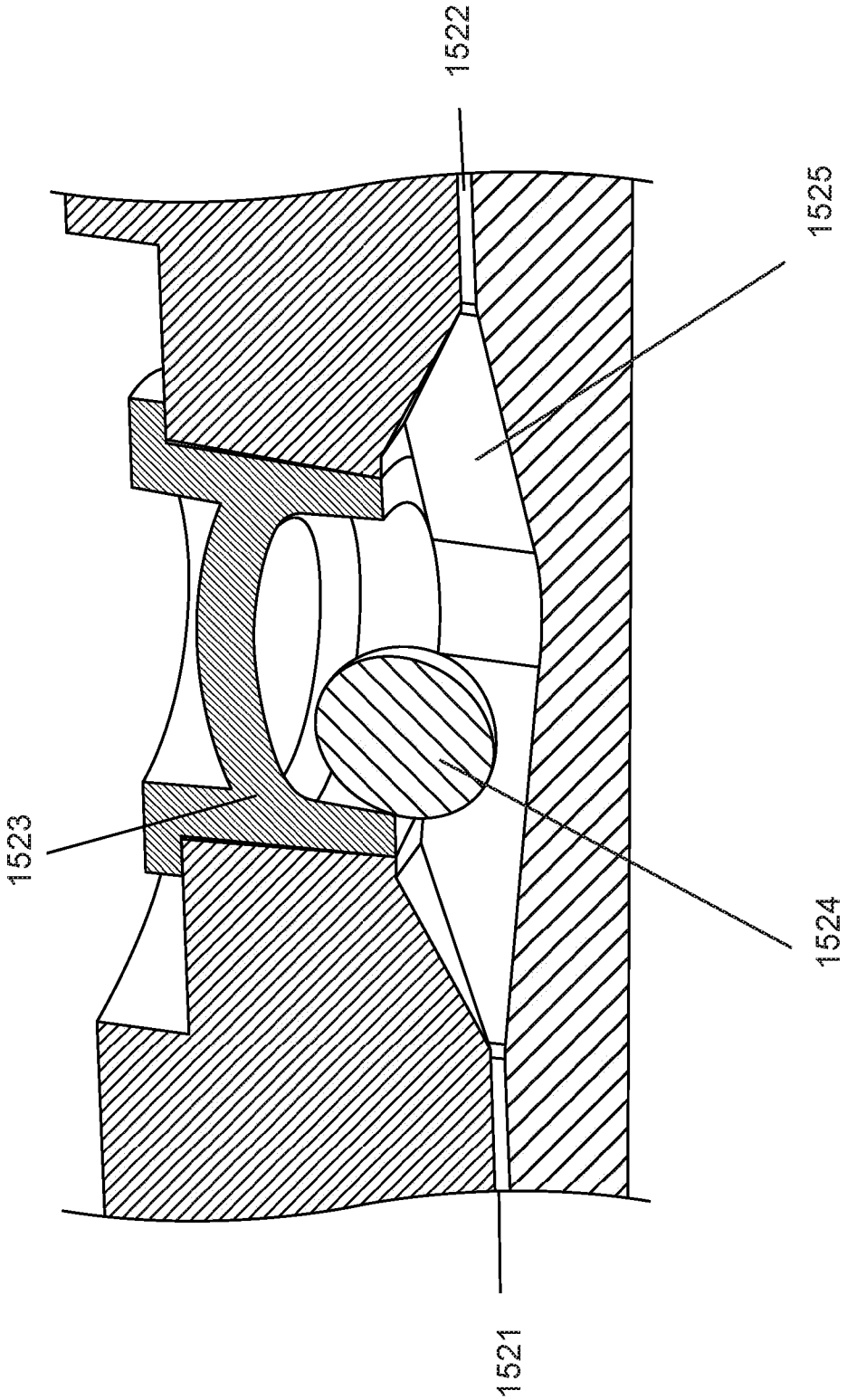


FIG. 82

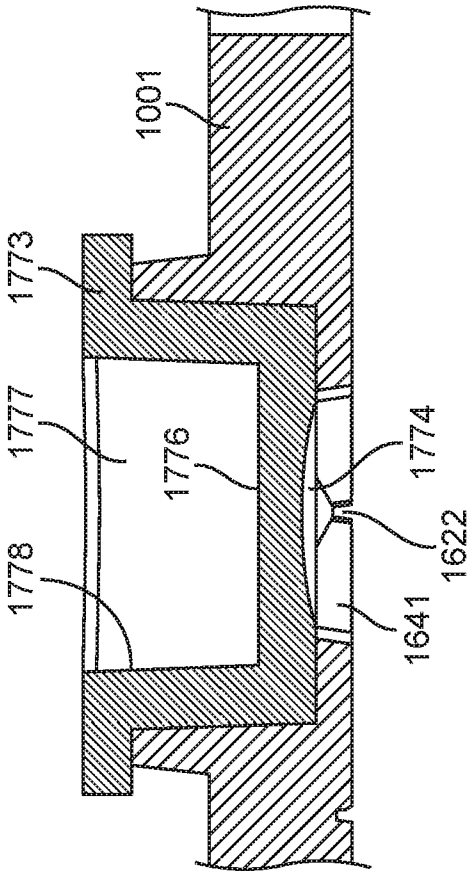


FIG. 83B

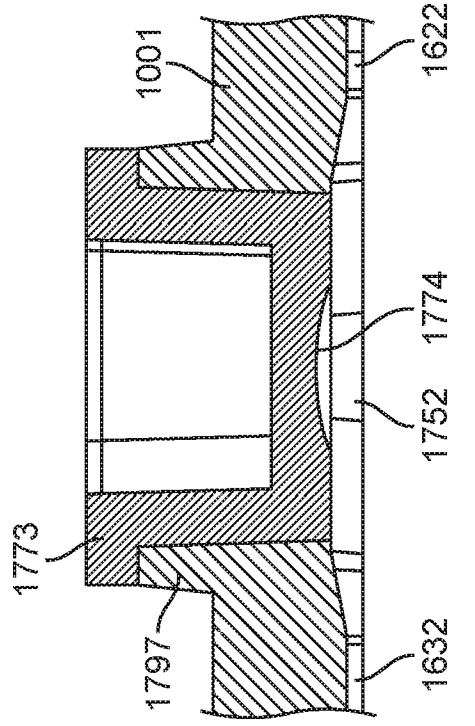


FIG. 83A

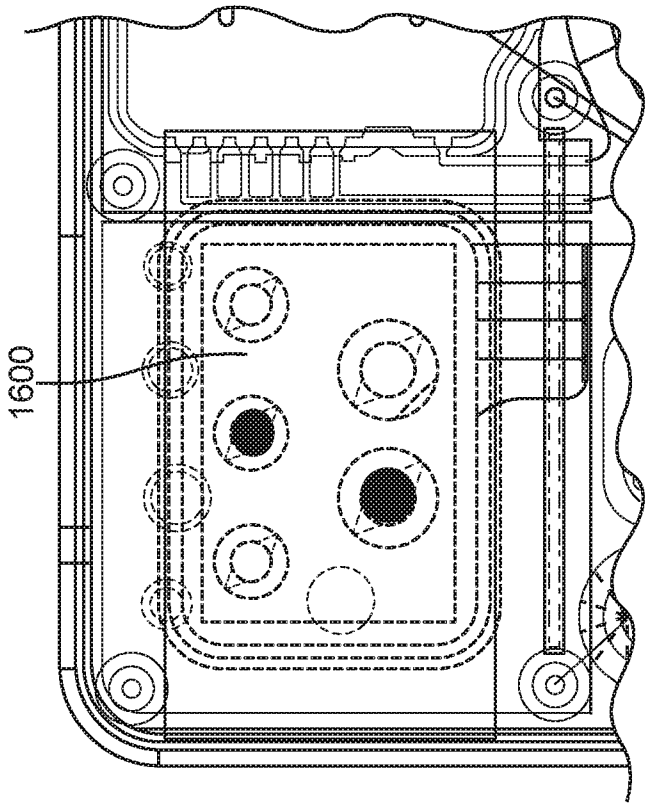


FIG. 84

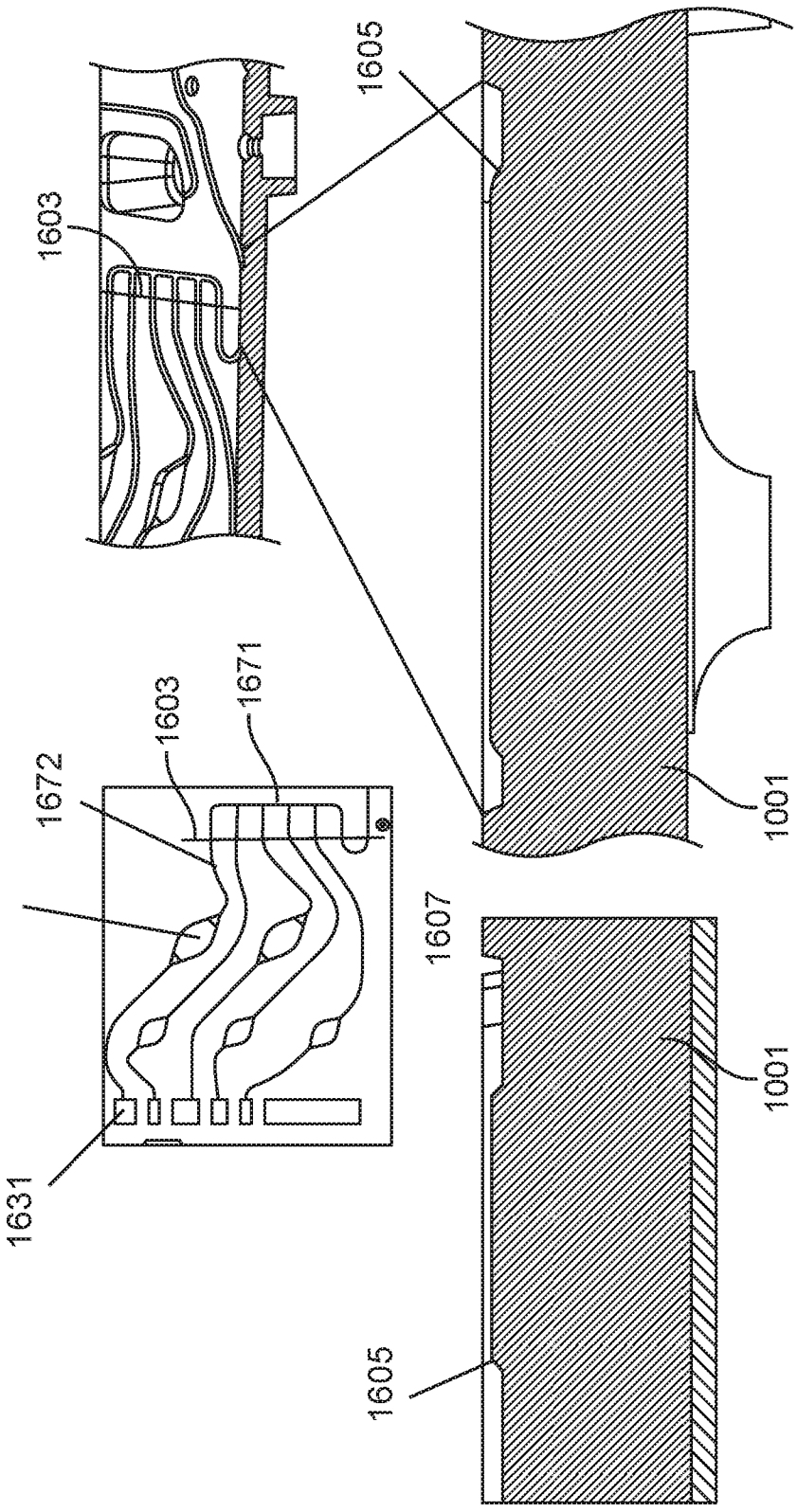


FIG. 86

FIG. 85

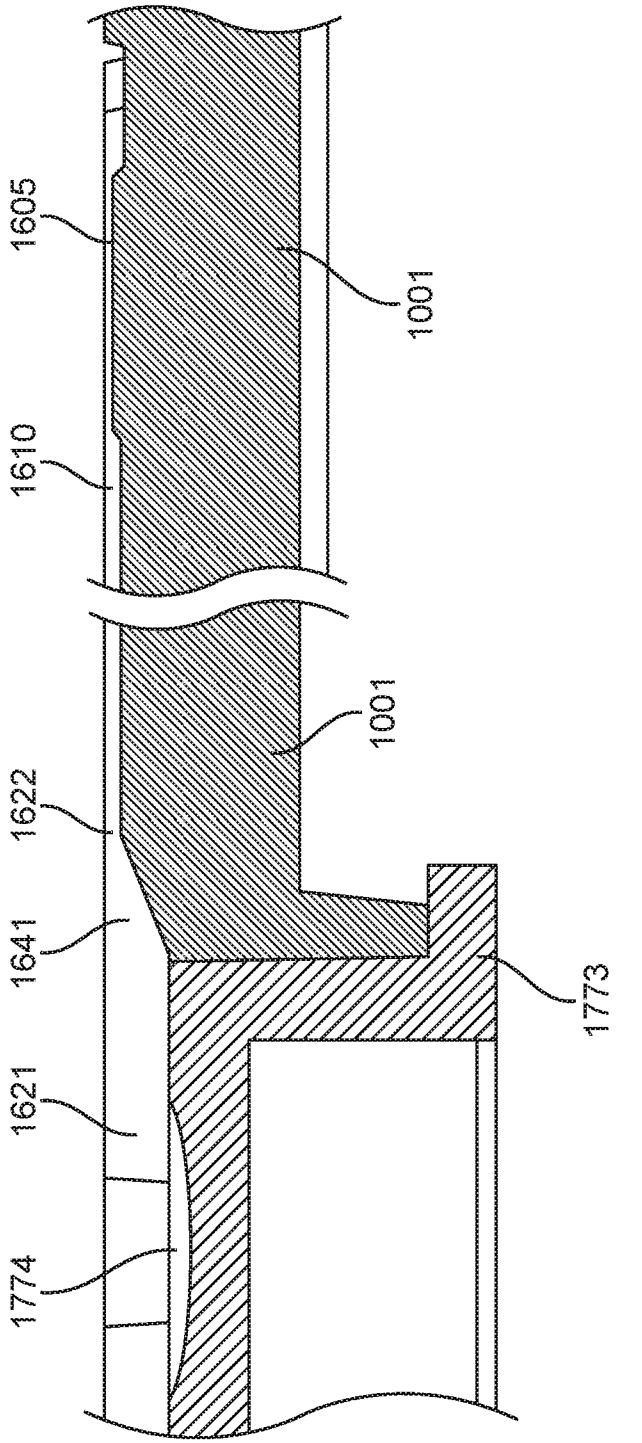


FIG. 87

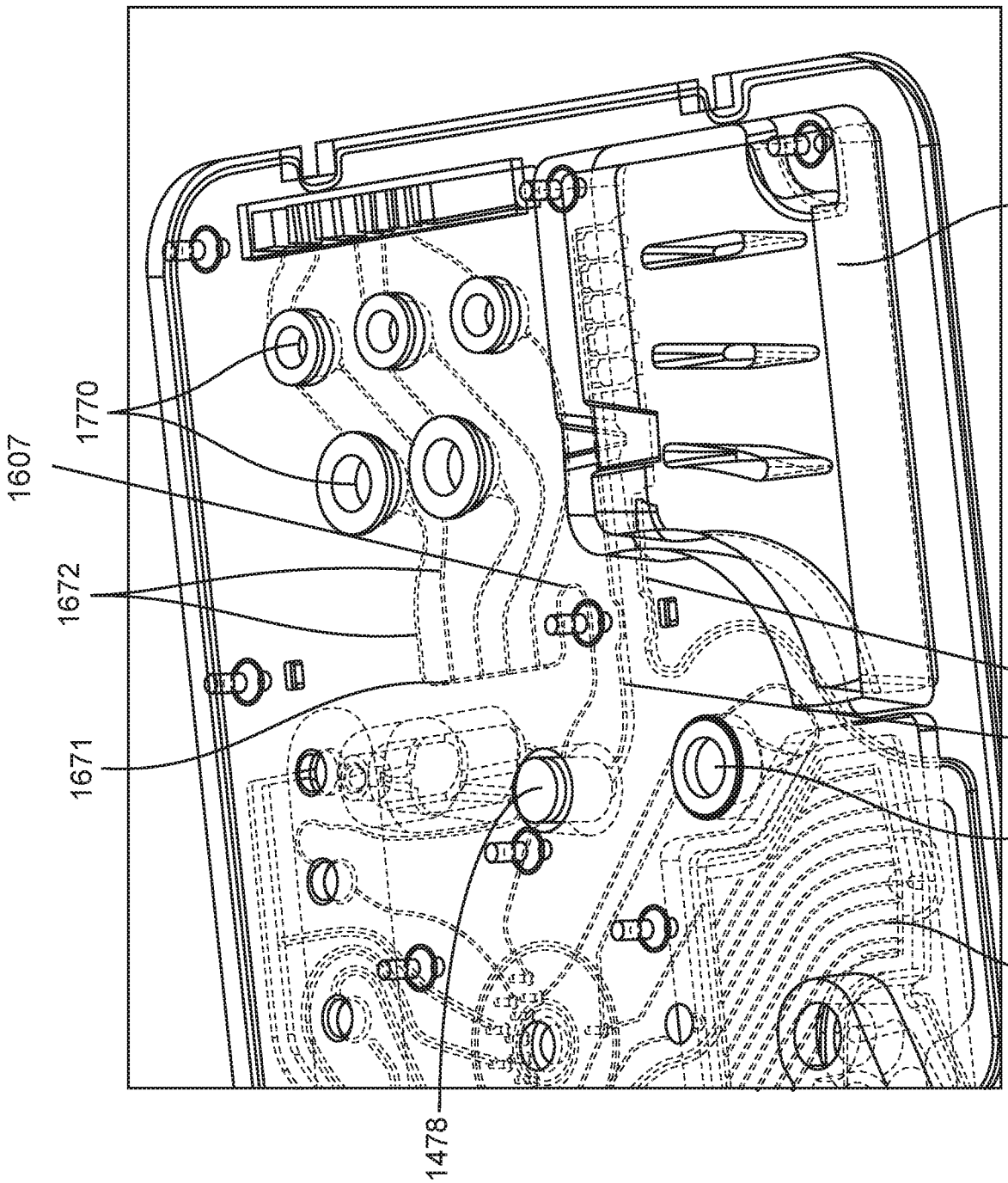


FIG. 88

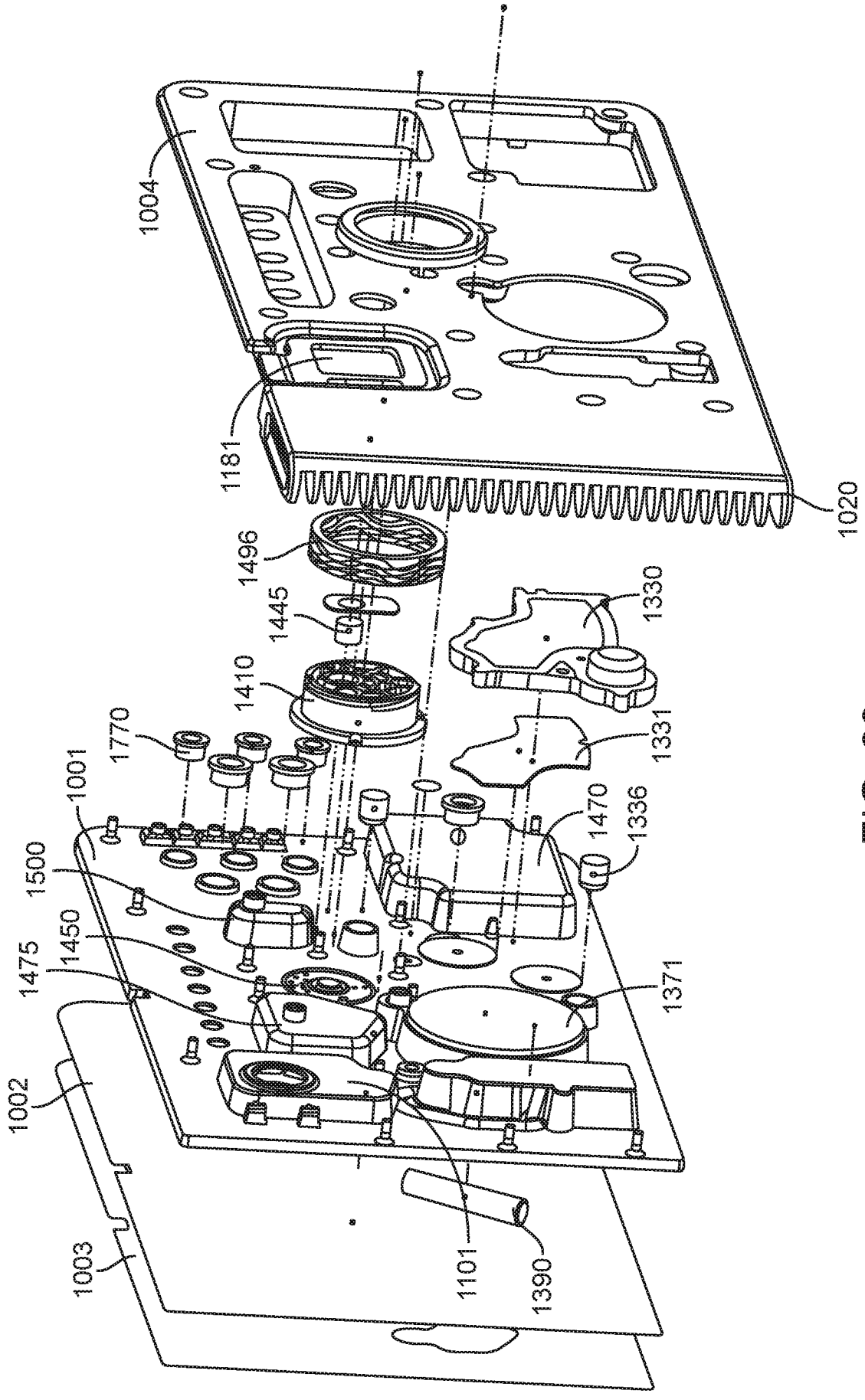

1004
1181
1020
