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(54) Title: COMBINED DETECTION INSTRUMENT FOR CULTURE SPECIMEN CONTAINERS AND INSTRUMENT FOR IDENTIFICATION AND/OR CHARACTERIZATION OF A MICROBIAL AGENT IN A SAMPLE

(57) Abstract: A detection instrument determines whether a specimen container (e.g., blood culture bottle) is positive for presence of microbial agent growth therein. When the container is deemed positive it is made available (e.g. transferred or exposed to) to an automated instrument performing identification and/or characterization of the microbial agent. The identification and/or characterization instrument removes a portion of the sample from the specimen container and places it into a disposable separation and concentration device. The microbial agent is concentrated via optional selective lysis of non-microbial agent cellular material which may be present and centrifugation. A reading module reads the concentrated microbial agent using spectroscopic methods, e.g., measurements of intrinsic fluorescence. Such interrogation may occur while the microbial agent remains concentrated in the disposable device.



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**COMBINED DETECTION INSTRUMENT FOR CULTURE SPECIMEN
CONTAINERS AND INSTRUMENT FOR IDENTIFICATION AND/OR
CHARACTERIZATION OF A MICROBIAL AGENT IN A SAMPLE**

5 **CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority under 35 U.S.C. § 119(e) to (1) US provisional application serial no. 61/216,339, entitled "System for Combining a Non-invasive Rapid Detection Blood Culture System with an Invasive Microbial Separation and Characterization System", filed May 15, 2009; (2) US provisional
10 application serial no. 61/277,862, entitled "Automated Loading Mechanism for Microbial Detection Apparatus", filed September 30, 2009; and (3) US provisional application serial no. 61/337,597, entitled "Automated Microbial Detection Apparatus", filed February 8, 2010, the content of which are incorporated by reference herein.

15 [0002] This application is also related to the following US patent applications, the content of which is incorporated by reference herein:

 [0003] US Serial No. 12/589,929, entitled "Methods for the isolation and identification of microorganisms", filed October 30, 2009.

 [0004] US Serial No. 12/589,969, entitled "Separation device for use in the
20 separation, identification and/or characterization of microorganisms", filed October 30, 2009.

 [0005] US Serial No. 12/589,952, entitled "Method for separation, identification and/or characterization of microorganisms using spectroscopy", filed October 30, 2009.

25 [0006] US Serial No. 12/589,936, entitled "Method for separation, identification and/or characterization of microorganisms using mass spectrometry", filed October 30, 2009.

 [0007] U.S. Serial No. 12/589,985, entitled "Method for separation and characterization of microorganisms using identifier agents", filed October 30, 2009.

30 [0008] US Serial No. 12/589,968, entitled "Method for detection, identification and/or characterization of microorganisms in a sealed container", filed October 30, 2009.

 [0009] US Serial No. 12/589,976, entitled "Method for separation, identification and/or characterization of microorganisms using Raman spectroscopy",
35 filed October 30, 2009.

[0010] This application is also related to the following applications filed on the same date as this application, the content of which is incorporated by reference herein:

[0011] Docket no. 01121, serial no. _____ “Automated microbial detection apparatus.”

[0012] Docket no. 09-271-A, serial no. _____, “Methods for rapid identification and/or characterization of a microbial agent in a sample.”

[0013] Docket no. 09-271-B, serial no. _____, “System for rapid identification and/or characterization of a microbial agent in a sample.”

STATEMENT OF FEDERALLY SPONSORED RESEARCH

[0014] Not applicable.

BACKGROUND

[0015] This invention solves a long-felt need in the art for an automated instrument and method for rapidly characterizing and/or identifying a microbial agent in a sample, such as blood or other biological sample, stored in a specimen container. As an example, the instrument of this disclosure provides information as to Gram type (positive or negative), morphology, species or other relevant clinical information of the microbial agent rapidly and automatically.

[0016] Instruments currently exist on the market in the U.S. that detect the growth and therefore the presence of a microorganism in a blood sample. One such instrument is the BacT/ALERT 3D instrument of the present assignee bioMérieux, Inc. The instrument receives a blood culture bottle containing a blood sample, e.g., from a human patient. The instrument incubates the bottle. Periodically during incubation an optical detection unit in the incubator analyzes a colorimetric sensor incorporated into the bottle to detect whether microbial growth has occurred within the bottle. The optical detection unit, specimen containers and sensors are described in the patent literature, see U.S. patents 4,945,060; 5,094,955; 5,162,229; 5,164,796; 5,217,876; 5,795,773; and 5,856,175, the entire content of each of which is incorporated by reference herein. Other prior art of interest relating generally to the detection of microorganisms in a biological sample includes the following patents:

U.S. 5,770,394, U.S. 5,518,923; U.S. 5,498,543, U.S. 5,432,061, U.S. 5,371,016, U.S. 5,397,709, U.S. 5,344,417, U.S. 5,374,264, U.S. 6,709,857; and U.S. 7,211,430.

[0017] In detection instruments such as the BacT/ALERT 3D and similar instruments, once the blood culture bottle has been tested positive for microorganism presence, it is difficult to obtain a high level of characterization of the microbial agent, or identification of the species of the microbial agent, due to the interference of blood components and artifacts of the disposable system (e.g., bottle) containing the sample. Therefore, current methods use a bottle or other suitable disposable container and a related instrument for natural growth and detection of a microorganism in the sample, as described above. Once the instrument indicates that the bottle is positive for presence of a microbial agent, according to current methods the “positive” bottle is manually retrieved from the instrument and a portion of the sample is manually removed from the bottle and cultured on an agar plate. There are instruments in the art that automate the streaking of a sample medium on a culture plate and incubating the plate. One such instrument is described in U.S. Patent 6,617,146. After streaking, the plate is manually placed in an incubator and periodically inspected for growth of a subculture of the microorganism. After the subculture has grown sufficiently, a sample of the culture is taken from the plate and placed in a test tube. The test tube is then introduced into yet another instrument for identification testing via a disposable test sample card having a multitude of individual wells. The disposable test cards are known in the patent literature, see e.g., U.S. Patents 4,118,280, 3,963,355, 4,018,65; 4,116,775 and 4,038,151, 5,609,828, 5,746,980, 5,766,553, 5,843,380, 5,869,005, 5,916,812, 5,932,177, 5,951,952, and 6,045,758, the entire content of which is incorporated by reference herein.

[0018] The test sample card is then processed in an analytical instrument known in the art as the VITEK 2 instrument of the assignee. The VITEK 2 instrument incubates and periodically reads the wells of the test sample card with a reader unit. Growth of the sample in one or more of the wells of the cards results in identification of the microbial agent. The VITEK 2 instrument is described in the patent literature, see e.g., U.S. Patents 5,762,873 and 6,086,824, the content of which is incorporated by reference herein.

[0019] This entire process from the time of introducing the sample into the blood collection bottle to culture, detection of microorganism presence, and then identification of the microorganism by the VITEK 2 instrument typically takes 2-5

days. The identification steps alone, occurring after positive bottle detection, typically occupy 1-3 of these days.

[0020] Substantial, and potentially life saving, clinical benefits for a patient are possible if the time it takes for detection and identification of a microbial agent in a blood sample and reporting the results to a clinician could be reduced from the current 2-5 days to less than one day. A system that meets this need has heretofore eluded the art. However, such rapid identification and/or characterization of a microbial agent in a biological sample such as a blood sample is made possible by this invention.

[0021] The system for rapidly identifying and/or characterizing a microbial agent set forth herein can be advantageously combined with an automated detection instrument for detecting the presence of an agent in the specimen container, as described in embodiments disclosed herein. In this combination, the inventive system and methods combine a detection instrument operative to detect a container containing a blood or other sample as being positive for microbial agent presence, and rapid and automated identification of the agent. In one embodiment, the detection instrument may be coupled to or integrated with an automated identification and/or characterization instrument as described herein performing additional steps necessary for identification and/or characterization of a microbial agent at the time of detection. The resulting combined system presents a unique automated solution for rapid identification and/or characterization at the time of detection, providing a complete system solution. The total time from first loading a biological sample into a detection container (e.g., bottle) to identification and/or characterization is typically less than 24 hours in most cases. Moreover, instead of it taking one to three additional days to obtain the identification and/or characterization of the microbial agent after a bottle is tested positive, as in the prior art, such results can potentially be obtained in less than one hour with the present inventive system and methods. The instrument of this disclosure also provides the ability to provide a rapid and automated identification and/or characterization result at any time of the day or night.

[0022] The systems and methods of this disclosure have other incidental benefits and features, including the potential to: (a) reduce exposure of lab personnel to sharps and biohazard materials; (b) reduce laboratory labor and user errors; (c) improve sample tracking, traceability and information management; (d) interface to laboratory automation systems; (e) improve workflow and ergonomics; (f) improve

patient care by delivering clinically relevant actionable information; and (g) provide faster results thereby potentially decreasing costs by focusing earlier on appropriate antimicrobial therapy and reducing hospital stay.

5 [0023] Many further advantages and benefits over the prior art will be explained below in the following detailed description.

SUMMARY

10 [0024] In general, an automated system is disclosed for rapid detection of a microbial agent in a sample and identifying and/or characterizing the microbial agent. In one form, the system includes a detection instrument receiving a specimen container containing a sample. The detection instrument includes a heated enclosure for incubating the specimen container and a detection unit interrogating the specimen container to detect whether the specimen container is positive for the presence of a
15 microbial agent in the sample. The system includes a supply of disposable separation devices. The system further includes and an automated identification/characterization instrument. This instrument receives a specimen container detected positive by the detection instrument. The identification/characterization instrument includes (a) a sample removal apparatus operative to remove a portion of the sample from the
20 specimen container and add the portion to a separation device obtained from the supply of separation devices; (b) a separation and concentration station operative on the separation device after receiving the portion of the sample so as to separate the microbial agent from other products in the sample and concentrate the microbial agent within the separation device; and (c) an identification and/or characterization module
25 interrogating the concentrated microbial agent to identify and/or characterize the microbial agent.

[0025] In another aspect, the invention can be viewed as a method for rapid identification and/or characterization of a microbial agent present in a sample, the sample loaded into a specimen container, comprising performing the following steps
30 in an automated manner:

[0026] (a) incubating the specimen container;

[0027] (b) periodically interrogating the specimen container to determine whether it is positive for presence of a microbial agent within the specimen container;

[0028] (c) when the specimen container is determined positive, withdrawing a portion of the sample from the specimen container;

[0029] (d) introducing the portion of the biological sample into a disposable separation device;

5 [0030] (e) lysing components in the portion of the biological sample, either before or after performing step (d);

[0031] (f) separating and concentrating the microbial agent within the separation device; and (g) analyzing the concentrated microbial agent to identify and/or characterize the microbial agent.

10 [0032] Steps (a)-(g) can be performed automatically within an integrated detection and identification/characterization instrument as described herein.

[0033] In one embodiment the analyzing step (g) comprises the step of performing a spectroscopic analysis of the concentrated microbial agent. In another embodiment the spectroscopic analysis comprise one of (i) intrinsic fluorescence
15 spectroscopy or (ii) Raman spectroscopy.

[0034] The method may further include step (f)(1) withdrawing the concentrated microbial agent from the separation device and wherein step (g) is performed on the concentrated microbial agent after withdrawal from the separation device.

20 [0035] The analyzing step (g) may include at least one of (i) performing a spectroscopic analysis on the concentrated microbial agent, (ii) performing mass spectrometry on the concentrated microbial agent, (iii) performing molecular testing of the concentrated microbial agent and (iv) introducing the concentrated microbial agent into a microbial characterization test device and subjecting the test device to
25 analysis.

[0036] In one configuration, steps (a) and (b) are performed in an automated detection instrument and wherein steps (c), (d), (e) and (f) are performed in an automated identification and/or characterization instrument.

[0037] In yet another aspect, the invention can be viewed as an automated
30 method for rapid characterization and/or identification of a microbial agent has been disclosed, comprising the steps of: (a) providing a detection subsystem having an entrance location for receiving specimen containers containing samples to be tested, and detection apparatus for detecting when a specimen container has become positive for presence of a microbial agent in a sample within the specimen container; (b) when

a specimen container has become positive, automatically moving the specimen container to an identification and/or characterization subsystem remote from the detection subsystem; (c) within the characterization and/or identification subsystem, automatically performing the steps of: (1) withdrawing a portion of the sample from the specimen container; (2) dispensing the withdrawn portion of the sample into a disposable separation device; (3) concentrating the microbial agent within the withdrawn portion of the sample within the separation device; and (4) analyzing the concentrated microbial agent to characterize and/or identify the microbial agent.

[0038] In still another aspect, an automated method for rapid characterization and/or identification of a microbial agent has been disclosed, comprising the steps of: (a) receiving a specimen container containing a sample to be tested in a detection instrument; (b) detecting when the specimen container has become positive for presence of a microbial agent in the sample; (c) when such detection has occurred, automatically removing a portion of the sample from the specimen container and placing the portion of the sample into a disposable separation device; (d) separating and concentrating the microbial agent in a disposable separation device, and (e) analyzing the concentrated microbial agent to identify and/or characterize the microbial agent.

[0039] In still another aspect, a sample testing system has been disclosed, comprising, in combination: a detection subsystem having an entrance location for receiving specimen containers containing samples to be tested, and detection apparatus for detecting when a specimen container has become positive for presence of a microbial agent in the sample; an identification and/or characterization subsystem receiving a positive specimen container and having modules for automatically characterizing and/or identifying the microbial agent in the positive specimen container; and apparatus for automatically transferring a positive detection container from the detection subsystem to the identification and/or characterization subsystem.

[0040] The apparatus for automatically transferring can take the form of a conveyor system as described in several embodiments below. The automatically transferring can also take the form of a robotic arm assembly. In still another embodiment, the conveyor system includes a first conveyor and a second conveyor, and wherein the system comprises two detection subsystems daisy chained and coupled to each other by the first conveyor, and wherein the one of the detection subsystems is coupled to the identification and/or characterization subsystem by the

second conveyor, and wherein a positive specimen detected in either the two detection subsystems is moved to the identification and/or characterization subsystem by means of the second conveyor.

5 **[0041]** In some embodiments described herein, the identification module in the automated identification and/or characterization instrument interrogates the concentrated microbial agent while it is contained within the separation device, and to that end the separation device may be made from suitable materials and optically transparent to facilitate an optical interrogation. For example, the identification
10 module may include the features of US Serial No. 12/589,929, entitled "Methods for the isolation and identification of microorganisms", filed October 30, 2009; US Serial No. 12/589,969; entitled "Separation device for use in the separation, identification and/or characterization of microorganisms", filed October 30, 2009; US Serial No. 12/589,952, entitled "Method for separation, identification and/or characterization of
15 microorganisms using spectroscopy", filed October 30, 2009; US Serial No. 12/589,936, entitled "Method for separation, identification and/or characterization of microorganisms using mass spectrometry", filed October 30, 2009; U.S. Serial No. 12/589,985, entitled "Method for separation and characterization of microorganisms using identifier agents", filed October 30, 2009; US Serial No. 12/589,968, entitled
20 "Method for detection, identification and/or characterization of microorganisms in a sealed container", filed October 30, 2009 and US Serial No. 12/589,976, entitled "Method for separation, identification and/or characterization of microorganisms using raman spectroscopy", filed October 30, 2009, the entire contents of which are incorporated by reference herein. A variety of optical technologies are envisioned for
25 use in the identification instrument, including for example, spectroscopic measurements such as fluorescence spectroscopy measuring intrinsic fluorescence from the concentrated microbial agent, diffuse reflectance spectroscopy, Raman spectroscopy, or other optical technique capable of characterizing and/or identifying a microorganism based on its chemical or physical make-up. In other embodiments, the
30 identification instrument may include a further instrument that removes all or a portion of the concentrated microbial agent from the separation device and analyzes the concentrated microbial agent directly, e.g., via a mass spectrometer, or via another disposable testing device (e.g., test strip or test card).

[0042] In applications where a lysis of the sample is desirable as an incident to separation and concentration of the microbial agent in the separation device, a particular selective lytic buffer (“lytic agent”) may not be optimal for all organisms expected to be encountered in the sample. In this case where a positive sample does not yield an identification or characterization result due to a non-optimal lysis process, the system can be configured to automatically re-process the sample using an alternative lytic buffer formula. Information on the growth rates measured in an associated detection instrument could be used to select the most optimal lysis buffer formula for re-processing. Upon re-processing, it is expected that a true positive will yield a result. Otherwise, the sample is considered to be a false positive determination from the detection subsystem. Accordingly, in one configuration of the instrument several containers of different lysis buffers are included in the instrument and a selected lysis buffer is obtained, e.g., loaded into a sampling device used to withdraw a sample from the specimen container.

[0043] False positives occur with detection technology known in the art (e.g., in the BacT/ALERT instrument) at a very low rate. However, until the culture sample has been tested under incubation conditions for five days, a final negative determination of the sample cannot be made. Therefore, in one possible embodiment, the identification/characterization instrument may continue to test a false “positive” culture sample by automatically returning the specimen container to the incubation/agitation/detection mode of testing. In accordance with this embodiment continued testing occurs within a incubation rack housed in the identification/characterization instrument. An alternative embodiment is to return the specimen container to an associated detection instrument. Automation to re-initiate the incubation of the specimen container is thus additional optional aspect of the present disclosure. Operator intervention to re-initiate incubation could be employed, but would require vigilance on the part of the institution that operates the identification instrument.

[0044] These and many more aspects and features of the disclosure will be discussed below in conjunction with the appended drawing figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] The following detailed description makes reference to the appended drawing figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative and offered by way of example rather than restrictive.

5 [0046] Figure 1 is a perspective view of an automated system for rapid non-invasive detection of a microbial agent in a test sample. As shown, the system includes an automated loading mechanism.

[0047] Figure 2 is a perspective view of the detection system of Figure 1, showing a close-up view of the automated loading mechanism.

10 [0048] Figure 3 is a perspective view of the detection system of Figure 1, which shows an automated loading mechanism and a lower drawer that opens to reveal a waste container for containers that tested negative for presence of a microbial agent.

[0049] Figure 4 is a side view of one of the specimen containers processed in the detection system of Figure 1-3. While the detection container can take a variety of forms, in one embodiment it is configured as a blood culture bottle.

[0050] Figure 5A is a side elevation view of one configuration of the detection system of Figure 1., with the upper and lower doors open showing the interior chambers and racks for holding multiple containers of the type shown in Figure 4.

20 [0051] Figure 5B is a perspective view of the detection system shown in Figure 5A, with the upper and lower doors open showing the interior chambers and racks for holding multiple containers of the type shown in Figure 4.