1496
1445
1330
1410
1331
1770
1001
1470
1336
1500
1450
1475
1371
1002
1003
1101
1390

FIG. 89

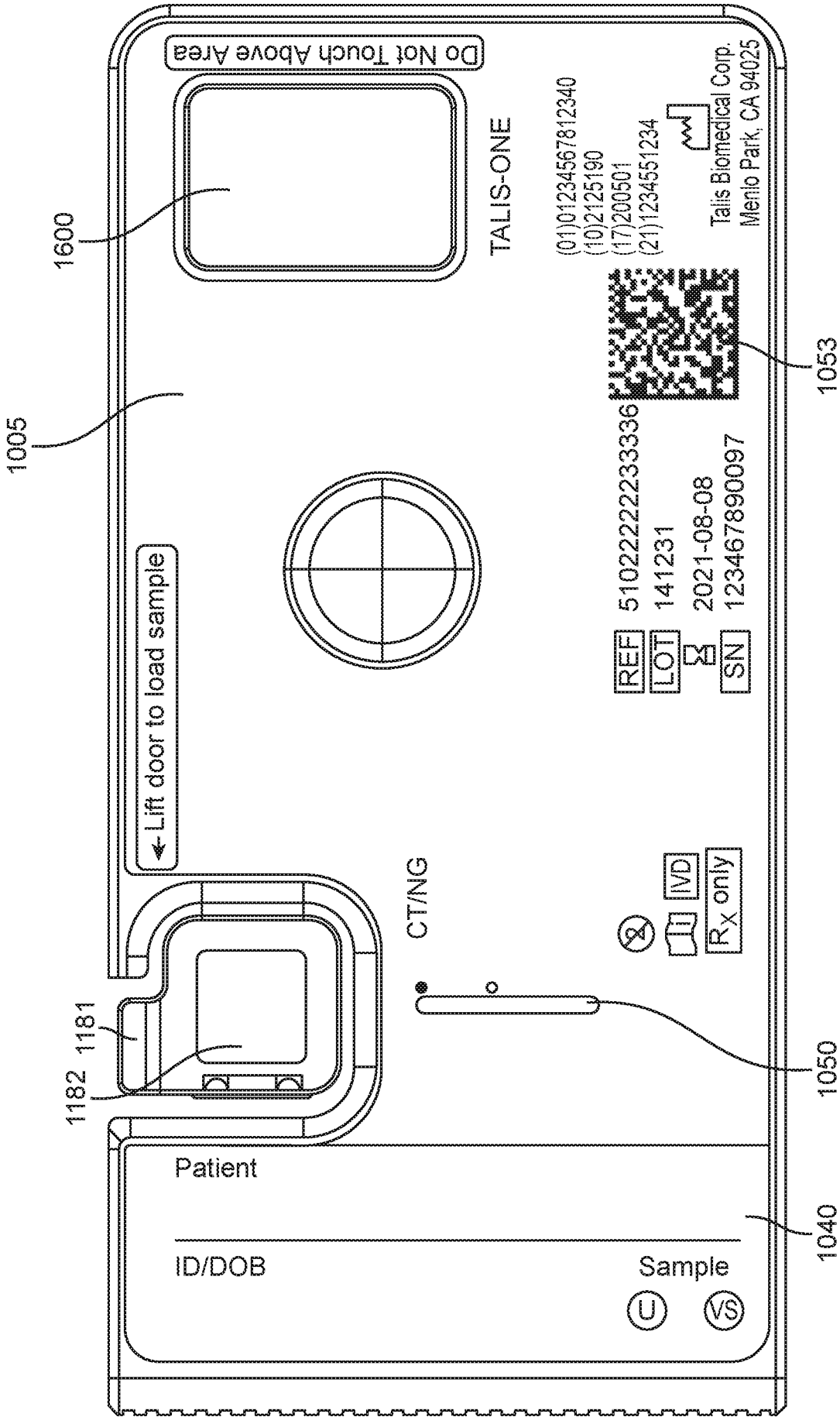

Do Not Touch Above Area
TALIS-ONE
(01)01234567812340
(10)2125190
(17)200501
(21)1234551234
Talis Biomedical Corp.
Menlo Park, CA 94025
1600
1005
1053
Lift door to load sample
510222222233336
REF
141231
LOT
2021-08-08
123467890097
SN
CT/NG
IVD
Rx only
1181
1182
1050
Patient
ID/DOB
Sample
U
VS
1040