[0052] Figure 6 is a perspective view of the transfer mechanism shown in Figures 5A and 5B, showing the horizontal and vertical support rails. Also shown are first and second rotational mechanisms, which are operable to rotate the transfer mechanism about one or more axes.

25 [0053] Figure 7A is a perspective view of the robotic head and vertical support rail shown in Figures 5A and 5B. As shown in Figure 7A, the robotic head is positioned in a vertical orientation, such that a specimen container held within the robotic head is also in a vertical orientation.

30 [0054] Figure 7B is another perspective view of the robotic head and vertical support rail shown in Figures 5A and 5B. As shown in Figure 7B, the robotic head is positioned in a horizontal orientation, such that the container held within the robotic head is also in a horizontal orientation.

[0055] Figures 8A-C shows a time-elapsd loading of a specimen container into the holding chamber of the robotic head shown in Figures 5A and 5B. As shown in Figure 8A, the gripping mechanism grips the top or cap of the container. Figure 8B shows the container in an intermediate position in the loading process. Figure 8B, 5 shows the container after being loaded into the robotic head.

[0056] Figures 9A and 9B are perspective and side views, respectively, of an alternative configuration of the detection system of Figures 1-3 and 5A-5B, with the upper and lower doors open showing an alternative configuration of the container holding structures. In the embodiment of Figures 9A and 9B, the racks are arranged 10 in a drum or cylinder-type configuration.

[0057] Figure 10 is a perspective view of another configuration of the automated loading mechanism, showing a first conveyor belt operable in a horizontal plane and a second conveyor belt operable in a vertical plane.

[0058] Figure 11 is a perspective view of yet another configuration of the 15 automated loading mechanism, showing a first conveyor belt operable in a horizontal plane and a second conveyor belt having a plurality of paddles and operable in a vertical plane.

[0059] Figure 12 is a perspective view of a casing and cover provided with an automated loading mechanism.

20 [0060] Figure 13 is a perspective view of one embodiment of an automated loading mechanism shown isolated from the detection system. In accordance with this embodiment, the automated loading mechanism comprises a loading station or area, a transport mechanism and an entrance location, for the fully automated loading of a specimen container. A portion of one side of the loading area has been removed 25 to show additional details of the automated loading mechanism of this embodiment.

[0061] Figure 14 is another perspective view of the automated loading mechanism shown in Figure 14. The container loading area is shown as a see through feature to reveal other features of the automated loading mechanism, as described herein.

30 [0062] Figure 15 is a close up perspective view of the drum-like loading mechanism, vertical chute, locating device and system transfer device in Figure 14. The drum-like loading mechanism, vertical chute, locating device and system transfer device are shown isolated from the detection system.

[0063] Figure 16 is a cross-sectional view of the automated loading mechanism shown in Figures 14-15. More specifically, Figure 16 is a cross-sectional view of the drum-like loading mechanism and vertical chute showing a specimen container falling through the chute. As shown in Figure 16, the top or cap of the specimen container is held in place briefly by the tapered ledge as the bottom of the container falls through the chute, thereby up-righting the specimen container.

[0064] Figure 17 is a perspective view of the automated detection apparatus comprising the automated loading mechanism shown in Figure 14. The container loading area of the automated loading mechanism is shown in a user accessible location on the front of an automated system for rapid non-invasive detection of a microbial agent. The automated detection system and the container loading area are shown with side panels removed and/or as see through features to reveal other features, as described herein.

[0065] Figure 18 is a perspective view of the automated detection apparatus comprising an alternative loading mechanism. The container loading area of the automated loading mechanism is shown in a user accessible location on the front of an automated system for rapid non-invasive detection of a microbial agent. The automated detection system and the container loading area are shown with side panels removed and/or as see through features to reveal other features, as described herein.

[0066] Figure 19 is a side view of the lower portion of the automated system for rapid non-invasive detection of a microbial agent shown in Figure 17. The automated detection system is shown with side panel removed to reveal other features of the system, as described herein.

[0067] Figure 20 is a perspective view of the holding structure and automated transfer mechanism shown in Figures 17-19. As shown, in this embodiment, the automated transfer mechanism comprises a lower horizontal support, a vertical support, a pivot plate and a robotic head for transferring a specimen container within a detection apparatus. For clarity, the holding structure and automated transfer mechanism are shown isolated from the detection apparatus.

[0068] Figures 21A-B are perspective views of the pivot plate and robotic head of the automated transfer mechanism shown in Figure 20. The robotic head is shown with a cross-sectional view of the gripping mechanism and specimen container to reveal the features of the gripping mechanism. As shown in Figure 21A, the robotic head is located at a first end of the pivot plate and in a horizontal orientation,

such that the specimen container is also orientated in a horizontal orientation. In Figure 21B, the robotic head is shown located at a second end of the pivot plate and in a vertical orientation, such that the specimen container is also orientated in a vertical orientation.

5 [0069] Figure 22 is a perspective view of an alternative configuration of the automated detection apparatus showing a user interface, a status screen, a locator device cover and two positive container ports.

 [0070] Figure 23 is a perspective view showing another design configuration of the detection apparatus. As shown in Figure 23, the detection system comprises a
10 first detection apparatus and a second detection instrument.

 [0071] Figure 24 is a perspective view of yet another embodiment of the automated detection system. As shown, the automated detection system comprises a first detection apparatus having an automated loading mechanism and a second or down-stream detection apparatus linked or “daisy-chained” to the first detection
15 apparatus, as described herein.

 [0072] Figures 25A-C show a time-elapsd pusher arm mechanism for pushing a specimen container from a first detection apparatus to a second or down-stream detection apparatus.

 [0073] Figure 26 shows a perspective view of the holding structure and agitation assembly shown isolated from the detection system.
20

 [0074] Figure 27A is a perspective view of a rack holding structure and retention feature for holding a specimen container securely within the rack holding structure.

 [0075] Figure 27B shows a cross-sectional view of the rack holding structure and retention feature shown in Figure 27A.
25

 [0076] Figure 27C is a top cross-sectional view of the rack holding structure and retention feature of Figure 27A, showing a schematic representation of a canted coil spring.

 [0077] Figure 28A-B show first and second perspective views of a carrier for
30 carrying a plurality of specimen containers to the detection apparatus. As shown, the carrier comprises a plurality of holding wells for holding a plurality of specimen containers. Figure 28A also shows two opposed gripping features or handles and a

release mechanism for releasing the plurality of specimen containers at the loading station, as described herein.

[0078] Figure 29 shows a perspective view of another possible configuration for the detection system. As shown in Figure 29, the detection system includes a
5 release mechanism for releasing one or more specimen containers from the carrier shown in Figures 28A-B.

[0079] Figure 30 is a flow chart showing the steps performed in the operation of the detection system.

[0080] Figure 31 is a block diagram of an automated instrument for rapid
10 identification and/or characterization of a microbial agent present in a sample.

[0081] Figure 32 is a perspective view of one possible configuration of the instrument shown in Figure 31. The instrument includes a rack for holding specimen containers, a cassette of disposables (including sampling devices and separation devices), a robotic transfer mechanism, sample removal apparatus, a separation and
15 concentration device in the form of a centrifuge, and a identification and/or characterization module (read station) operating to interrogate a separation device containing a concentrated microbial agent for identification and/or characterization of the microbial agent. In one possible embodiment, the instrument of Figure 32 could be integrated with an automated detection instrument (See Figure 58, 77-78 below), in
20 which case the rack for the specimen containers is the same structure holding the specimen containers during the detection operations. Alternatively, the identification and/or characterization instrument is located remotely from but coupled to an automated detection instrument as shown in Figure 77 and described in our prior provisional application and in conjunction with Figures 1-30.

[0082] Figure 33 is top plan view of the identification and/or characterization
25 instrument of Figure 32, showing the rack of positive specimen containers in one position for incubation.

[0083] Figure 34 is a top plan view of the instrument of Figure 32, showing the rack for the positive specimen containers moved to a position for withdrawal of
30 the sample from the bottle for identification and/or characterization testing.

[0084] Figure 35 is another perspective view of the embodiment of Figures 32-34.

[0085] Figure 36 is a perspective view of a separation device which is used in conjunction with the identification/characterization instrument of Figure 31. The

separation device receives a portion of the sample from a positive specimen container. The microbial agent is concentrated at the bottom of a capillary tube located in the separation device in the manner described herein. The concentrated microbial agent is then interrogated by a identification and/or characterization reading module to
5 characterize and/or identify the microbial agent.

[0086] Figure 37 is a perspective view of the separation device of Figure 36.

[0087] Figure 38 is a cross-sectional view of the separation device of Figures 36 and 37.

[0088] Figure 39 is a cross-sectional view of an end-cap which is fitted to the
10 lower end of the separation device of Figures 36-38.

[0089] Figure 40 is a cross-sectional view of the separation device of Figure 36, showing the concentrated microbial agent in the capillary tube of the separation device after centrifugation.

[0090] Figure 41 is a schematic illustration of the concentrated microbial
15 agent in the separation device of Figure 36 being interrogated by the identification/characterization or reading module.

[0091] Figure 42 is a perspective view of an alternative embodiment of the separation device of Figure 36.

[0092] Figure 43 is a cross-section of the separation device of Figure 42.

[0093] Figure 44 is an illustration of one embodiment of a disposable
20 sampling device which is used within the identification and/or characterization instrument.

[0094] Figure 45 is a detailed perspective view showing the operation of the sample removal apparatus in the identification and/or characterization instrument to
25 pick up one of the sampling devices of Figure 44 from a cassette of disposable devices. The cassette includes a multitude of the separation devices of Figure 46 or 42 and a multitude of the sampling devices of Figure 44.