FIG. 90

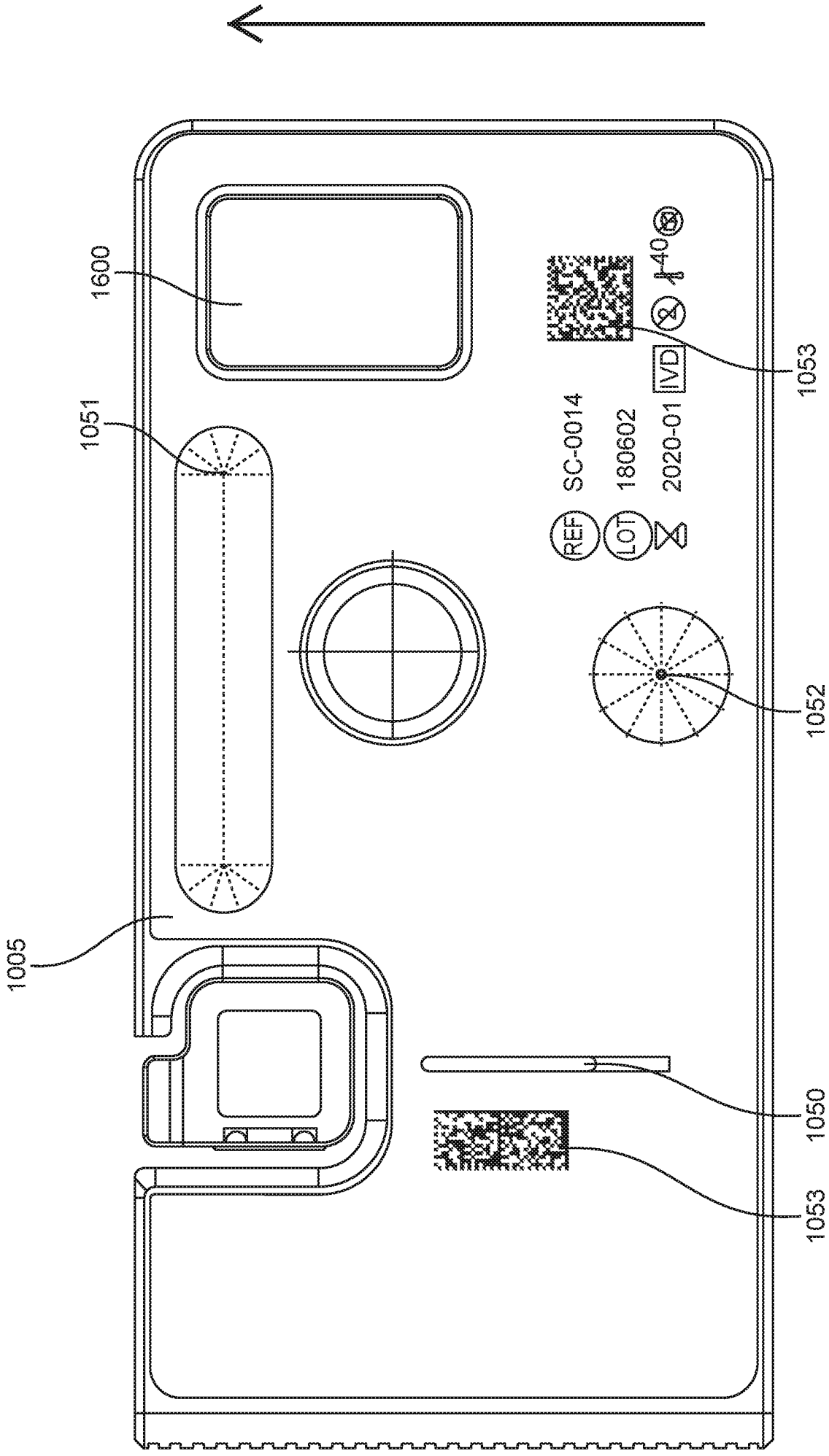

1600
1051
1005
1053
1052
1050
1053
SC-0014
180602
2020-01
REF
LOT
IVD

FIG. 91

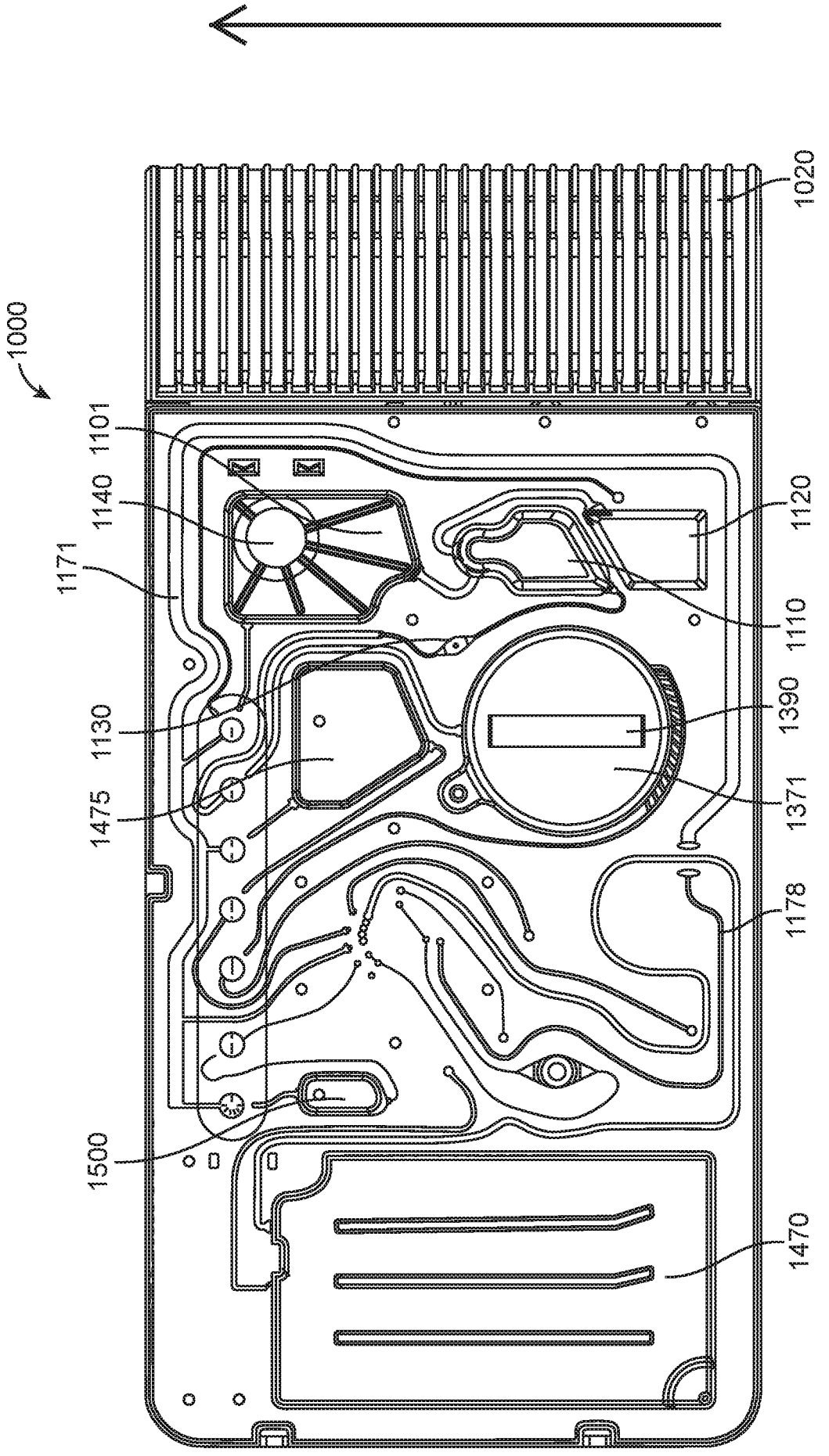

1000
1020
1101
1140
1171
1120
1110
1130
1390
1475
1371
1178
1500
1470

FIG. 92

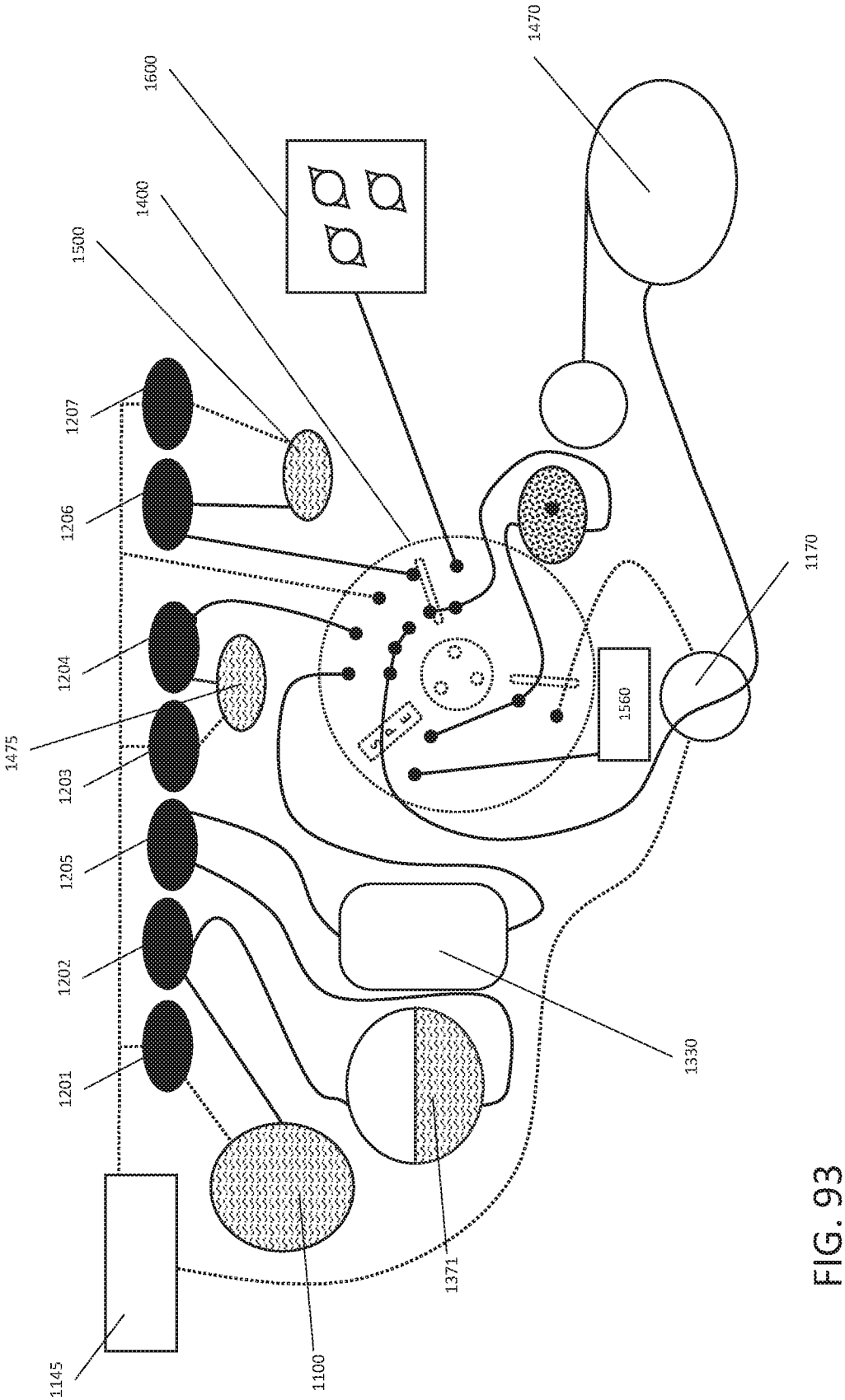

1600
1470
1400
1500
1207
1206
1204
1475
1203
1205
1202
1201
1560
1170
1330
1371
1100
1145

FIG. 93

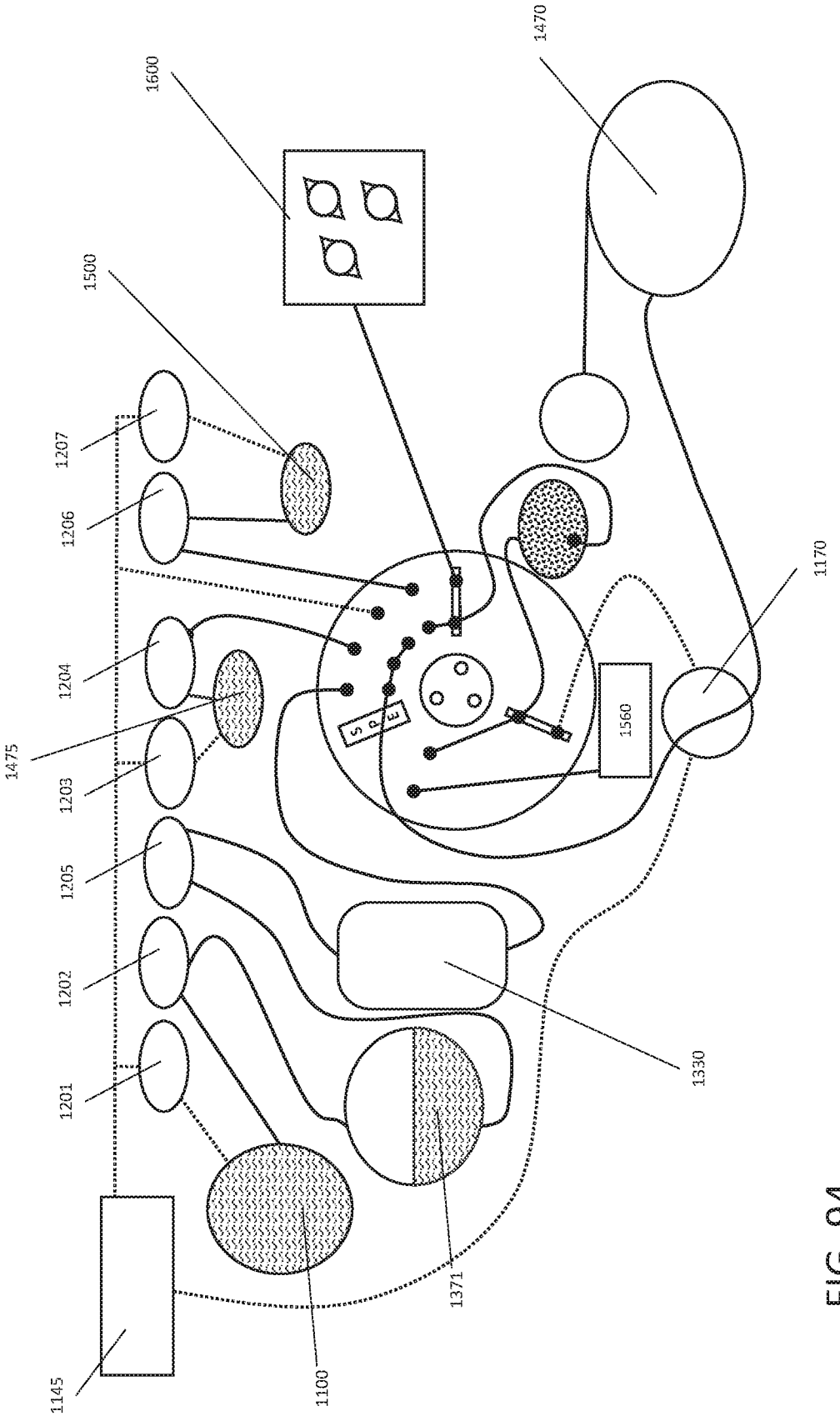

1600
1470
1500
1207
1206
1204
1475
1203
1205
1202
1201
1145
1100
1371
1330
1560
1170