[0095] Figure 46 is a detailed perspective view showing the operation of the sample removal apparatus to sterilize the stopper at the top of the detection container
30 and vent the detection container.

[0096] Figure 47 is a more detailed illustration of the sample removing apparatus in the position shown in Figure 46.

[0097] Figure 48 is a detailed perspective view showing the operation of the sample removal apparatus to withdraw a portion of the sample within the detection container into one of the disposable sampling devices of Figure 44.

5 [0098] Figure 49 is a more detailed illustration of the sample removal apparatus in the position of Figure 48.

[0099] Figures 50A-50C are three perspective views of the sample removal apparatus showing the operations of dispensing the sample into one of the separation devices and transfer the sampling device to the waste container.

10 [0100] Figure 51 is sequence of perspective views of the sample removal apparatus showing the operations of transferring the separation device to the separation and concentration station and optical interrogation of the separation device in the identification and/or characterization module.

[0101] Figure 52 is a more detailed illustration of the separation and concentration station and the identification and/or characterization module.

15 [0102] Figure 53 is a sequence of three perspective views of the identification and/or characterization instrument showing the operations of picking up the separation device, transferring the separation device to the waste container and placing the separation device in the waste container.

20 [0103] Figure 54 is a more detailed illustration of the operation of placing the separation device into the waste container.

[0104] Figure 55 is a block diagram of an alternative configuration of the identification and/or characterization instrument in which the concentrated microbial agent is removed from the separation device and analyzed after removal. The analysis could be performed by any one of a number of different types of systems or units, including a molecular diagnostic test unit, a mass spectrometry unit, or a microbial identification test device and associated processing instrument.

25 [0105] Figures 56A-56C are a flow chart showing the steps performed in the operation of both automatically detecting the presence of a microbial agent in a specimen container (Figure 56A) and automatically identifying and/or characterizing the microbial agent (Figure 56B and 56C).

30 [0106] Figure 57 is a perspective view of a second embodiment of an instrument for rapid and automated identification and/or characterization of a microbial agent in a sample.

[0107] Figure 58 is a perspective view of the instrument of Figure 57, showing one possible configuration of the racks for holding the specimen containers. In the embodiment of Figure 58, the racks include features for incubation of the specimen containers, agitation of the specimen containers, and automated detection of microbial growth within the specimen containers. Thus, Figure 58 shows one embodiment in which the automated detection and identification instruments can be combined into a single instrument.

[0108] Figure 59 is another perspective view of the embodiments of Figures 57 and 58. A multiple axis robot is used access the specimen containers and perform the sampling operation using disposable sampling devices.

[0109] Figure 60 is a perspective view of cassettes holding disposable sampling devices and disposable separation devices, which can be used in the instruments of either Figures 32-35 or 57-59.

[0110] Figure 61 is a perspective view of a multiple axis robot used in the embodiment of Figure 59.

[0111] Figure 62 is a perspective view of an alternative embodiment of a disposable sampling device, presenting a variation on the general design shown in Figure 44.

[0112] Figure 63 is a cross sectional view of the sampling device of Figure 62.

[0113] Figure 64 is a detailed perspective view of the distal end of the arm of the robot of Figure 61 shown gripping the sampling device of Figure 62.

[0114] Figure 65 is another detailed perspective view of the distal end of the arm of the robot of Figure 61 shown gripping the sampling device of Figure 62.

[0115] Figure 66 is a perspective view of the pump assembly on the robot of Figure 61 which operates to provide vacuum and positive pressure to the sampling device in order to (a) withdraw a small portion of the sample from one of the specimen containers and (b) dispense the sample (optionally after lysing the sample) into one of the disposable separation devices of Figures 36 and 60.

[0116] Figure 67 is a perspective view of the robot of Figure 61 performing a sampling operation on one of the specimen containers using the sampling device of Figure 62.

[0117] Figure 68 is a more detailed view of the sampling operation shown in Figure 67.

[0118] Figure 69 is a perspective view of the sampling device of Figure 62 being placed into a vortexer shown in Figures 56 and 57 in order to facilitate lysing of cellular components in the sample withdrawn from one of the specimen containers.

5 [0119] Figure 69A is a perspective view of the vortexer having an optional coil heater around the holder of the sampling device in order to heat the holder and maintain the sample within the sampling device at 37 degrees C.

[0120] Figure 70 is a perspective view of the sampling device of Figure 62 being held by the robot hand during the vortexing operation.

10 [0121] Figures 71A and 71B are side and cross-sectional views of the vortexer and sampling device.

[0122] Figures 72A and 72B are cross-sectional and perspective views of a holder for the sampling device incorporated into the vortexer.

15 [0123] Figures 73A, 73B and 73C are cross-sectional, side and perspective views, respectively, of the sampling device and the separation device prior to introduction of the sample from the sampling device into the separation device. Figure 73D is another perspective view of the sampling and separation device, with the rubber needle sheath of the sampling device shown partially removed in order to show the needle of the sampling device.

20 [0124] Figure 74 is a perspective view of the sampling device in position to inject a portion of the sample into the separation device.

[0125] Figure 75 is a side view of the injection operation shown in Figure 74.

[0126] Figure 76 is a cross-section view of the sampling and separation devices showing the injection operation.

25 [0127] Figure 76A is a detailed view of the cup and cup holder of Figure 57, showing the cup receiving one of the separation devices; Figure 76B shows a separation device being inserted into the cup of Figure 76A; Figure 76C is a cross-section of the cup holder, cup and separation device of Figure 76A.

30 [0128] Figure 77 is a schematic representation of a detection instrument for detection of a microbial agent in a biological sample coupled to an automated identification and/or characterization instrument via a conveyor.

[0129] Figure 78 is a schematic representation of a combined automated detection and identification instrument which receives specimen containers in an automated fashion e.g., via a conveyor.

[0130] Figure 79 is a schematic representation of a combined automated detection and identification instrument which receives specimen containers manually from a user, e.g., via opening a door in a front panel of the instrument. The embodiment of Figure 79 could be implemented, for example, using the arrangement shown in Figure 57.

[0131] Figure 80 is schematic block diagram showing a computer system controlling the operation of the instrument of Figures 57-86.

[0132] Figures 81A-C are a flow chart showing a sequence of processing instructions which perform identification and/or characterization of the concentrated microbial agent using intrinsic fluorescence measurements.

[0133] Figures 82-57 are plots of intrinsic fluorescence (IF) measurements, and transforms thereof which illustrate the benefit of the pre-processing instructions of Figure 51A in terms of minimizing strain-to-strain variations within an organism group.

[0134] Figures 88 and 89 are plots of first derivative of logarithm transformed IF measurements showing the discrimination potential between a subset of species for excitation wavelengths of 315 and 415 nm.

[0135] Figure 90 is a perspective view of a second embodiment of a separation device which can be used in conjunction with the identification/characterization instrument of Figure 1. The separation device of this embodiment has a separate lytic chamber and a separate separation chamber which are connected by a fluid flow-channel.

[0136] Figure 91 is a perspective view of the separation device embodiment of Figure 90, showing a top plate and base plate separated from the separation device.

[0137] Figure 92 is a top view of the body portion of the separation device embodiment shown in Figure 90.

[0138] Figure 93 is a cross-sectional view along line A-A of the separation device embodiment shown in Figure 92.

[0139] Figure 94 is a cross-sectional view along line B-B of the separation device embodiment shown in Figure 92.

[0140] Figure 95 is a perspective view of a combined sampling and separation device which can be used in conjunction with the identification/characterization instrument of Figure 31.

[0141] Figure 96 is a front view of the combined sampling and separation device shown in Figure 65 with a pinch valve shown in the open position.

[0142] Figure 97 is a cross-sectional view of the combined sampling and separation device shown in Figure 96 with the pinch valve shown in the open position.

[0143] Figure 98 is a front view of the combined sampling and separation device shown in Figure 95 with a pinch valve shown in the closed position.

[0144] Figure 99 is a cross-sectional view of the combined sampling and separation device shown in Figure 97 with the pinch valve shown in the closed position.

[0145] Figure 100 is a perspective view of a second embodiment of a combined sampling and separation device.

[0146] Figure 101 is a cross-sectional view of the combined sampling and separation device shown in Figure 100.

[0147] Figure 102 is a cross-sectional view of the valve shown in Figure 101.

[0148] Figure 103 is a side view of a third embodiment of a combined sampling and separation device which can be used in conjunction with the identification/characterization instrument of Figure 31.

[0149] Figure 104 is a cross-sectional view of the combined sampling and separation device shown in Figure 103.

[0150] Figure 105 is an exploded view of the combined sampling and separation device shown in Figure 103.

[0151] Figure 106 is a perspective view of a third embodiment of a combined sampling and separation device which can be used in conjunction with the identification/characterization instrument of Figure 31.

[0152] Figure 107A is a side view of the combined sampling and separation device shown in Figure 106.

[0153] Figure 107B is a cross-sectional view of the combined sampling and separation device shown in Figure 107A.

[0154] Figure 108A is a side view of the combined sampling and separation device shown in Figure 106.

[0155] Figure 108B is a cross-sectional view of the combined sampling and separation device shown in Figure 108A.