FIG. 94

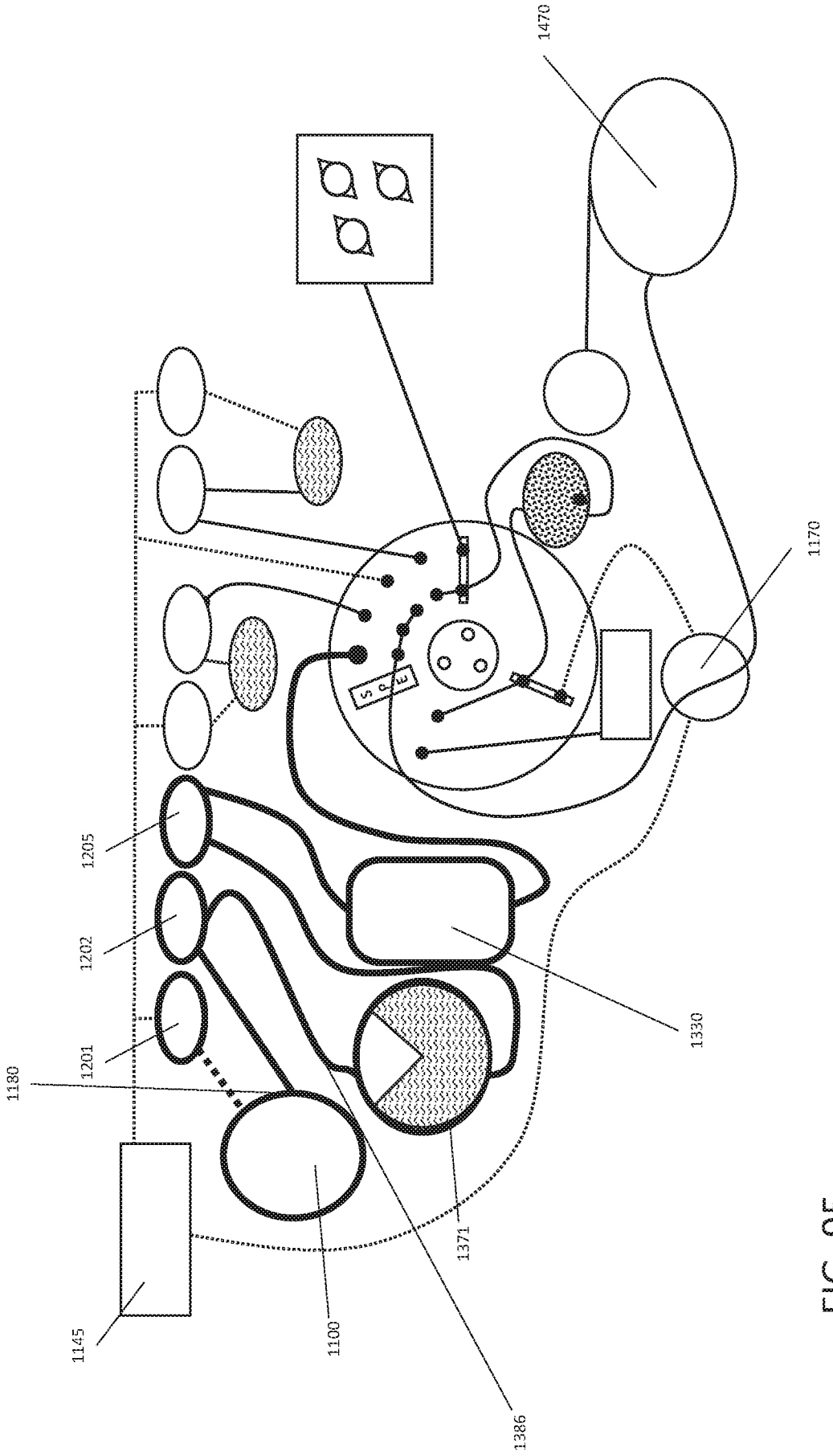


FIG. 95

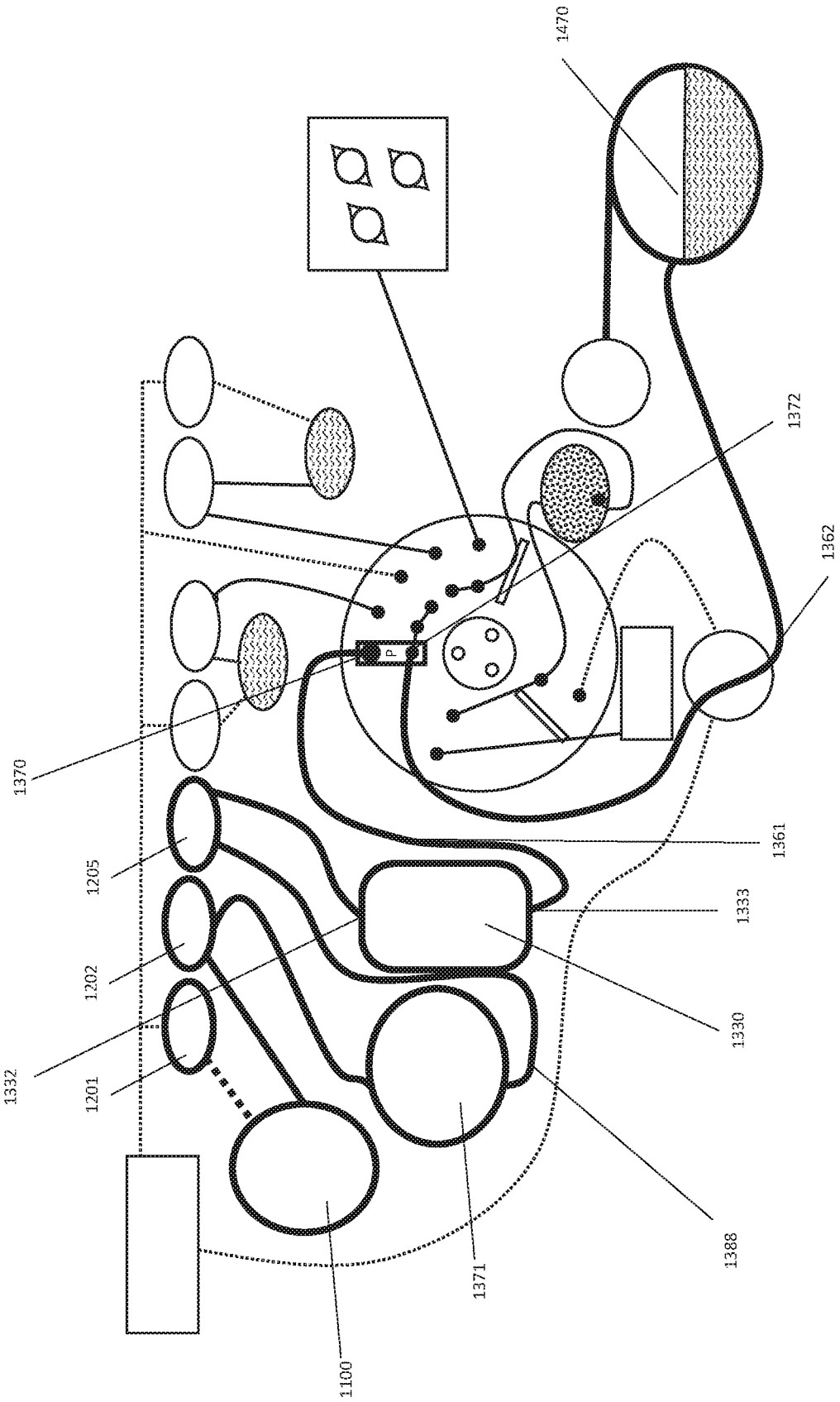

1470
1372
1362
1370
1361
1205
1333
1202
1330
1332
1201
1388
1371
1100

FIG. 96

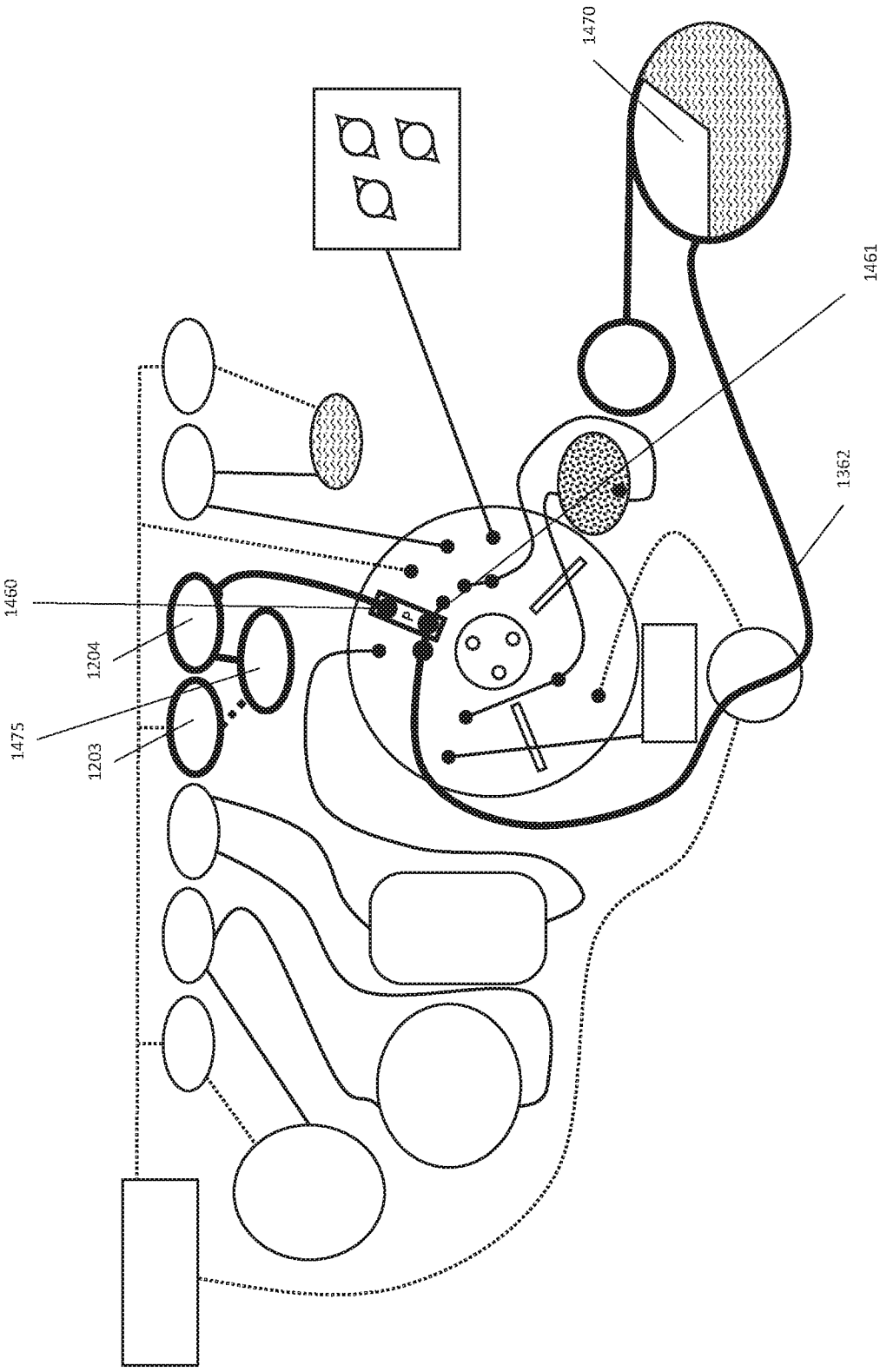


FIG. 97

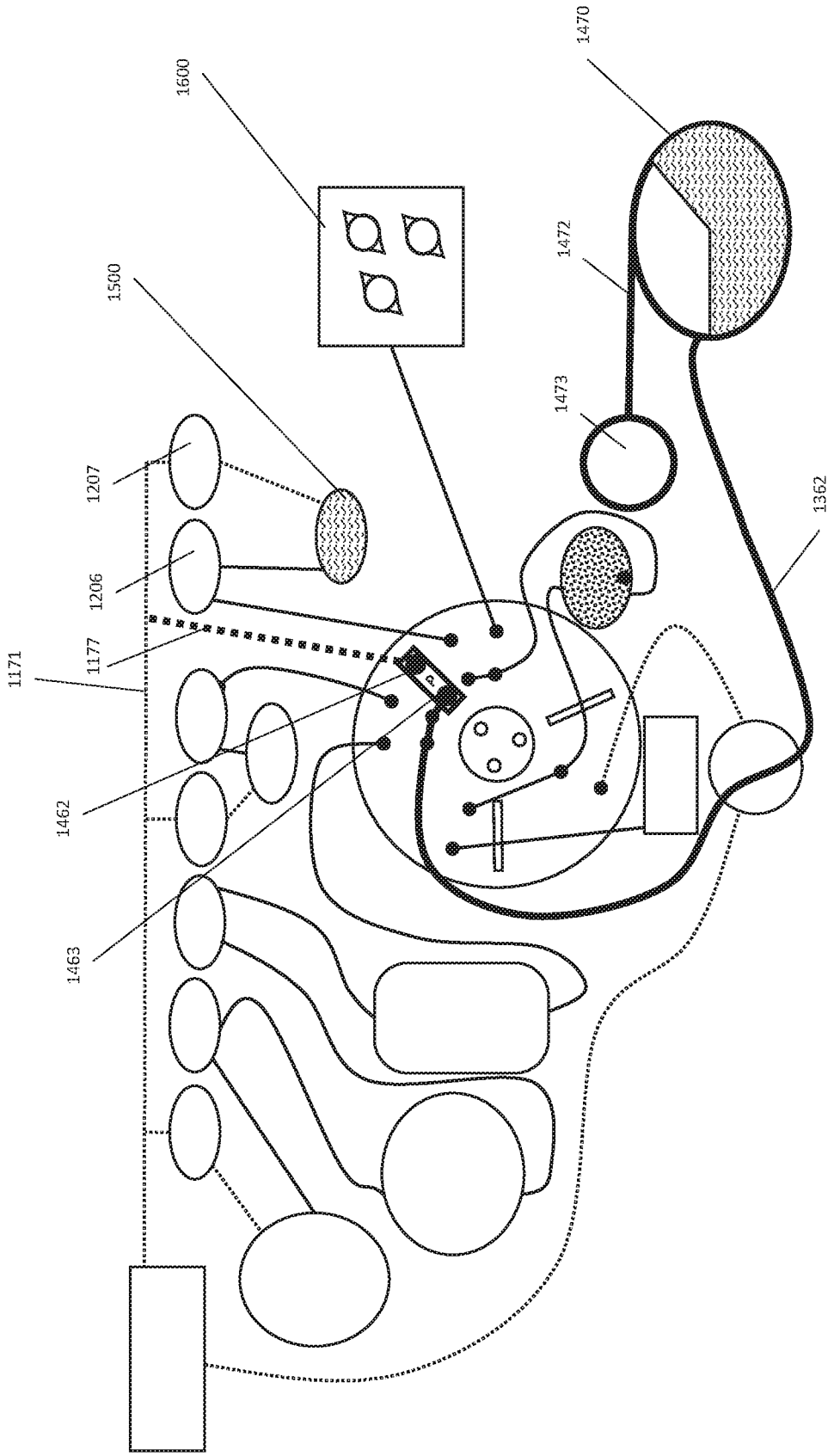


FIG. 98

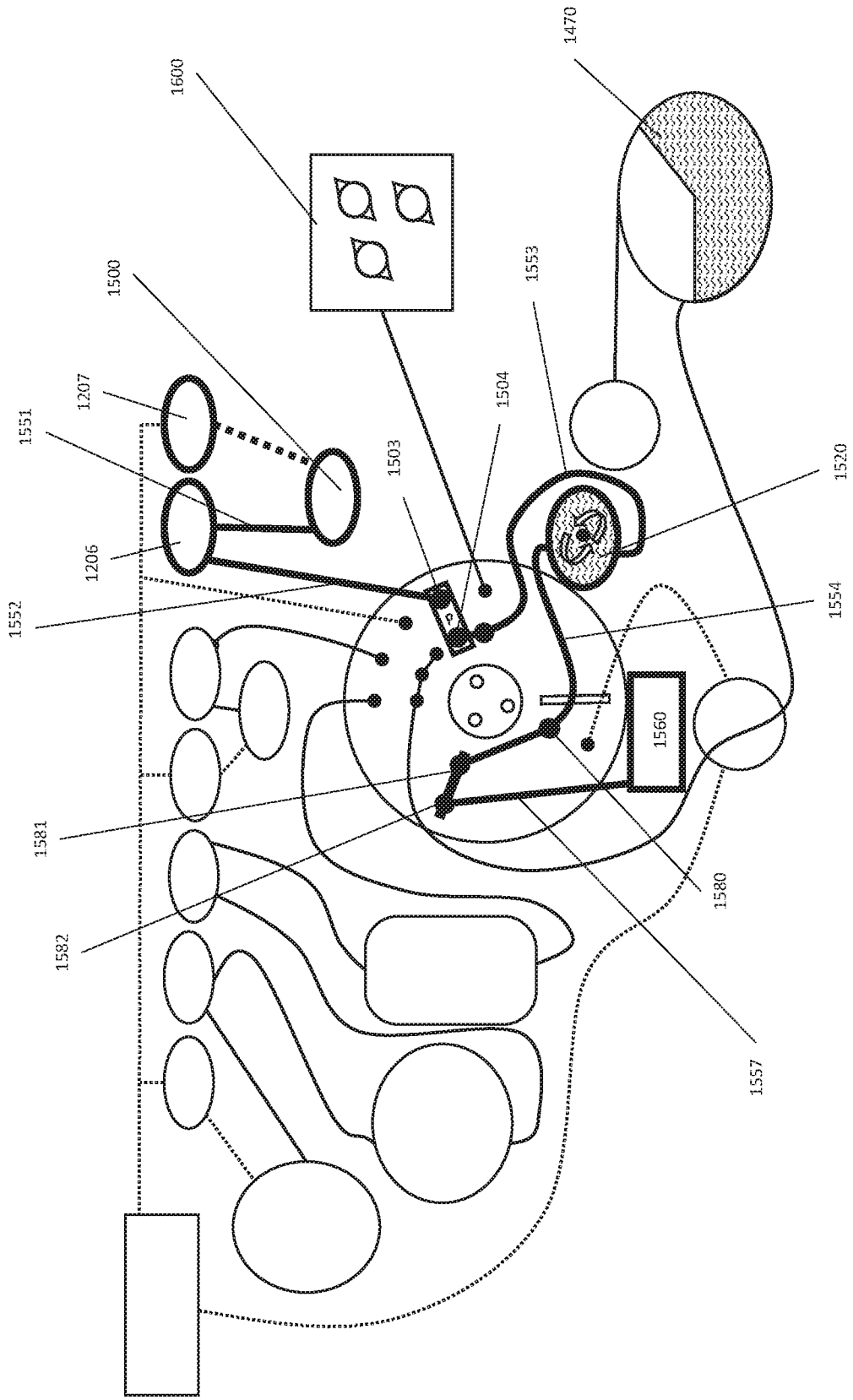

1600
1470
1500
1553
1207
1504
1551
1503
1520
1206
1552
1554
1560
1581
1580
1582
1557