DETAILED DESCRIPTION

[0156] As noted in the summary section, this document describes an automated system for and instrument and methodology for detection of microbial agent presence within a specimen container and an instrument and methodology for automated identification and/or characterization of the microbial agent. The instrument and methodology for detection of microbial agent presence will be set forth in Part 1 (Figures 1-30). The instrument and methodology for identification and/or characterization of the microbial agent will be described in Part 2 (Figures 31-108). Feature for integrating the two instrument into either separate instruments one in one combined instrument will be described at various points in this document. These features may include automated loading and unloading features, conveyor features, and robotic handling features that operate on specimen containers, including but not limited to specimen containers in the form of bottles or the like.

[0157] Part 1 Automated Instrument for Detection of Specimen Container for Microbial Agent Growth (Figures 1-30)

[0158] An automated system or instrument for non-invasive detection of the presence of a microbial agent (e.g., a microorganism) in a test sample contained within a sample container, e.g., a culture bottle, is described herein. One embodiment of the automated system or instrument is described herein in conjunction with FIGS. 1-8C. Other possible embodiments and design alternatives are shown in conjunction with FIGS. 9A-30, and described herein. The automated system can include one or more of the following features: (1) a housing, enclosing an interior chamber; (2) an automated loading mechanism for loading one or more containers into the interior chamber of the system; (3) an automated container management mechanism or locator device for moving or locating a container among various work-flow stations within the system; (4) an automated transfer mechanism, for transfer of a container within the system; (5) one or more container holding structures for holding a plurality of specimen containers, optionally provided with an agitation assembly; (6) a detection unit for detection of microbial growth; and/or (7) mechanism for automated unloading of a specimen container from the system. In order to better appreciate how the illustrated embodiment of the detection system operates, this specification may describe the automated detection apparatus in the context of a particular detection

instrument (a blood culture instrument) and specimen container (a blood culture bottle). However, persons skilled in the art will readily appreciate that the detection apparatus can be practiced in other embodiments, that variations from the specific embodiments disclosed herein can be arrived at to suit particular implementations, and that therefore the present description of a preferred embodiment and best mode for practicing the invention is provided by way of illustration and not limitation.

System Overview

[0159] An automated detection system 100 (for example, as illustrated in FIGS. 1-3 and 5A-5B) is described herein that provides a new architecture and method for automated detection of a microbial agent (e.g., a microorganism) that may be present in a test sample or specimen sample. In general, any known test sample (e.g., a biological sample) can be used. For example, the test sample can be a clinical or non-clinical sample suspected of containing one or more microbial agents. Clinical samples, such as a bodily fluid, include, but are not limited to, blood, serum, plasma, blood fractions, joint fluid, urine, semen, saliva, feces, cerebrospinal fluid, gastric contents, vaginal secretions, tissue homogenates, bone marrow aspirates, bone homogenates, sputum, aspirates, swabs and swab rinsates, other body fluids, and the like. Non-clinical samples that may be tested include, but not limited to, foodstuffs, beverages, pharmaceuticals, cosmetics, water (e.g., drinking water, non-potable water, and waste water), seawater ballasts, air, soil, sewage, plant material (e.g., seeds, leaves, stems, roots, flowers, fruit), blood products (e.g., platelets, serum, plasma, white blood cell fractions, *etc.*), donor organ or tissue samples, biowarfare samples, and the like. In one embodiment, the biological sample tested is a blood sample.

[0160] Referring now to the Figures 1-30, several configurations are possible for the detection system 100. As shown, for example, in FIGS. 1-3 and 5A-5B, the automated detection system 100 comprises a housing 102 and one or more automated mechanisms for loading (see, e.g., 200, FIG. 1), moving or locating (not shown), transferring (see, e.g., 650, FIGS. 5A-5B), agitating (not shown) and/or unloading of specimen containers 500 within or from the detection system 100. The housing 102 comprises front and back panels 104A and 104B, opposing side panels (e.g., left-side and right-side panels) 106A and 106B, a top or roof panel 108A and a bottom or floor panel 108B, which form an enclosure, enclosing an interior chamber 620 (see, e.g.,

FIGS. 5A-5B) of the detection system 100. In one embodiment, the interior chamber 620 of the detection system 100 is a climate-controlled chamber (e.g., a temperature-controlled incubation chamber wherein the temperature is maintained at approximately 37° C.) to promote or enhance microbial growth. As shown in FIGS. 1-3, the housing also may include a first port or container entrance location 110, a second port or misread/error location 120, a third port or positive container exit location 130, a lower access panel 140 (FIG. 1) or drawer 142 (FIG. 3), and/or a user interface display 150. As known in the art, the lower access panel 140 or drawer 142 may include a handle 144. Also as shown in FIG. 1, the housing 102 may also comprise upper and lower sections 160 and 170, optionally each comprising an operable door (i.e., upper and lower doors) 162 and 172 (see, e.g., FIG. 5B). The upper door 162 and lower door 172 are operable to allow access to the interior chamber 620 of the detection system 100. However, as one of skill in the art would appreciate other design configurations are possible. For example, in another possible embodiment, the entire front panel may comprise a single operable door (not shown).

[0161] In one design possibility, as shown for example in FIGS. 1-3, the lower section 170 may have a larger profile or footprint than the upper section 160. In accordance with this embodiment the housing of the larger lower section 170 forms a shelf 180 on a top surface of the lower section 170 and adjacent to or in front of the upper section 160. This shelf 180 may provide a user workstation and/or workflow access points to the detection system 100. Furthermore, the shelf 180 may comprise an automated loading means or mechanism 200. The shelf 180 may further provide access locations for the first port or container entrance location 110, the second port or misread/error location 120, and the third port or positive container exit location 130.

[0162] In one embodiment, as shown for example in FIGS. 1-3 and 5A-5B, the detection system 100 may comprise an automated loading mechanism 200, for the automated loading of a specimen container 500 into the detection system 100. The automated loading mechanism 200 may comprise a container loading station or area 202, a transport mechanism 204 and a first port or container entrance location 110. In operation, a user or technician can place one or more specimen containers 500 (see, e.g., FIG. 4) at the container loading station or area 202. A transport mechanism 204, for example, a conveyor belt 206, will transport the specimen container to the first port or container entrance location 110, and subsequently through the entrance

location 110 and into the detection system 100, thereby loading the container into the system. The automated loading mechanism 200 is described in greater detail herein.

[0163] As one of skill in the art would appreciate, other designs may be employed for the automated loading mechanism and are described elsewhere herein.

5 For example, alternative automated loading mechanisms are shown in FIGS. 10-16. In one embodiment, as shown in FIGS. 13-16, and as described in greater detail herein, the detection system 100 may employ a container loading area or reservoir 302 and a drum-like loading device 308 for the automated loading of a specimen container into the detection system 100.

10 [0164] In another embodiment, as shown for example in FIGS. 14-15 and 18, the automated detection system 100 may contain one or more work-flow stations 404 for obtaining one or more measurements, readings, scans and/or images of a specimen container, thereby providing information, such as, container type, container lot number, container expiration date, patient information, sample type, test type, fill
15 level, weight measurement, etc. Furthermore, the one or more work-flow stations 404 may comprise one or more container management stations, such as, a container pick-up station or a container transfer station. For example, the automated detection system may contain one or more of the following work-flow stations: (1) a bar code reading station; (2) a container scanning stations; (3) a container imaging station; (4) a
20 container weighing station; (5) container pick-up station; and/or (6) a container transfer station. In accordance with this embodiment, the detection system 100 may further have a container management means or container locator device 400, as shown, for example, in FIGS. 13-15, 18 and 24. In operation, the container management device or locator device 400, operates to move or otherwise locate a
25 specimen container 500 to one or more work-flow stations 404. In one design configuration, one or more of the work-flow stations are included within the housing 102 of the detection system 100. In one embodiment, as best shown in FIGS. 14-15, the drum or drum-like loading device 308 and vertically orientated chute 332 of automated loading mechanism 300 can operated to deposit or place a specimen
30 container into a locator well 402, as described elsewhere herein. In another embodiment, as best shown, in FIGS. 18 and 24, the transport mechanism 204, or conveyor belt 206, of automated loading mechanism 200 can operate to deposit or place a specimen container into a locator well 402, as described elsewhere herein. As known in the art, the detection system 100 may further comprise one or more guide

rails (not shown) to guide the specimen container into the locator well 402. In accordance with both of these embodiments, the container management device or locating device 400 can then rotate to move or locate the specimen container among various work-flow stations 404 within the system, such as for example, a bar code reading station, a container scanning stations, a container imaging station, a container weighing station, container pick-up station, and/or a container transfer station. The container management device or locator device 400 is described in greater detail herein.

[0165] As shown, for example, in FIGS. 5A-8C the detection system 100 may also comprise an automated transfer means or mechanism 650 for transferring the specimen containers 500 within the housing 102 of the detection system 100. For example, the transfer mechanism 650 may transfer the specimen container 500 from an entrance location or port 110 (see, e.g., FIGS. 1-3), into the interior chamber 620 of the detection system 100, and place the container 500 into one of the receiving structures or wells 602 contained in one of a plurality of holding structures or racks 600. In another embodiment, the transfer mechanism 650 may also be used to rearrange, transfer or otherwise manage specimen containers 500 within the system. For example, in one embodiment, the transfer mechanism 650 can be used to transfer a specimen container 500, detected as positive for microbial growth (referred to herein as a “positive” container), from the holding structure or rack 600 to a positive container location, such as a positive container exit location or port 130 (see, e.g., FIG. 1) where a user or technician can easily remove the positive container 500 from the detection system 100. In another embodiment, the transfer mechanism 650 can be used to transfer a container 500 determined as negative for microbial growth after a designated time has passed (referred to herein as a “negative” container), from the holding structure or rack 600 to a negative container location within the system (e.g., a negative container waste bin 146 (see, e.g., FIG. 1)) where a user or technician can easily access the waste bin 146 for removal and disposal of the container 500. As one of skill in the art would appreciate, other designs may be employed for the automated transfer mechanism and are described elsewhere herein. For example, another design configuration is described herein in conjunction with FIGS. 17-21B.