FIG. 99

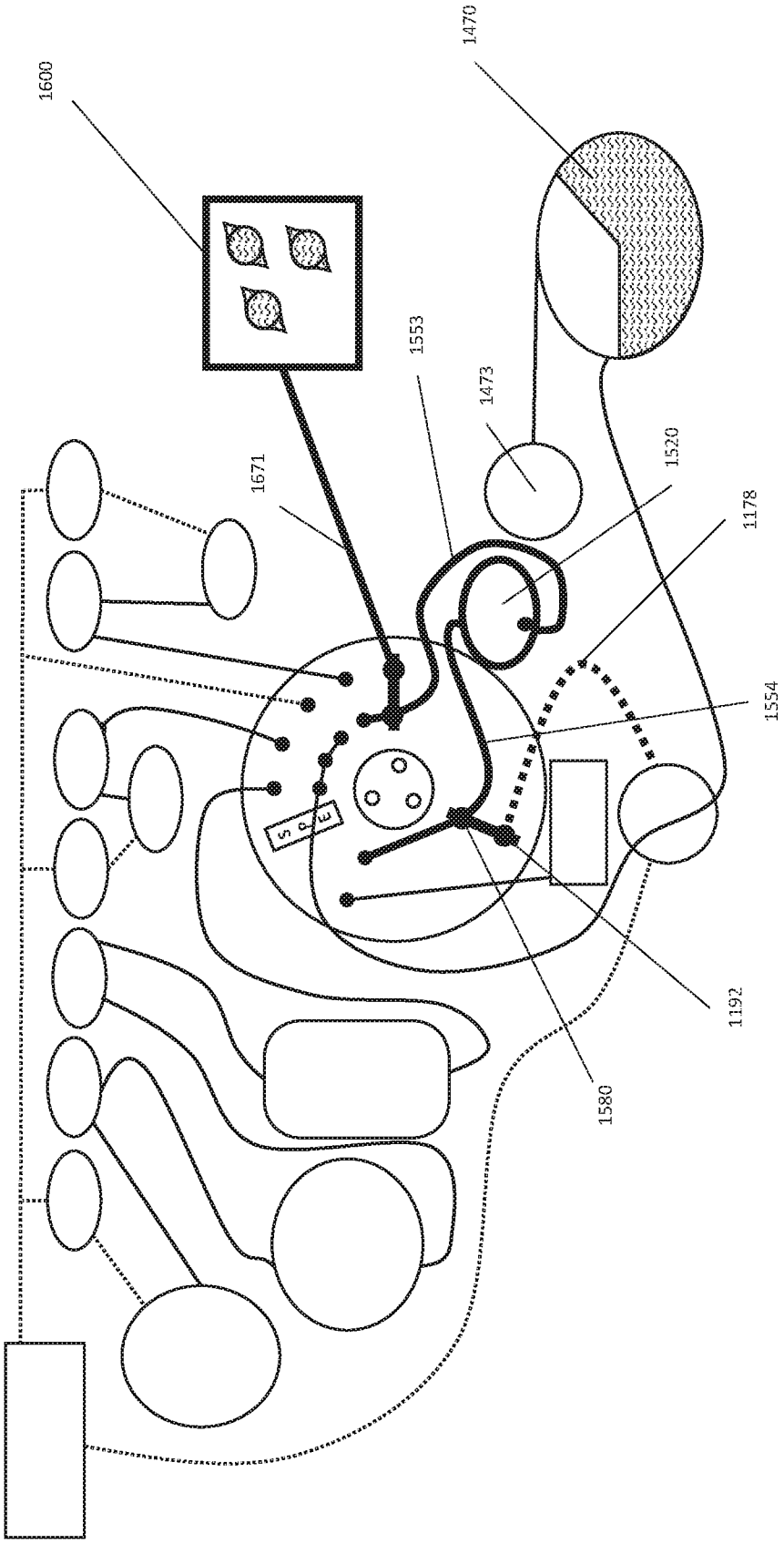


FIG. 100

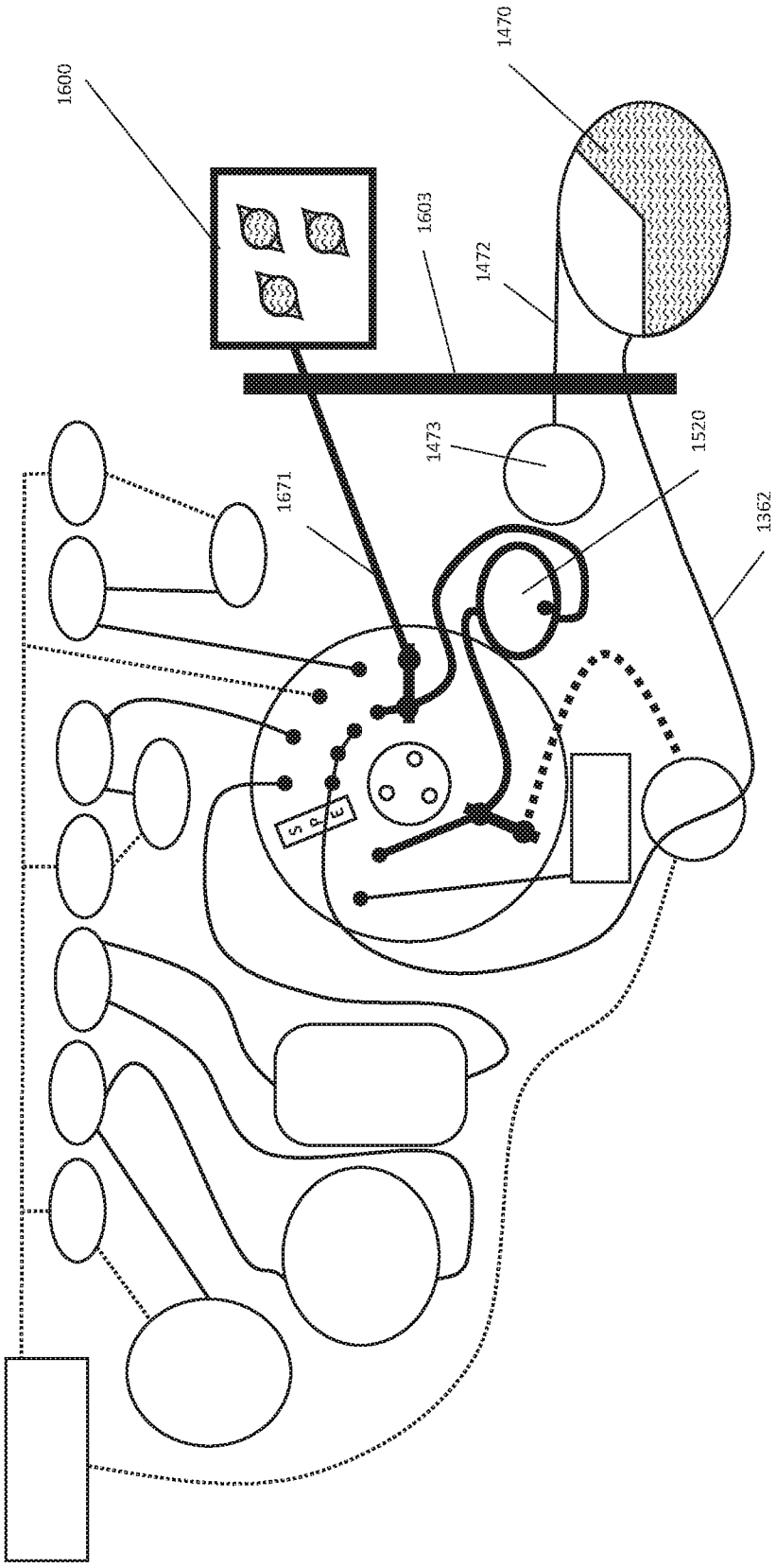


FIG. 101

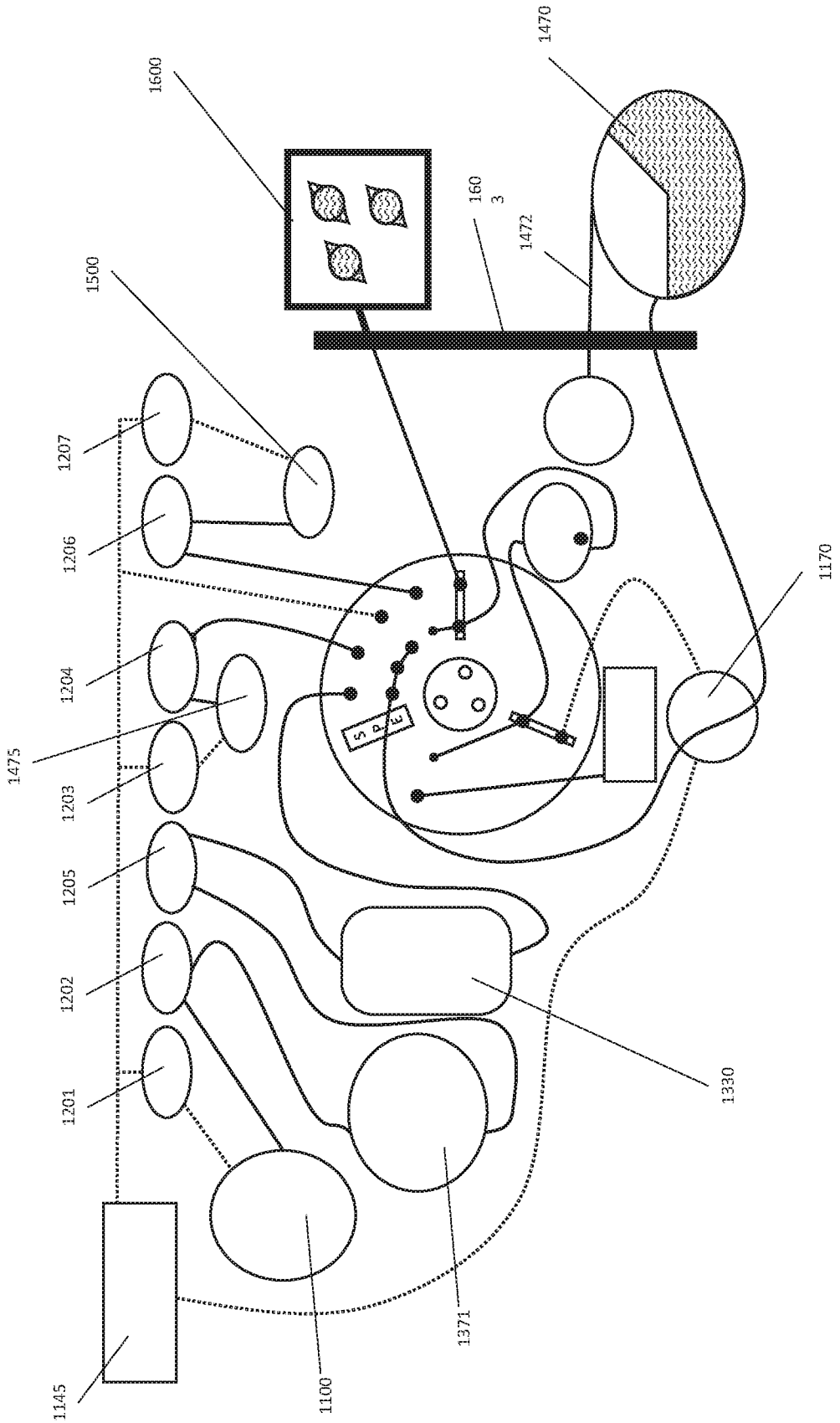

1600
1470
1603
1472
1500
1207
1206
1204
1475
1203
1205
1202
1201
1170
1330
1145
1100
1371

FIG. 102

cartridge	1000
fluidics card	1001
first film	1002
second film	1003
cartridge cover	1004
cartridge label	1005
fluidic side	1006
feature side	1007
user	1010
handle	1020
notch	1021
interference feature	1022
patient label area	1040
sample window	1050
seal area label perforations	1051
pneumatic interface label perforation	1052
computer readable visual code	1053
pipette	1060
sample port assembly	1100
fill chamber	1101
fill chamber outlet	1102
metering chamber	1110
metering chamber inlet	1111
metering channel	1113
ball	1114
overflow chamber	1120
overflow chamber inlet	1121
overflow channel	1122
overflow channel inlet	1123

process control chamber	1130
process control chamber inlet	1131
process control chamber outlet	1132
process control plug	1133
entry port	1140
liquid trap	1145
gas conduit	1150
channel	1160
channel	1161
vent	1165
cartridge pneumatic interface	1170
main pneumatic line	1171
pneumatic interface cover adaptor	1172
inlet filter plug	1173
pneumatic inlets	1174
pneumatic line	1175
sample chamber pneumatic inlet	1176
pneumatic line	1177
pneumatic line	1178
metering chamber exit port	1180
cap	1181
top of the cap	1182
air inlet via	1190
air outlet via	1191
via	1192

main pneumatic via	1193
pneumatic via	1194
input via	1195
frangible seal area	1200
first frangible seal	1201
second frangible seal	1202
third frangible seal	1203
fourth frangible seal	1204
fifth frangible seal	1205
sixth frangible seal	1206
seventh frangible seal	1207
filter assembly	1330
filter	1331
inlet via	1332
outlet via	1333
flow directors	1334
deformation space	1335
filter plug	1336
channel	1360
channel	1361
channel	1362
via	1370
lysis chamber	1371
via	1372
mixing chamber inlet	1373
sample transfer channel	1386
bead filter channels	1387
sample exit channel	1388
stir bar	1390
rotary valve	1400
rotor stator interface	1402
gasket stator interface	1404

rotor	1410
rotor threaded portion	1411
outer face	1413
rotor central opening	1415
propulsion engagement openings	1417
compression limiter	1418
peripheral lip	1422
rotor cap	1430
inlet	1441
outlet	1442
1st conduit	1443
2nd conduit	1444
solid support	1445
solid support chamber	1446
exit from solid support chamber	1448
flow channel spacer	1449
stator	1450
stator valving face	1452
stator port	1453
passages	1454
wash inlet via	1460
wash outlet via	1461
inlet via	1462
outlet via	1463
waste collection element	1470
waste inlet	1471
vent channel	1472
vent	1473
waste outlet	1474
wash buffer reservoir	1475

FIG. 103

wash inlet	1476
wash outlet	1477
outlet filter plug	1478
gasket	1480
gasket inlet	1484
gasket outlet	1485
retention element	1490
retention ring	1491
threaded portion of retention ring	1492
retention ring lip	1493
biasing element	1496
sloping feature	1497
elution buffer reservoir	1500
elution reservoir inlet	1501
elution reservoir outlet	1502
eluent inlet via	1503
eluate outlet via	1504
rehydration chamber	1520
rehydration chamber inlet	1521
rehydration chamber outlet	1522
reagent plug	1523
magnetic ball	1524
channel	1550
channel	1551
channel	1552
channel	1553
channel	1554
channel	1555
channel	1556

metering channel	1557
metering vent	1560
via	1580
via	1581
metering via	1582
vent	1583
reaction area	1600
heat stake	1603
raised platform	1605
u-bend	1607
independent fluidic pathway	1610
assay chamber	1621
entry conduit	1622
air chamber	1631
pneumatic conduit	1632
tapered inlet	1641
tapered outlet	1642
channel	1670
main channel	1671
loading channels	1672
assay inlet via	1680
assay outlet via	1681
thermal clamping area	1690
recess	1752
plug	1770
plug cap flange	1773
plug cap internal cavity	1774
plug bottom surface	1776
central opening	1777
central opening side wall	1778
raised annulus	1797

instrument	2000
fixed bracket assembly	2010
fixed support bracket	2011
first surface of fixed support bracket	2012
second surface of fixed support bracket	2013
linear actuator	2014
notch	2015
lead screw	2016
sensor	2017
pins	2018
hard stop sensor	2019
moving bracket assembly	2040
clamp block	2041
first surface of clamp block	2042
linear slide	2043
lead nut	2044
extension springs	2045
ledge	2046
flag	2047
enclosure	2070
metal sheet	2071
slot	2072
front	2073
foot	2074
pneumatic interface	2100
steel plunger	2101
compression spring	2102
extension stop	2103
plunger surface	2104
shim	2105

housing	2106
outer surface of plunger	2107
inner surface of housing	2108
step-up feature	2109
clearance	2110
pneumatic subsystem	2130
pump	2131
pressure regulator	2132
proportional valve	2133
pressure sensor	2134
accumulator	2135
output selector valve	2136
manifold block	2137
control board	2138
pump filter	2160
regulator inlet	2161
pump outlet filter	2162
tubing	2190
bleed orifice	2191
grommets	2194
latch and pin assembly	2210
clamp hard stop	2211
latch	2212
spring	2213
latch release arm	2214
latch release arm slot	2215
pin	2216
tab	2217
loading assembly	2230
rails	2231
rack	2232
pinion	2233

FIG. 104

pusher carriage	2234
ejection spring	2235
load position sensor	2236
flag	2237
damper	2238
post	2239
guide	2240s
frangible seal block	2260
frangible seal pins	2261
frangible seal pocket	2262
hard stop	2263
linear slide	2264
gap	2265
sensor	2266
door support assembly	2280
door support	2281
spring	2282
magnetic mixing assembly	2300
driving magnet system	2310
first driving magnet	2311
first magnet field focuser	2312
second driving magnet	2316
second driving magnet field focuser	2317
driving magnet spindle	2321
driving magnet holder / spacer	2325
drive motor	2330
drive belt	2332
driven magnet system	2350
first driven magnet	2351

first driven magnet field focuser	2352
second driven magnet	2356
second driven magnet field focuser	2357
driven magnet spindle	2361
driven magnet holder / spacer	2365
perforations	2377
microphone	2380
valve drive assembly	2400
valve drive	2401
valve drive pins	2402
motor	2403
interference sensor	2404
end of valve drive shaft	2405
pulley	2406
belt	2407
valve drive shaft	2408
homing sensor	2409
rehydration motor	2510
cartridge heater assembly	2550
cartridge heater	2551
cartridge heater zone	2552
insulator	2553
cutouts	2554
chemistry heater assembly	2600
chemistry heater	2601
chemistry heater plate	2602
chemistry heater fan	2603
fan plenum	2604

flow vane	2605
flow guide frame	2606
heater plenum	2607
reaction well zone	2620
first surface of chemistry heater plate	2621
second surface of chemistry heater plate	2622
machined pocket geometry	2623
grooves	2624
heat staker assembly	2640
staker bar assembly	2641
linear actuation motor	2642
spring	2643
heat staker fan	2644
inductive linear sensor	2645
staker blade	2660
wire heater	2661
depth stop	2662
thermal clamp assembly	2680
clamp plate	2681
thermal clamp posts	2682
springs	2683
shoulder screw	2684
bushing	2685
light frame	2686
reaction imaging assembly	2700
reaction camera	2701
dichroic beam splitter	2702
light trap	2703

fold mirror	2704
camera mount adaptor	2705
objective lens	2706
beam splitter block	2707
optical block	2710
pocket	2711
excitation lens cell	2730
excitation LED	2731
aperture	2732
plano-convex lens	2733
aspheric lens	2734
bandpass filter	2735
heat sink	2736
thermal isolation spacer	2737
temperature sensor	2738
photodiode	2739
emission lens cell	2750
image lens	2751
longpass filter	2752
image plane	2760
label imaging assembly	2770
camera	2771
tri color LED	2772
aperture	2773
diffuser	2774
cellular antenna	2800
antenna ground plate	2810
display	2820