[0166] The detection system 100 will also include a means for detecting growth (e.g., a detection unit) in the specimen containers 500 (see, e.g., Figure 27). In general, any known means in the art for detecting microbial growth in a container can

be used. For example, as is well known in the art, each holding station or rack 600 may contain a linear scanning optical system that has the capability of non-invasive monitoring of microorganism growth in each specimen container 500. In one embodiment, the optical system can interrogate a sensor (e.g., a Liquid Emulsion Sensor (LES) sensor) 514 (see, e.g., FIG. 4) in the containers 500, thereby detecting for microorganism growth within the container.

[0167] The detection system 100 may also include an automated unloading mechanism for the unloading of “positive” and/or “negative” specimen containers 500. This automated unloading mechanism can operate to ensure that once a “positive” or “negative” reading has been made for each specimen container 500, the container 500 is removed from the container receiving structures or wells 602 (see, e.g., FIGS. 5A and 5B), making room for another container to be loaded into the detection system 100, thereby increasing system through-put.

Specimen Container

[0168] The specimen container 500, shown for example in FIGS. 4 and 27B, and other figures, is shown in the form of a standard culture bottle (e.g., a blood culture bottle). However, the description of a culture bottle (e.g., a blood culture bottle) is offered by way of example and not limitation. As shown in FIG. 4, the specimen container 500 comprises a top portion 502, a body 504, and a base 506. The container 500 may include a bar code label 508 for automated reading of the container 500 within either the detection system or off-line equipment. As shown in FIGS. 4 and 27B, the top portion 502 of the container 500 typically comprises a narrow portion or neck 510 through which an opening 516 extends to provide communication with the interior chamber 518 of the container. As shown in FIG. 27B, the container also includes a closure device 512 (e.g., a stopper), optionally having a pierceable septum and may also have a sensor 514 (e.g., an LES sensor) formed or placed in the bottom of the container 500 for purposes of colorimetric detection of the presence of microbial growth in the container 500. The configuration of the container 500 is not particular important and the inventive system and methods can be adapted to a variety of containers designed for culturing a test sample (e.g., a biological test sample). Containers 500 of the type shown in FIGS. 4 and 27B are well known in the art and described in the patent literature cited in the Background section of this document.

[0169] In one embodiment, the specimen containers 500 are inoculated with a test sample (e.g., a clinical or non-clinical biological sample) and are loaded/unloaded into/out of the detection system 100. The container 500 may further comprise a growth or culture medium (not shown) for promoting and/or enhancing microbial or microorganism growth. The use of a growth or culture media (or medium) for the cultivation of microorganisms is well known. A suitable growth or culture medium provides the proper nutritional and environmental conditions for growth of microorganisms and should contain all the nutrients required by the microorganism which is to be cultivated in the specimen container 500. After a sufficient time interval to allow natural amplification of microorganisms (this time interval varies from species to species), the container 500 is tested within the detection system 100 for the presence of microbial or microorganism growth. The testing may occur continuously or on a periodic basis so that the container can be determined as positive for microorganism growth as soon as possible.

[0170] In one embodiment, once a container 500 is detected as positive in the detection system 100, the system will notify the operator through an indicator 190 (e.g., a visual prompt), and/or via a notification at the user interface display 150, or by other means.

Automated Loading Means or Mechanism

[0171] The detection system 100 may include a means or mechanism for automated loading of a specimen container 500 into the detection system 100. In one embodiment, as shown for example in FIGS. 1-3 and 5A-5B, the automated loading mechanism 200 may comprise a container loading station or area 202, a transport mechanism 204 and an entrance location or port 110. However, as would be appreciated by one of skill in the art, the automated loading mechanism can take on many different configurations. For example, another design configuration of an automated loading mechanism 300 is described herein in conjunction with FIGS. 13-16. The various design configurations described herein are by way of illustration and not limitation. The automated loading mechanisms shown herein (e.g., FIGS. 1-3, 5A-5B and 13-16) are shown schematically and the parts are not to scale.

[0172] A user or technician can transport one or more specimen containers 500 to the detection system 100 by any known means and place the containers 500 at a container loading station or area 202. For example, in one embodiment, a user or

technician can use a carrier designed to transport a plurality of specimen containers to the loading station or area 202 of the detection system 100.

[0173] One possible carrier design is shown in FIGS. 28A and 28B. As shown in FIGS. 28A and 28B, the carrier 350 comprises a body 351 having top and bottom surfaces 352A and 352B, respectively, front and back surfaces 354A and 354B, respectively, opposing side surfaces 356A and 356B (e.g., a right side surface and left side surface), respectively, and a pair of opposing user handles 358A and 358B, attached to said opposing side surfaces 356A, 356B. The body further comprises a plurality of through holes 360, each configured to hold a single specimen container 500 therein. The body 351 may also comprise a slide plate 362 operable within a slide joint 364 to slide back-and-forth (see, e.g., arrow 366 in FIG. 28A) between a “closed” position, to retain the specimen containers 500 loaded within the carrier 350, and an “open” position, to release the specimen containers 500 from the carrier 350, and deposit them onto or into an automated loading mechanism. The slide joint 364 may further comprise a spring, or like means, for locking the slide plate 362 in the “closed” position during transport by a user to a detection system.

[0174] As shown in FIGS. 28A-29, the carrier 350 may further comprise a pair of alignment arms 368A and 368B and a release tab 370 operable with a release mechanism 372 for releasing the specimen containers 500 at an automated loading mechanism 200 of a detection system 100. The release mechanism 372 comprises a pair of slots 374 that correspond to the pair of alignment arms 368A and 368B, to ensure the carrier 350 is properly aligned at the loading station or area 202 for depositing the specimen containers 300, and a release bar 376. In operation, a technician transports a carrier 350, containing one or more specimen containers 500, to the automated loading mechanism 200 and presses the carrier 350 against the release bar 376, with the alignment arms 368A and 368B aligned with the corresponding slots 374 of the release mechanism 372. By pressing the carrier 350 against the release bar 376, the release tab 370 is pushed in or depressed, thereby moving the slide plate 362 to the “open” position and allowing the specimen containers 500 to fall out of the through holes 360 and onto the loading station or area 202. The technician can then lift the carrier 350 upward until the carrier body 351 and plurality of through holes 360 clear the specimen containers 500, thereby depositing the containers at the automated loading mechanism 200 for automated

loading into the detection system 100. As one of skill in the art would appreciate other design configurations are possible.

[0175] As shown in FIGS. 1-3, the loading station or area 202 is typically an easily accessible location or area of the automated loading mechanism 200 where a user or technician can place one or more specimen containers 500 for loading into the detection system 100. Once at the loading station 202, the containers 500 will be transported, using a transport mechanism 204, from the loading station or area 202 to an entrance location or port 110, and subsequently through the entrance location or port 110 and into the detection system 100. Accordingly, a user or technician can simply place one or more specimen containers 500 at the loading station or area 202 and walk away, while the containers 500 are automatically loaded into the detection system 100. Once the specimen containers 500 have been transported into the system, they can be moved to one or more work-flow stations using a container management device or locator device, and/or transferred to a holding structure or rack, as described elsewhere herein.

[0176] In one embodiment, as shown in FIGS. 1-3, 5A and 5B, the transport mechanism 204 is a conveyor belt 206 operable to transport (e.g., convey) the containers 500 to an entrance location or port 110 and subsequently through the entrance location or port 110 and into the detection system 100. However, other means or mechanisms for transporting the specimen containers 500 from the loading station or area 202 to the entrance location or port 110 are envisioned, and may include, but are not limited to, feed screws, timing belts having grooves or molded plates, and the like. In other embodiments, the process of automated loading of a specimen container 500 into the detection system 100 may further comprise transferring the container to a holding structure or rack using a transfer mechanism 650 or moving the container to one or more work-flow stations using a container locator device (see, e.g., FIG. 24, 400A), as described below.

[0177] As shown in FIGS. 1-3, 5A and 5B, the loading station or area 202 and transport mechanism 204 comprise a conveyor belt 206. In accordance with this embodiment, the user or technician can place one or more specimen containers 500 at a specific location or area (i.e., the loading station or area 202) of the conveyor belt 206 for automated loading of the containers 500 into the detection system 100. The conveyor belt 206 may run continuously, or may be activated by the physical presence of the container 500 at the loading station or area 202. For example, a system

controller can be used to operate the conveyor belt 206 (i.e., turn it on or off) based on a signal (e.g., a light sensor) indicating the presence, or absence, of one or more specimen containers at the loading station 202. Similarly, one or more sensors can be used at the entrance location or port 110 to indicate if a container is improperly loaded and/or has fallen over and may cause jamming. The conveyor belt 206 operates to move or transport the containers 500 from the loading station or area 202 (e.g., the left portion of the conveyor belt 206, as shown in FIG. 1) to the entrance location or port 110, thereby accumulating one or more containers 500 at the entrance location or port 110 to be loaded into the detection system 100. Typically, as shown in FIGS. 1-3 and 5A-5B, the loading station or area 202, transport mechanism 204 or conveyor belt 206, and entrance location or port 110 are located outside, or on the housing 102 of the detection system 100. In one embodiment, the automated loading mechanism 200 is located on a shelf 180 located on top of the lower section 170 and adjacent to the upper section 160 of the system 100. Also, as shown, the transport mechanism or conveyor belt 206 typically operates in a horizontal plane, so as to maintain the specimen containers 500 in a vertical or up-right orientation (i.e., such that the top portion 506 of the container 500 is up) for loading into the detection system 100 (see, e.g., FIGS. 1-3 and 5A-5B). As shown in FIGS. 1-3, the transport mechanism or conveyor belt 206 moves, for example, from left-to-right, or from the loading station or area 202 towards the entrance location or port 110, to transport one or more free standing containers 500 (see, e.g., FIG. 2, arrow 208).