FIG. 105

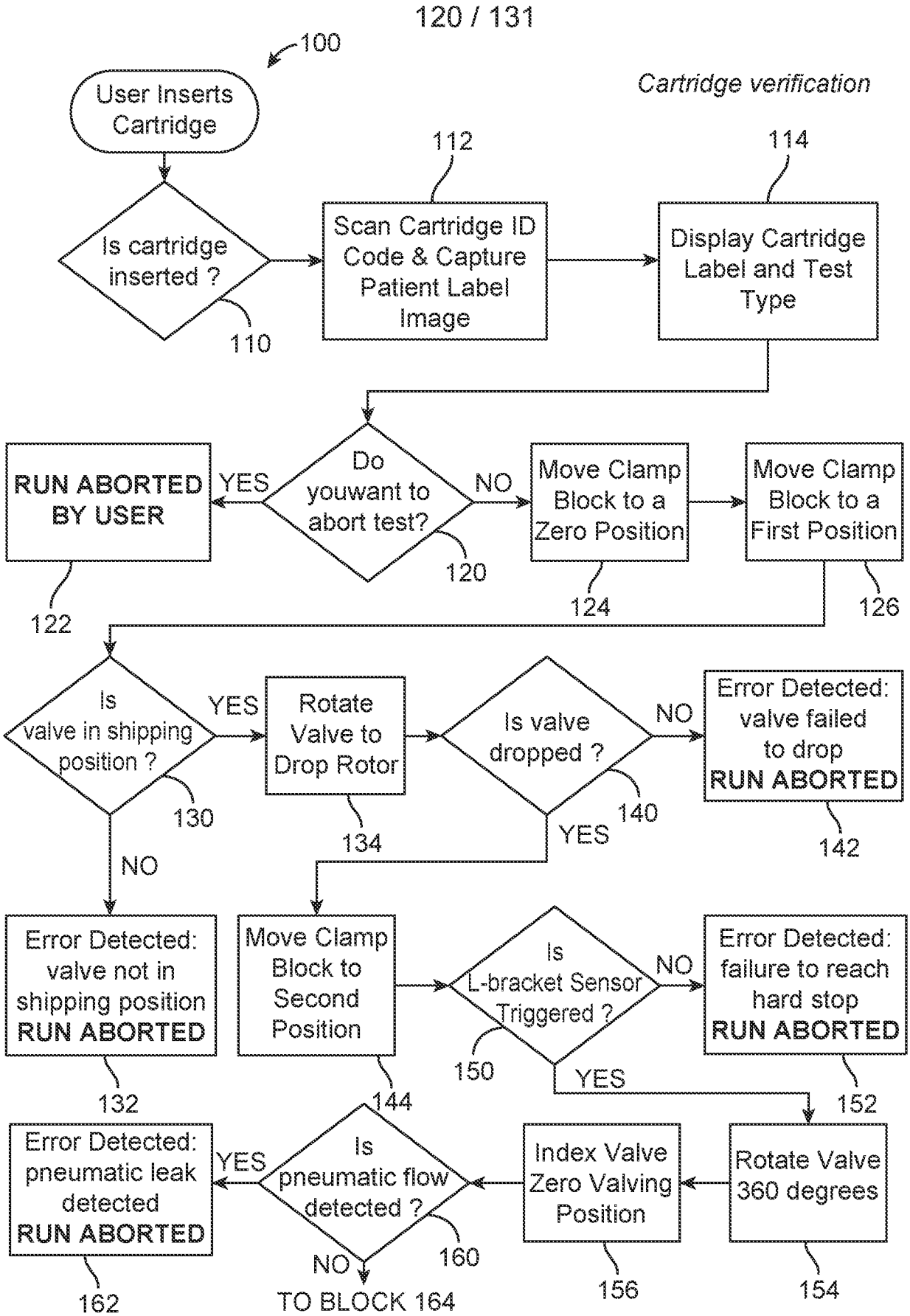

120 / 131
100
User Inserts Cartridge
Cartridge verification
Is cartridge inserted ?
110
112
Scan Cartridge ID Code & Capture Patient Label Image
114
Display Cartridge Label and Test Type
Do youwant to abort test?
120
YES
RUN ABORTED BY USER
122
NO
Move Clamp Block to a Zero Position
124
Move Clamp Block to a First Position
126
Is valve in shipping position ?
130
YES
Rotate Valve to Drop Rotor
134
Is valve dropped ?
140
NO
Error Detected: valve failed to drop RUN ABORTED
142
YES
NO
Error Detected: valve not in shipping position RUN ABORTED
132
Move Clamp Block to Second Position
144
Is L-bracket Sensor Triggered ?
150
NO
Error Detected: failure to reach hard stop RUN ABORTED
152
YES
Rotate Valve 360 degrees
154
Index Valve Zero Valving Position
156
Is pneumatic flow detected ?
160
YES
Error Detected: pneumatic leak detected RUN ABORTED
162
NO
TO BLOCK 164

FIG. 106A

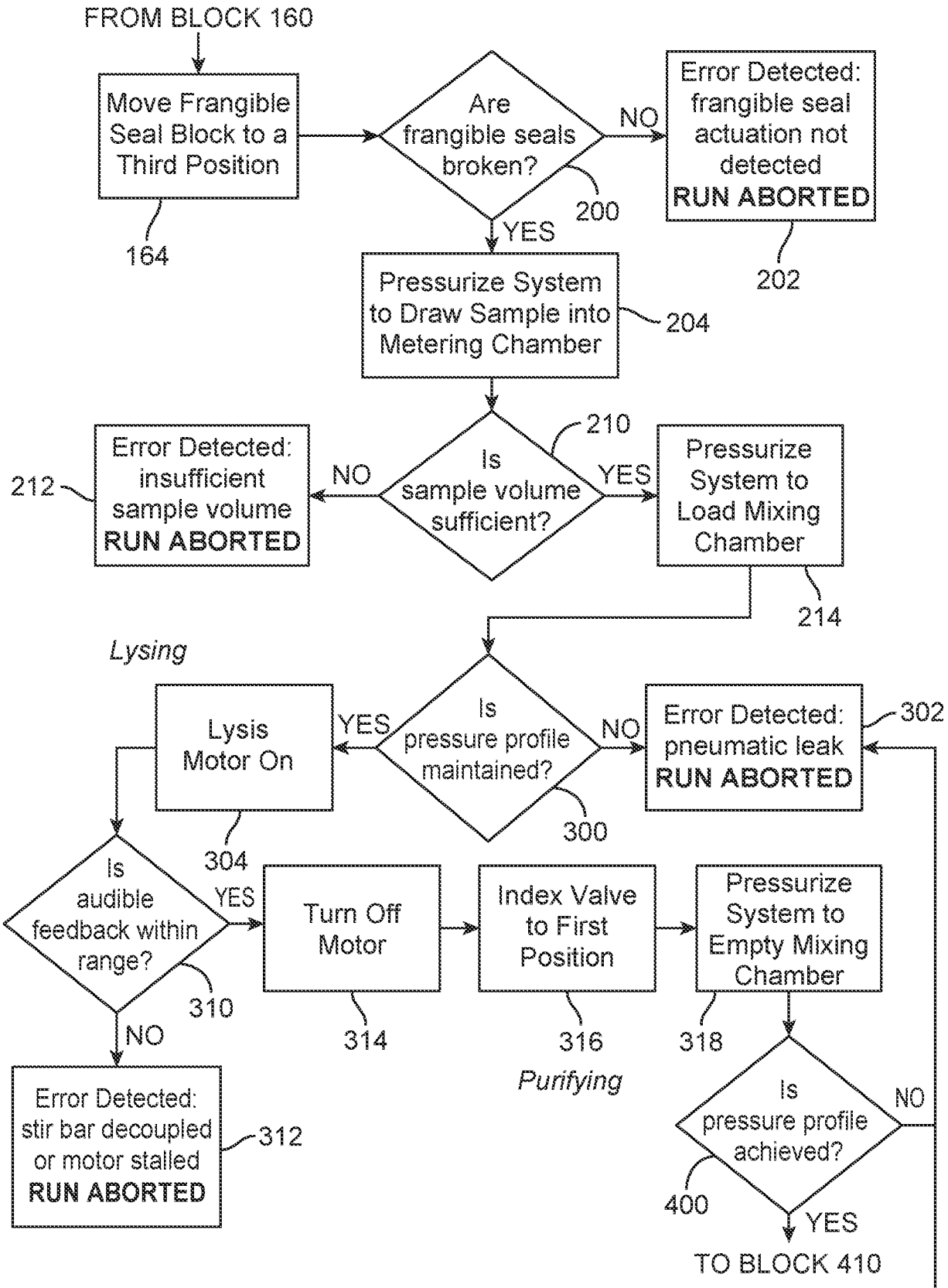

FROM BLOCK 160
Move Frangible Seal Block to a Third Position
164
Are frangible seals broken?
200
NO
Error Detected: frangible seal actuation not detected RUN ABORTED
202
YES
Pressurize System to Draw Sample into Metering Chamber
204
Is sample volume sufficient?
210
NO
Error Detected: insufficient sample volume RUN ABORTED
212
YES
Pressurize System to Load Mixing Chamber
214
Lysing
Is pressure profile maintained?
300
NO
Error Detected: pneumatic leak RUN ABORTED
302
YES
Lysis Motor On
304
Is audible feedback within range?
310
YES
NO
Error Detected: stir bar decoupled or motor stalled RUN ABORTED
312
Turn Off Motor
314
Index Valve to First Position
316
Purifying
Pressurize System to Empty Mixing Chamber
318
Is pressure profile achieved?
400
NO
YES
TO BLOCK 410

FIG. 106B

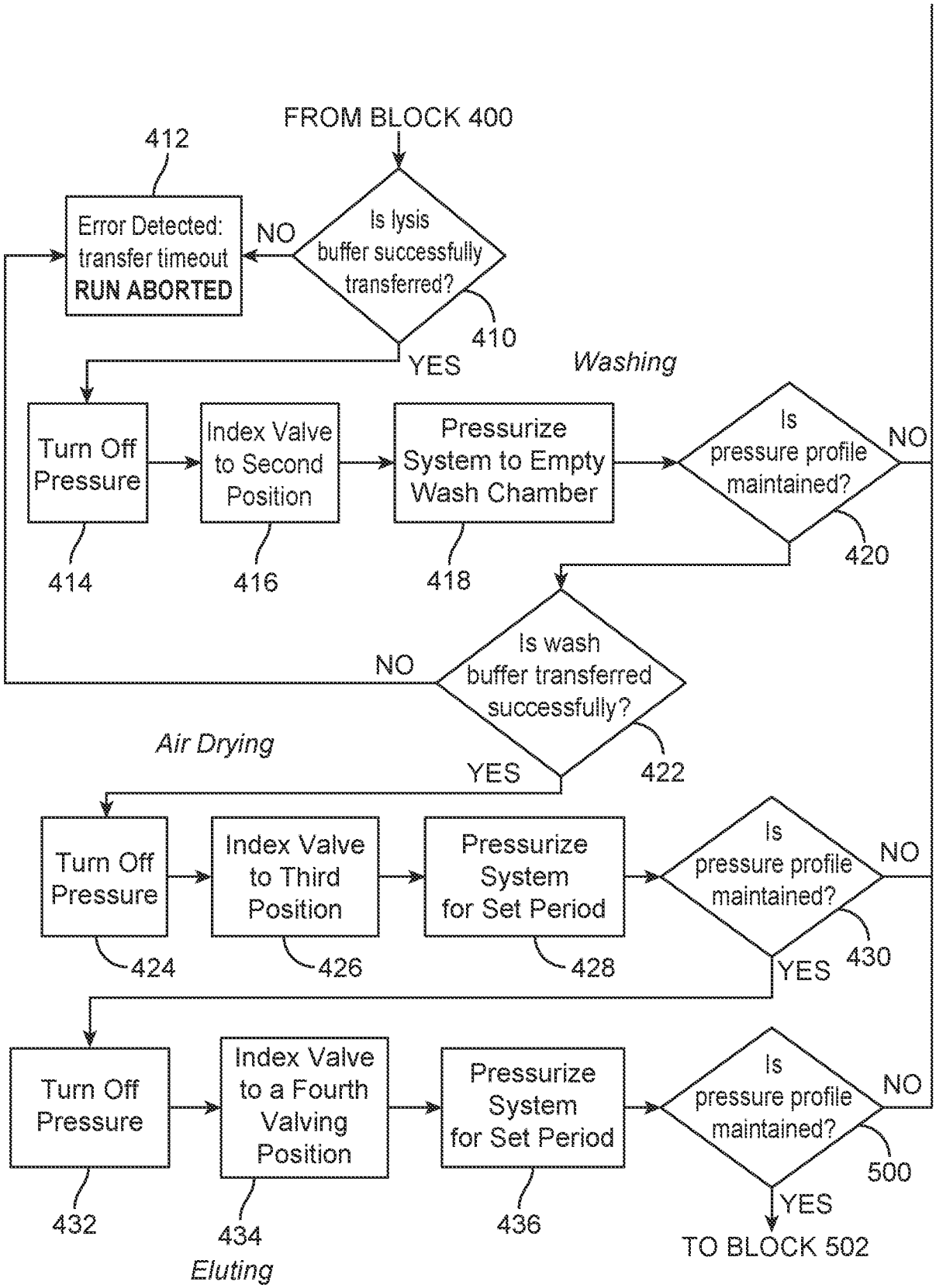

FROM BLOCK 400
412
Error Detected: transfer timeout RUN ABORTED
NO
Is lysis buffer successfully transferred?
410
YES
Washing
Turn Off Pressure
414
Index Valve to Second Position
416
Pressurize System to Empty Wash Chamber
418
Is pressure profile maintained?
NO
420
NO
Is wash buffer transferred successfully?
422
Air Drying
YES
Turn Off Pressure
424
Index Valve to Third Position
426
Pressurize System for Set Period
428
Is pressure profile maintained?
NO
430
YES
Turn Off Pressure
432
Index Valve to a Fourth Valving Position
434
Eluting
Pressurize System for Set Period
436
Is pressure profile maintained?
NO
500
YES
TO BLOCK 502

FIG. 106C

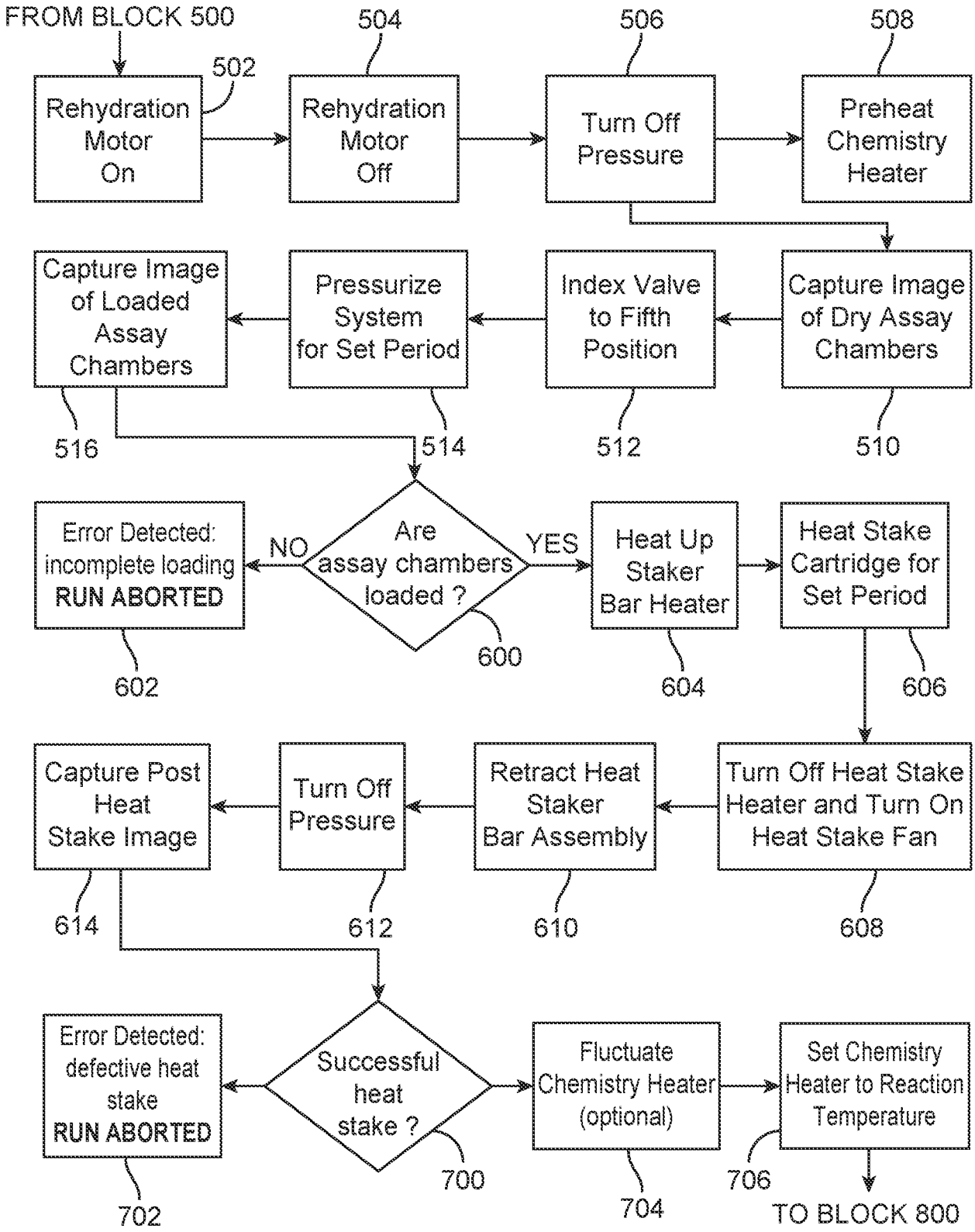

FROM BLOCK 500
502
Rehydration Motor On
504
Rehydration Motor Off
506
Turn Off Pressure
508
Preheat Chemistry Heater
Capture Image of Loaded Assay Chambers
Pressurize System for Set Period
Index Valve to Fifth Position
Capture Image of Dry Assay Chambers
516
514
512
510
Error Detected: incomplete loading RUN ABORTED
NO
Are assay chambers loaded ?
YES
Heat Up Staker Bar Heater
Heat Stake Cartridge for Set Period
602
600
604
606
Capture Post Heat Stake Image
Turn Off Pressure
Retract Heat Staker Bar Assembly
Turn Off Heat Stake Heater and Turn On Heat Stake Fan
614
612
610
608
Error Detected: defective heat stake RUN ABORTED
Successful heat stake ?
Fluctuate Chemistry Heater (optional)
Set Chemistry Heater to Reaction Temperature
702
700
704
706
TO BLOCK 800

FIG. 106D

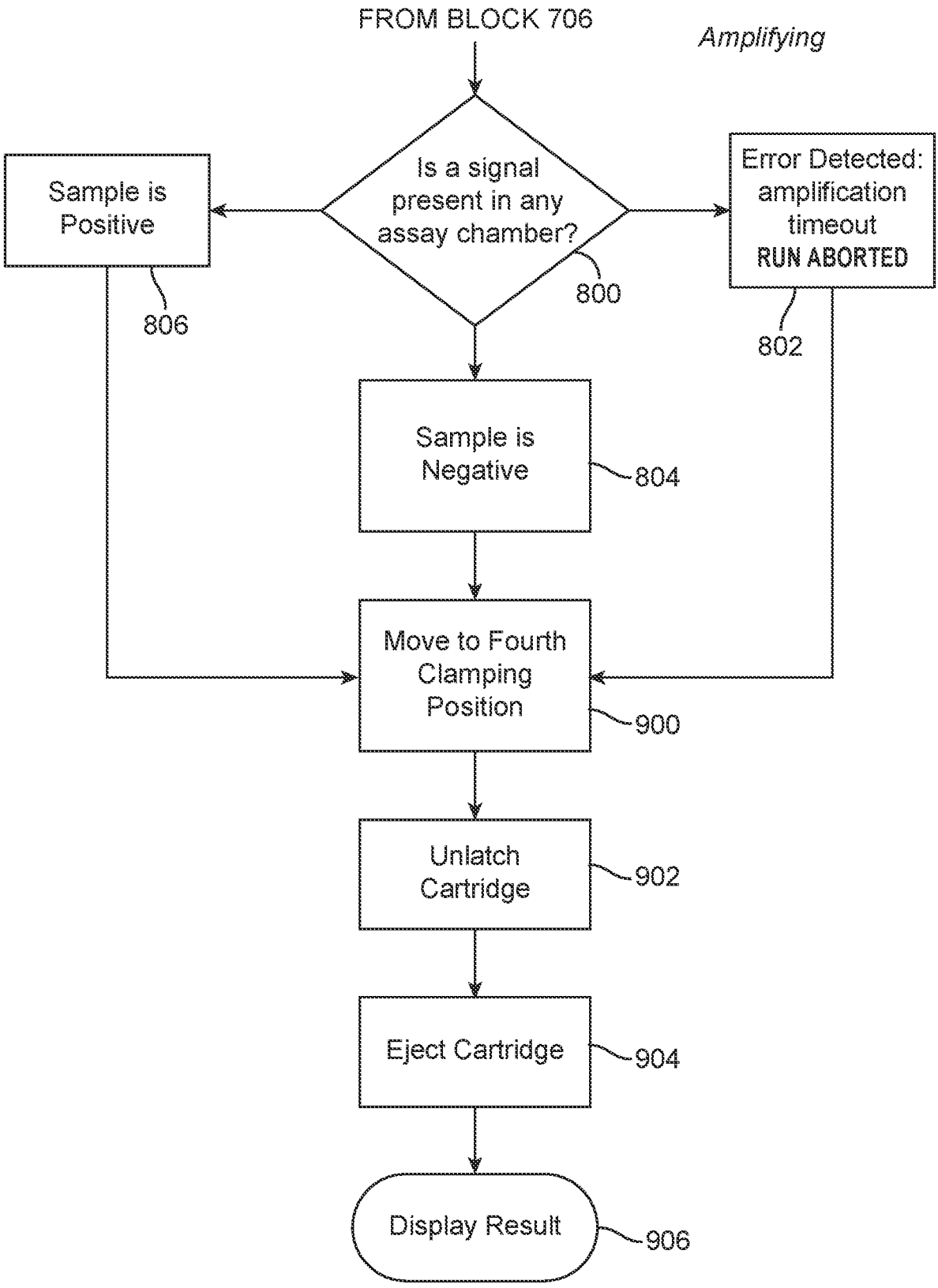

FROM BLOCK 706
Amplifying
Is a signal present in any assay chamber?
800
Sample is Positive
806
Error Detected: amplification timeout RUN ABORTED
802
Sample is Negative
804
Move to Fourth Clamping Position
900
Unlatch Cartridge
902
Eject Cartridge
904
Display Result
906

FIG. 106E

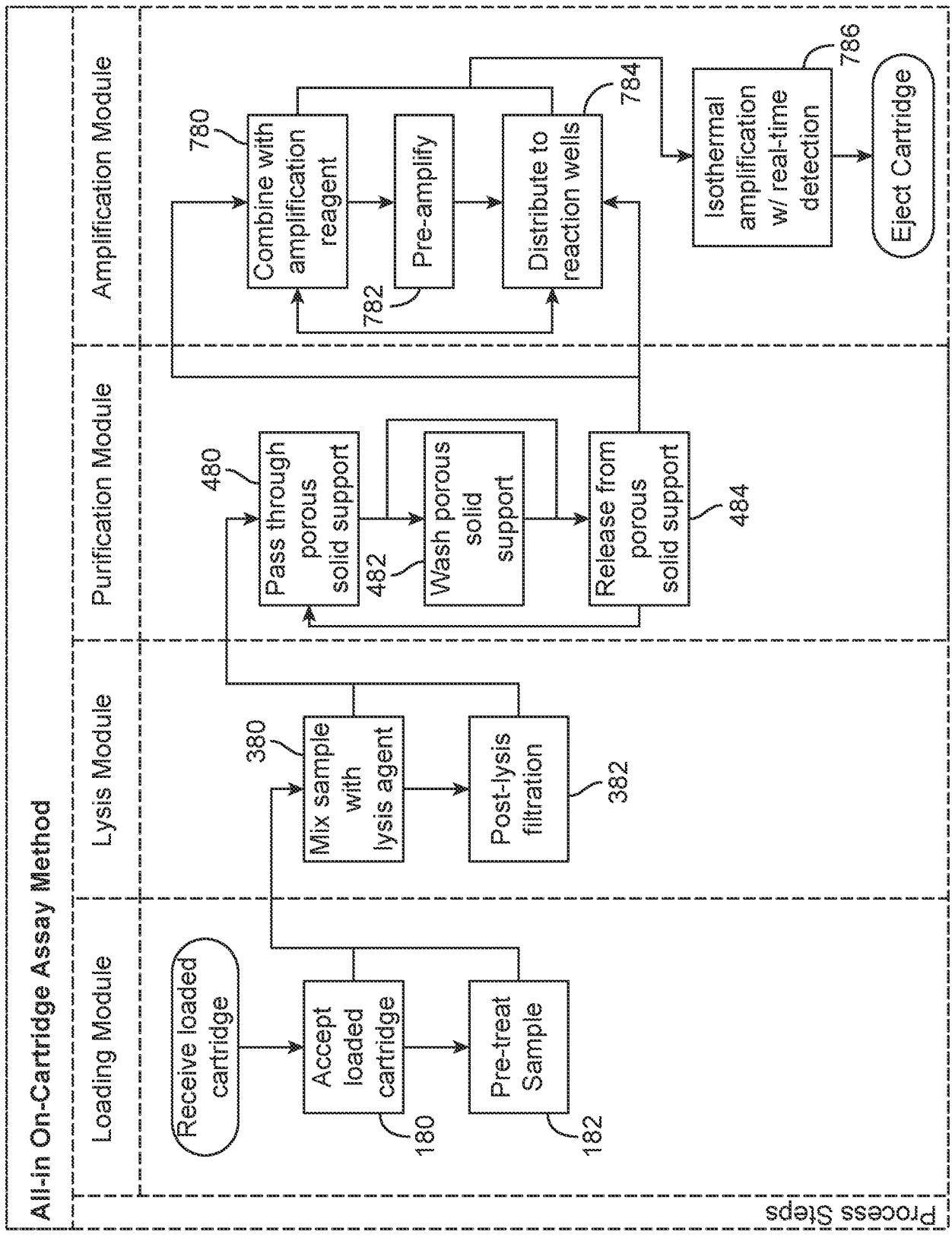

All-in On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
Pre-treat Sample
182
380
Mix sample with lysis agent
Post-lysis filtration
382
480
Pass through porous solid support
482
Wash porous solid support
Release from porous solid support
484
780
Combine with amplification reagent
782
Pre-amplify
Distribute to reaction wells
784
Isothermal amplification w/ real-time detection
786
Eject Cartridge

FIG. 107

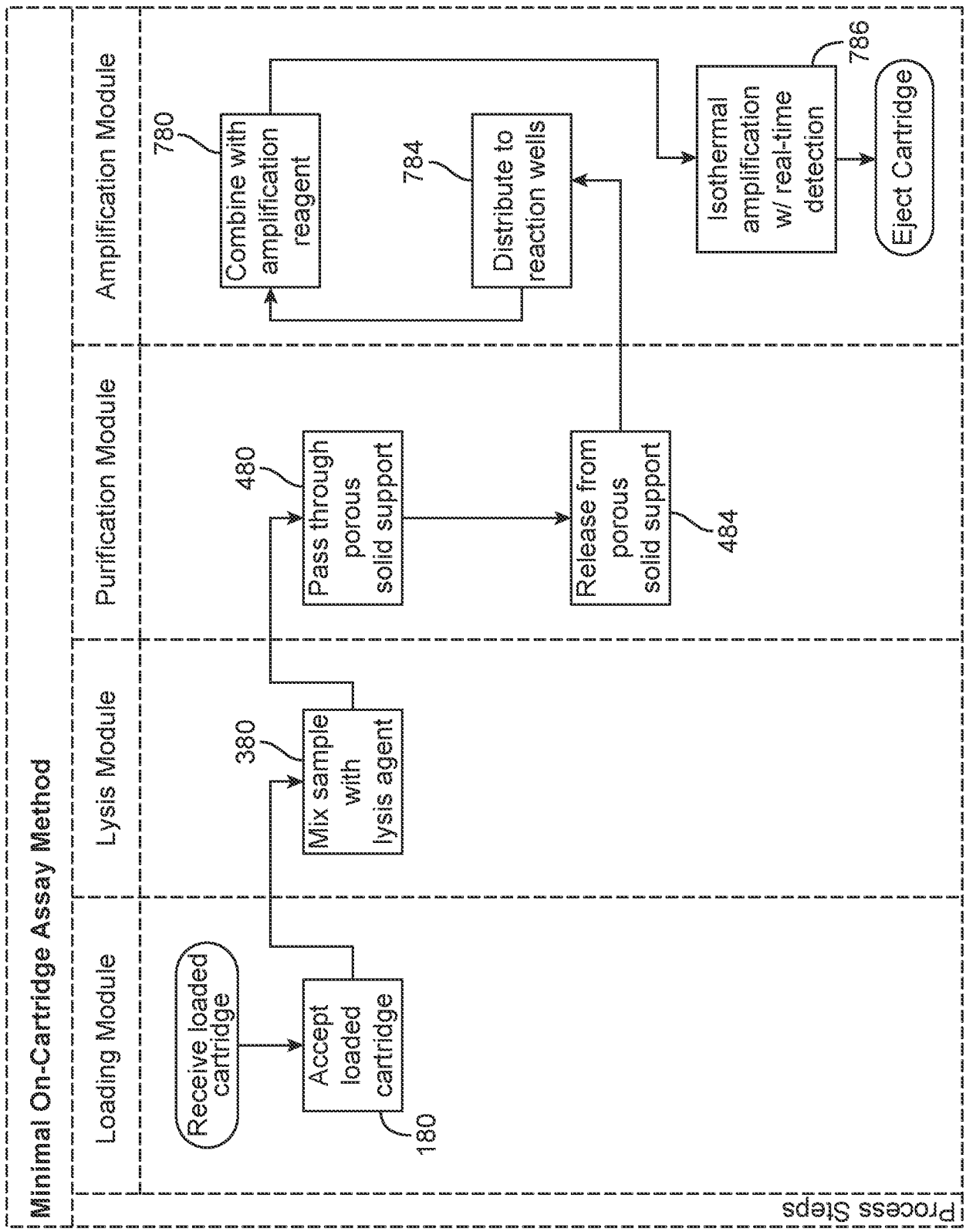

Minimal On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
380
Mix sample with lysis agent
480
Pass through porous solid support
484
Release from porous solid support
780
Combine with amplification reagent
784
Distribute to reaction wells
786
Isothermal amplification w/ real-time detection
Eject Cartridge

FIG. 108

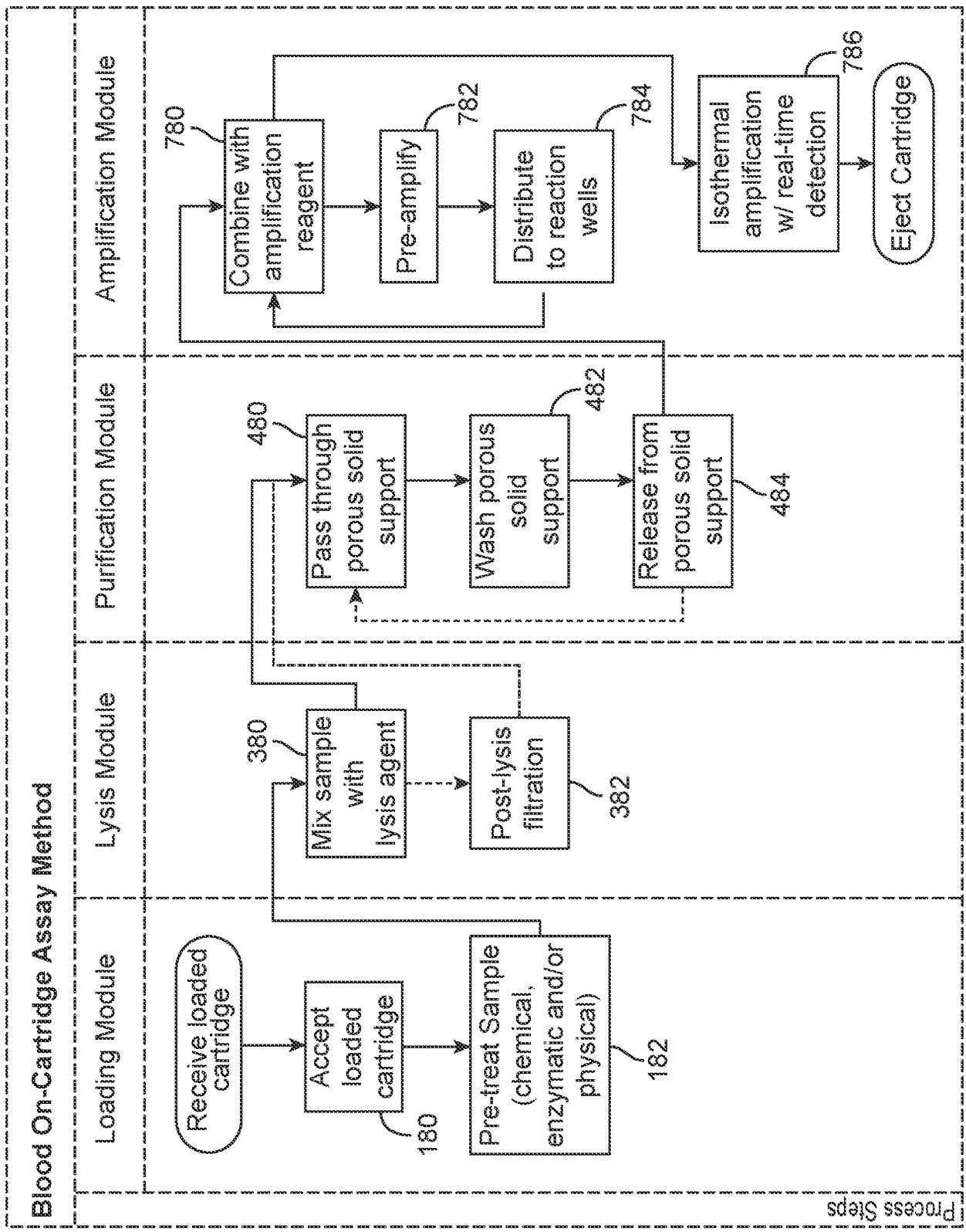

Blood On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
Pre-treat Sample (chemical, enzymatic and/or physical)
182
380
Mix sample with lysis agent
Post-lysis filtration
382
480
Pass through porous solid support
Wash porous solid support
482
Release from porous solid support
484
780
Combine with amplification reagent
Pre-amplify
782
Distribute to reaction wells
784
Isothermal amplification w/ real-time detection
786
Eject Cartridge