[0178] In one embodiment, as shown, for example in FIGS. 1-3 and 10-11, the automated loading mechanism 200 will further comprise one or more guide rails 210 located juxtaposed to one or both sides of the transport mechanism or conveyor belt 206. The one or more guide rails 210 function to guide or direct the specimen containers 500 to the entrance location or port 110 during operation of the transport mechanism or conveyor belt 206. In one embodiment, the guide rails operate to funnel or guide the specimen containers into a single file line at the back of the automated loading mechanism 200, where they await their turn to be loaded, one container at a time, into the detection system 100. In another design aspect, as shown for example in FIG. 22, the detection system 100 may further comprise a locator device cover 460 that covers a locator device (described elsewhere herein) and encloses an interior locator device chamber (not shown) therein. The locator device cover 460 may comprise one or more container guide rails 462 for guiding a specimen

container 500, as it is transported from the automated loading mechanism 200 to the entrance location or port 110, and subsequently into the interior chamber, thereby automatically loading the specimen contain into the system. In accordance with this embodiment, the interior locator device chamber (not shown) is considered to be a part of the interior chamber, which is described elsewhere herein.

[0179] In still another embodiment, the automated loading mechanism 200 may further comprise a means or device for reading or otherwise identifying the specimen containers 500 as the containers enter the detection system 100. For example, the containers 500 may include a bar code label 508 which can be read for container identification and tracking within the system. In accordance with this embodiment, the detection system 100 will include one or more bar code readers (see, e.g., 410 in FIGS. 14-15) at one or more locations within the system. For example, the detection system 100 may include a bar code reader at the entrance location or port 110 to read, identify and log the individual containers 500 into the detection system controller as they enter the system. In another embodiment, the entrance location or port 110 may also include a means or device (e.g., a container rotator or rotating turntable, as described elsewhere herein) for rotating the container within the entrance location or port 110 to enable reading of the bar code label 508. In another possible embodiment, the transfer mechanism (see, e.g., FIG. 5B, 650) may rotate the container 500 to enable reading of the bar code label 508. Once the bar code has been read, the transfer mechanism will typically transfer the container 500 from the entrance location or port 110 to one of a plurality of receiving structures or wells 602 in one of a plurality of holding structures or racks 600.

[0180] In yet another embodiment, if the bar code 508 cannot be properly read, (e.g., the label is misread or a reading error occurs) the detection system controller (not shown) can direct the container 500 to a misread/error location or port 120 for user access to the unreadable or misread container 500. The user can re-load the container using the automated loading mechanism 200 and/or at the user's discretion, may optionally manually load the container 500 and hand enter container 500 information into the system controller (e.g., using the user interface 150). In another embodiment, the detection system 100 may contain a high priority (or STAT) loading location (not shown) for the loading of high priority containers and/or for manual loading of containers where the label has been misread or a reading error has occurred.

[0181] Another design configuration of the automated loading mechanism is shown in FIG. 10. As shown in FIG. 10, the automated loading mechanism 200 comprises a loading station or area 202, a first conveyor belt 206, and an entrance location or port 110. The conveyor belt 206 operates to transport the specimen containers 500 from the left edge of the system 100 (i.e., the location of the loading station 202) to the entrance location or port 110. In this example, the movement is from left-to-right and is represented by arrow 220 in FIG. 10. The automated loading mechanism 200 may further comprise a guide rail 210 and a second conveyor belt 212, which operates around a set of gears or wheels 214, 216. In accordance with this embodiment, the second conveyor belt 212 is orientated and operable in a vertical plane above the first horizontal conveyor belt 206, and can operate in a clockwise or counter-clockwise manner (i.e., to move the belt from left-to-right or from right-to-left). The clockwise or counter-clockwise operation of the second vertically orientated conveyor belt 212 can provide the specimen container 500 with a counter-clockwise or clockwise rotation, respectively, about a vertical axis of the container. Applicants have found that providing a specimen container 500 with clockwise or counter-clockwise rotation can prevent and/or reduce jamming or clogging of the automated loading mechanism 200 as a plurality of specimen containers 500 accumulate at the entrance location or port 110. Once the containers 500 have arrived at the entrance location or port 110 they can be moved into the detection system 100.

[0182] In still another embodiment, the automated loading mechanism 200 may also contain a backer board (not shown) located in a horizontal plane underneath the first conveyor belt 206. As one of skill in the art would appreciate, the conveyor belt 206 may have some give, flexibility, or may otherwise be considered “springy”. This springy nature of the conveyor belt 206 may lead to instability of the specimen container 500 as the container is transported across the conveyor belt 206 from the loading station or area 202 to the first port or entrance location 110 and may result in specimen containers 500 tipping or falling over. Applicants have found that by including a rigid or semi-rigid backer board underneath the conveyor belt 206, this problem can be reduce and/or eliminate altogether, thereby, reducing and/or preventing jamming or clogging of the loading mechanism 200 (e.g., with containers 500 that have fallen over). In general, any known backer board material may be used. For example, the backer board can be a rigid or semi-rigid board made of plastic, wood, or metal.

[0183] Yet another configuration of the automated loading mechanism is shown in FIG. 11. As shown in FIG. 11, the automated loading mechanism 200 may comprise a loading station or area 202, a conveyor belt 206, and an entrance location or port 110. Also as shown, the conveyor belt 206 can operate to transport the specimen containers 500 from the front edge of the system 100 (i.e., the loading station 202) to the entrance location or port 110. In this example, the movement of the loading mechanism 200 is from front-to-back (i.e., from the front edge of the instrument to the loading port 110) and is represented by arrow 240 in FIG. 11. As shown, the automated loading mechanism 200 may further comprise one or more guide rails 210 to guide the one or more specimen containers 500 to the entrance location or port 110, as they are transported by the conveyor belt 206.

[0184] Optionally, as shown in FIG. 11, the automated loading mechanism 200, in accordance with this embodiment, may include a second transport mechanism 230. In one embodiment, the second transport mechanism 230 may comprise a second conveyor belt 232 located in, and operable in, a vertical plan above the first conveyor belt 206. As shown, the second transport mechanism 230 may further comprise a plurality of paddles or plates 236 attached to the second conveyor belt 232. In accordance with this embodiment, the first conveyor belt 206 operates to move or transport one or more specimen containers 500 from the loading station or area 202 to the second transport mechanism 230, where the containers 500 are individually moved or transported into a well or space 234 between the paddles or plates 236. The second conveyor belt 232 operates around a set of gears or drive wheels (not shown), and runs or moves, for example, from left-to-right across the back edge of the automated loading mechanism 200, thereby transporting the containers 500 from left-to-right along the back of the loading mechanism 200 and to the entrance location or port 110 (see, e.g., arrow 250). Once the containers 500 have arrived at the entrance location or port 110 they can be moved into the detection system 100.

[0185] In yet another embodiment, the automated loading mechanism 200 can be enclosed or encased in a protective housing or casing 260, as shown for example in FIG. 12. In accordance with this embodiment, the automated loading mechanism 200, or one or more components thereof (i.e., one or more of the loading area, transport means (e.g., conveyor belt 206) and/or entrance location or port (not shown)), can be housed or encased in a protective housing or casing 260. The protective housing or

casing 260 will have an opening 262 providing access to, and for loading specimen container 500 into/onto the automated loading mechanism 200 housed therein. Optionally, the protective housing or casing 260 can further include a cover means 264 that can be closed or shut to protect the automated loading mechanism 200, and/or containers 500, contained therein. The cover can be a closable lid 266, as shown, or other structure or means for closing the housing or casing 260. For example, in another embodiment, the cover 264 can be a lightweight curtain (not shown) that can be pulled shut over the opening 262. The protective housing or casing 260 may also provide a priority container loading port 270 for the loading or high priority containers (i.e., STAT container) and/or misread containers. In one embodiment, a container 500 can be manually loaded into the priority port 270.

[0186] Another embodiment of an automated loading mechanism is shown in FIGS. 13-15. Like the previously described automated loading mechanism, the automated loading mechanism 300 shown in FIGS. 13-15, comprises a container loading station or area 302, a transport mechanism 304 and a container entrance location 306, for the fully automated loading of one or more specimen containers 500 into the detection system 100.

[0187] The container loading area 302 is in an easily accessible location on the detection system 100 to allow a user to easily place one or more specimen containers 500 therein, as shown for example in FIG. 17. In accordance with this embodiment, the specimen containers 500 are loaded in a horizontal orientation, such that they are lying on their side, as shown for example in FIG. 13. Once at the container loading area 302, the specimen containers 500 can be transported by a transport mechanism 304 from the container loading area 302 to an entrance location 306, from where the containers 500 will enter the detection system 100, as described in more detail herein. Surprisingly, regardless of the specimen container 500 orientation in the loading area 302 (i.e., regardless of whether the top portion 506 of the container 500 is facing the detection system 100 or facing away from the detection system 100 (as shown, e.g., in FIG. 14)), the automated loading mechanism 300 of this embodiment is capable of loading the specimen containers 500 into the detection system 100.