FIG. 109

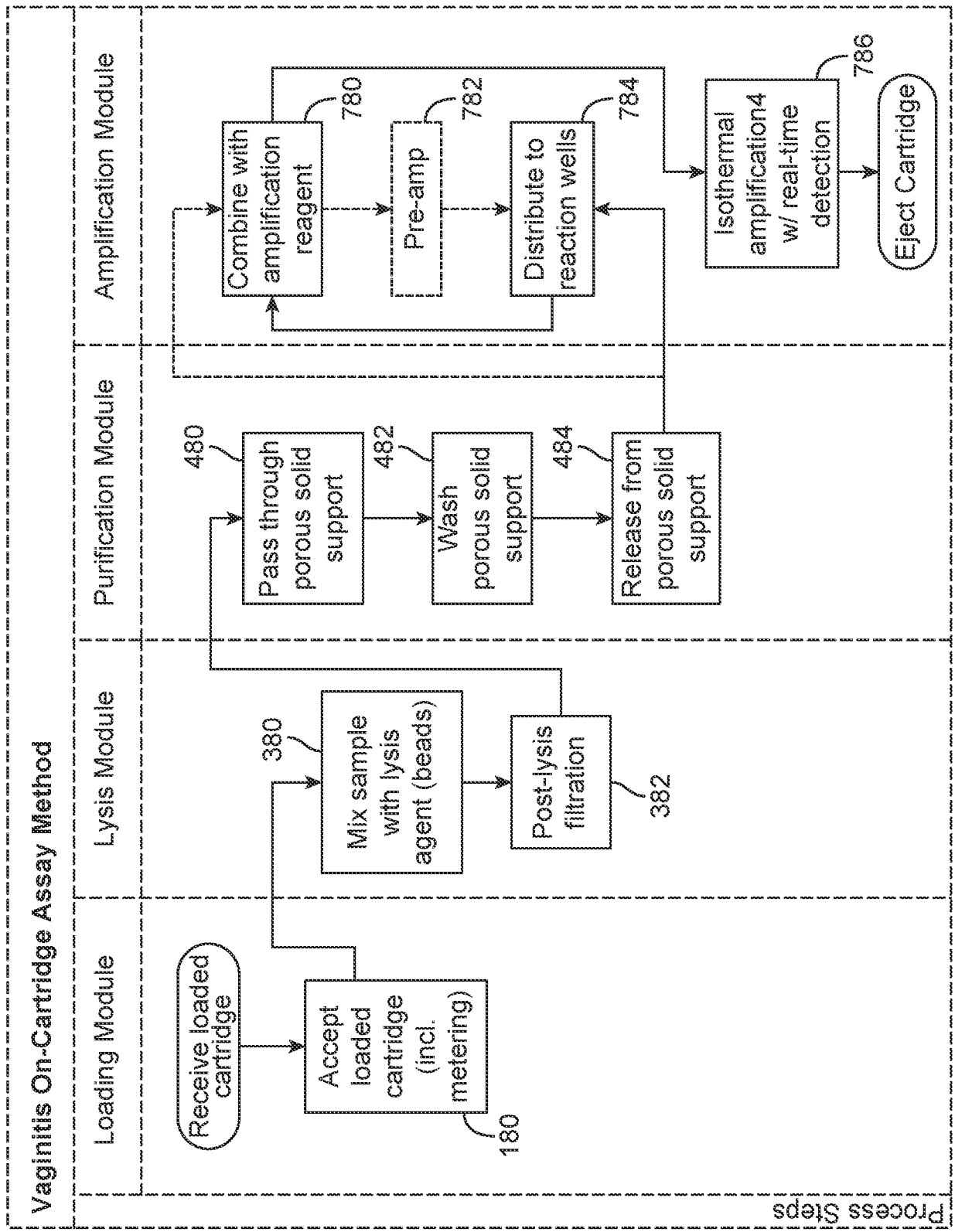

Vaginitis On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge (incl. metering)
180
380
Mix sample with lysis agent (beads)
Post-lysis filtration
382
480
Pass through porous solid support
482
Wash porous solid support
484
Release from porous solid support
Combine with amplification reagent
780
Pre-amp
782
Distribute to reaction wells
784
Isothermal amplification4 w/ real-time detection
786
Eject Cartridge

FIG. 110

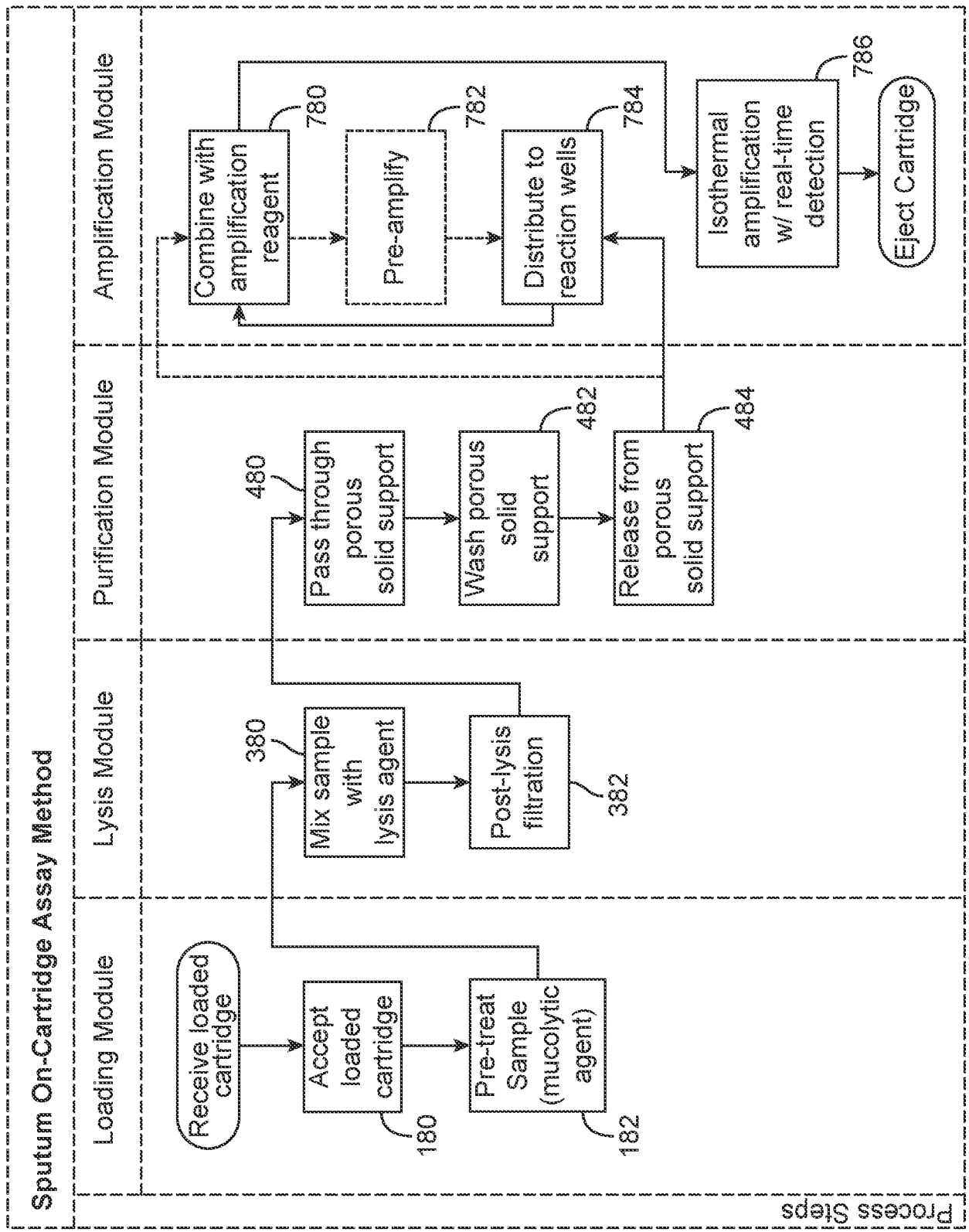

Sputum On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
Pre-treat Sample (mucolytic agent)
182
380
Mix sample with lysis agent
Post-lysis filtration
382
480
Pass through porous solid support
Wash porous solid support
482
Release from porous solid support
484
Combine with amplification reagent
780
Pre-amplify
782
Distribute to reaction wells
784
Isothermal amplification w/ real-time detection
786
Eject Cartridge

FIG. 111

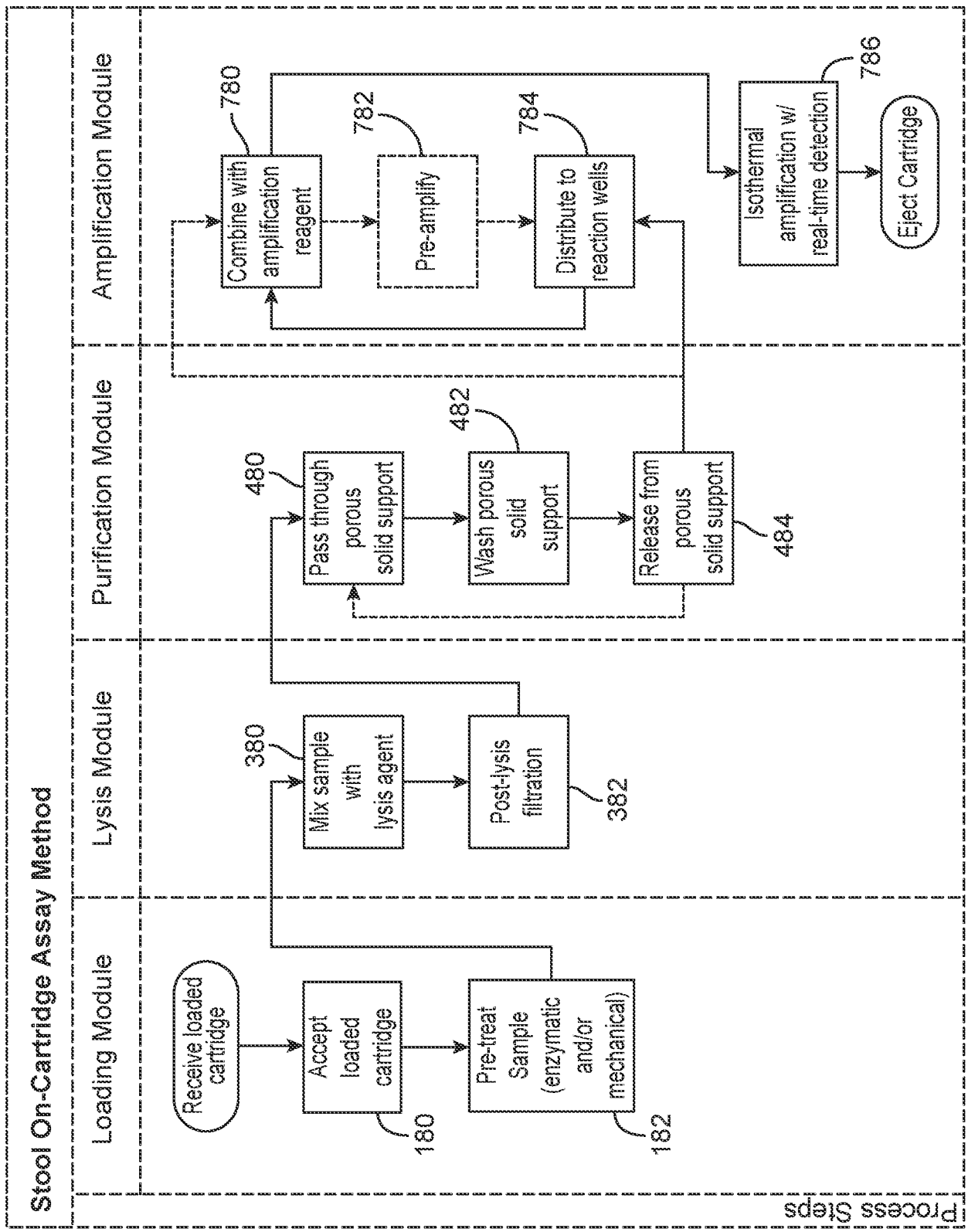

Stool On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
Pre-treat Sample (enzymatic and/or mechanical)
182
Mix sample with lysis agent
380
Post-lysis filtration
382
Pass through porous solid support
480
Wash porous solid support
482
Release from porous solid support
484
Combine with amplification reagent
780
Pre-amplify
782
Distribute to reaction wells
784
Isothermal amplification w/ real-time detection
786
Eject Cartridge

FIG. 112

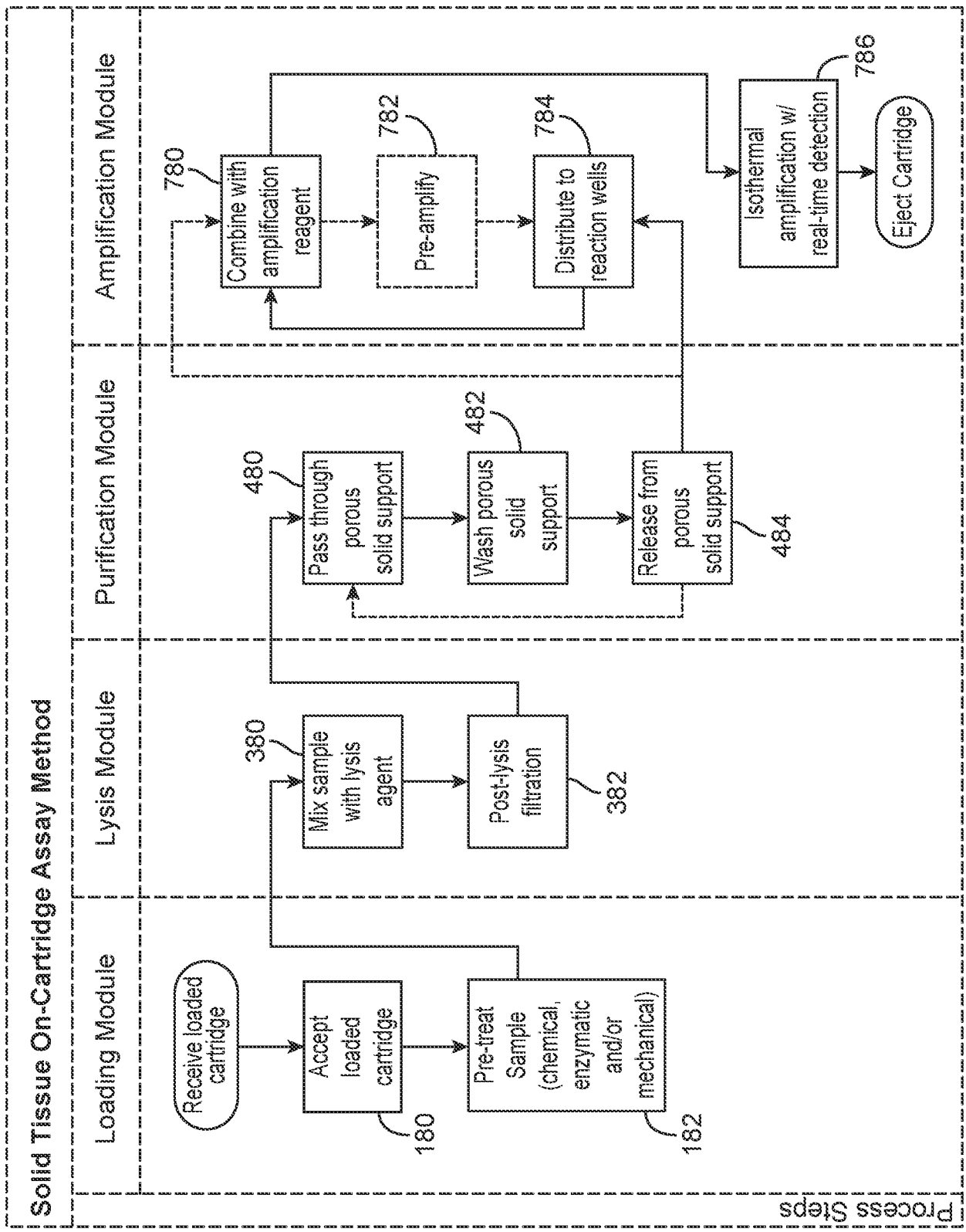

Solid Tissue On-Cartridge Assay Method
Process Steps
Loading Module
Lysis Module
Purification Module
Amplification Module
Receive loaded cartridge
Accept loaded cartridge
180
Pre-treat Sample (chemical, enzymatic and/or mechanical)
182
380
Mix sample with lysis agent
Post-lysis filtration
382
480
Pass through porous solid support
482
Wash porous solid support
Release from porous solid support
484
780
Combine with amplification reagent
782
Pre-amplify
784
Distribute to reaction wells
786
Isothermal amplification w/ real-time detection
Eject Cartridge

FIG. 113