[0188] In one embodiment, the container loading station or area 302 comprises a loading reservoir 303 that is capable of holding one or more specimen containers 500, as shown for example in FIG. 13. The loading reservoir 303 can be designed to hold from 1 to 100 specimen containers, from 1 to 80 specimen containers, or from 1

to 50 specimen containers. In other design concepts, the loading reservoir may hold 100 or more specimen containers 500. The automated loading mechanism 300 of this embodiment may further comprise a lid or cover (not shown), which the user or technician can optionally close to cover the loading reservoir 303 and loading area 302. Various designs are possible and contemplated for the lid or cover.

[0189] As show in FIGS. 13-14, the loading reservoir 303 contains a transport mechanism 304, for example, a sloped ramp that slopes downwards towards an entrance location 306 so as to transport the specimen containers 500 from the loading area 302 to the entrance location 306. In accordance with this embodiment, the sloped ramp will allow the specimen containers to roll or slide down the ramp to the entrance location 306. Although, a sloped ramp is exemplified in the figures other designs are possible and contemplated for the transport means or mechanism 304 for transporting the specimen containers to the entrance location 306. For example, in one alternative design concept the transport mechanism 304 may comprise a conveyor belt (not shown). In accordance with this design concept the conveyor belt can be designed to hold one or more specimen containers and may optionally be designed such that the conveyor belt slopes downward towards the entrance location 306.

[0190] Once at the entrance location 306, a drum or drum-like loading device 308 will be used for loading the specimen containers 500 into the detection system 100. As shown, the drum-like loading device 308 has one or more horizontally orientated slots 310 for holding one or more specimen containers therein. Each individual slot 310 is capable of holding a single specimen container 500. In one embodiment, the drum-like loading device 308 has a plurality of slots, for example, from 1 to 10 slots, from 1 to 8 slots, from 1 to 6 slots, from 1 to 5 slots, from 1 to 4 slots, or from 1 to 3 slots for holding specimen containers 500 therein. In another embodiment, the drum-like loading device 308 can be designed to have a single slot capable of holding a single specimen container 500 therein.

[0191] The drum-like loading device 308 is capable of rotating (either in a clock-wise direction, or counter-clock wise direction) about a horizontal axis, and is capable of picking-up and loading individual specimen container 500 into the detection system 100. In operation, the rotation of the drum or drum-like loading device 308 picks up a horizontally orientated specimen container 500 in one of a plurality of horizontally orientated slots 310, and moves the container 500, by rotation of the drum or drum-like loading device to a tumbler device 330 (see, e.g., FIG. 16).

Any known means in the art can be used for rotation of the drum or drum-like loading device 308. For example, the system may employ the use of a motor (not shown) and drive belt 316 for rotation of the drum-like loading device 308.

[0192] In another embodiment, as shown in FIG. 13, the automated loading mechanism 300 of this embodiment may further comprise a single container loading port 312. In operation, a user or technician can place a single specimen container into the single container loading port 312 for quick, or immediate loading, for example of a STAT specimen container. Once placed in the single container loading port 312, the container will drop or fall via gravity onto a second transport mechanism 314, for example, a sloped ramp that slopes downward toward the drum-like loading device 308 for quick or immediate automated loading of the specimen container into the detection system 100.

[0193] As shown in FIGS. 13-16, the drum or drum-like loading device 308 rotates in a vertical plane (i.e., around or about a horizontal axis) to move the specimen container 500 from the entrance location 306 to a tumbler device 330. The tumbler device comprises an open slot at the top of a vertically orientated chute 332. Once moved to the tumbler device 330, the specimen containers are up-righted (i.e., the specimen containers are re-positioned from a horizontal container orientation to an up-right vertical container orientation) by a cam mechanism and vertically orientated chute 332. In operation, the cam mechanism (not shown) is capable of sensing the top and/or bottom of the specimen container, and pushing the specimen container 500 in a horizontal direction from the base of the specimen container, thereby allowing the base to drop or fall through the opening of a vertically orientated chute 332. Accordingly, the tumbler device 330 operates to allow the container 500 to drop (via gravity) bottom first through the vertical chute 332 and into a first locator well of a container locator device 400 (described elsewhere herein), thereby re-orientating the container 500 in a vertical, up-right orientation.

[0194] As shown for example in FIG. 16, the tumbler device 330 has two tapered ledges 334, one on each side of the drum, each being narrow at a front edge and thicker at a back edge. The ledges 334 are aligned so that the cap portion 502 of the container 500 will be caught or held by the ledge (i.e., the cap will move over the top side of the ledge such that the cap will rest on the top of ledge 334) as the drum rotates. The ledge 334 only holds the cap portion 502 of the container 500 in place briefly, as the bottom of the container falls through the vertical chute 332.

Furthermore, the bottom or base 506 of the container will not be caught or held by the ledge. Instead, the tapered ledge 334 will act to push or slide the bottom or base 506 of the container 500 in a horizontal direction, from the bottom 506 of the container 500 towards the top or cap portion 502 of the container (see FIG. 4), as the drum or drum-like loading device 308 rotates. This action helps to ensure that the cap end 502 of the container is held by the top edge of the ledge 334, thereby allowing the bottom 506 of the container 500 to fall freely through the vertical chute 332 and into the container locator device 400. By having a ledge 334 on each side of the drum or drum-like loading device 308, container 500 orientation in the rotating drum is not essential. The container 500 will be up-right by the tumbler device 330 regardless of whether the cap end 502 of the container is on the right or left side (see, e.g., FIG. 16) of the drum-like loading device 308, as the corresponding ledges 334 will function to hold the cap or top 502 of the container as the bottom 506 falls through the vertical chute 332. In another embodiment, the vertical chute 332 may further comprise a narrower section 333 that helps direct the falling container 500 into the container locating device 400. In operation, as the drum or drum-like loading device 308 rotates over the open slot at the top of the vertically orientated chute 332, the cap or top portion 502 of the container 500 is held at the outer edge of the drum by one or more ledges 334 (see, e.g., FIG. 16). The ledges 334 hold the cap or top portion 502 of the container 500 in place while allowing the bottom 506 of the container to swing or fall freely out of the drum or drum-like loading device 308 and into the vertically orientated chute 332, thereby up-righting or vertically orientating the container 500 as it drops or falls via gravity through the vertically orientated chute 332 bottom first, as previously described.

Container Management Means or Locator Device

[0195] As shown, for example in FIGS. 13-15 18, and 25A-25C the detection system 100 may further comprise a container management device or locator device 400. The container management device or locator device 400 can be used to manage, move or otherwise locate a container 500, once inside the housing 102 of the detection system 100, among various work-flow stations 404. In one embodiment, the container management device or locator device 400 can be used in combination with the automated loading mechanism 300 shown in FIGS. 13-15, as shown. In another embodiment, the container management device or locator device 400 can be

used in combination with the automated loading mechanism 200 shown, for example, in FIG. 18. The container management device or locator device 400 in FIGS. 13-15 and 18 is shown schematically and the parts not to scale.

[0196] The container management device or locator device 400 comprises a
5 rotatable wheel-like device or rotatable disk that contains one or more locator wells 402, for example 1 to 10 locator wells, 1 to 8 locator wells, 1 to 5 locator wells, 1 to 4 locator wells, or 1 to 3 locator wells 402. In one embodiment, the locator device comprises opposable parallel plates or discs (see, e.g., FIGS. 25A-25C). Each individual locator well 402 is capable of holding a single specimen container 500. In
10 operation, the locator device 400 rotates (either clock-wise or counter clock-wise) in a horizontal plane (and around or about a vertical axis) to move an individual container 500 to or among various work-flow stations 404 (i.e., from station-to-station). In one embodiment, the work-flow station 404 is operable to obtain one or more measurements or readings of the specimen container, thereby providing information
15 about the container, such as, container lot number, container expiration date, patient information, sample type, fill level, etc. In another embodiment, the one or more work-flow stations 404 may comprise one or more container management stations, such as, a container pick-up station or a container transfer station. For example, the locator device 400 is capable of moving an individual specimen container 500 to one
20 or more work-flow stations 404, such as: (1) a bar code reading station; (2) a container scanning stations; (3) a container imaging station; (4) a container weighing station; (4) container pick-up station; and/or (5) a container transfer station. In another embodiment, one or more of these measurements and/or readings can occur at the same station. For example, container weight, scanning, imaging and/or pick-up
25 may occur at a single station location. In yet another embodiment, the detection system may contain a separate pick-up station. A container can be picked-up by a transfer mechanism (as described herein) at the pick-up location, and transferred to other locations (e.g., to a holding structure and/or agitation assembly) within the detection system 100. In still another embodiment, the detection system 100 may
30 contain a transfer station for the transfer of a specimen container 500 to another instrument, e.g., a second automated detection instrument. In accordance with this embodiment, the transfer station may communicate with a system transfer device 440. For example, as shown, the system transfer device 440 may be a conveyor belt that allows the specimen container to be transferred to another location within the

detection system 100, or in another embodiment, to another instrument (e.g., a second detection system (e.g., as shown in FIG. 24)). As shown in FIG. 14-15, the locator device 400 comprises: (1) an entrance station 412; (2) a bar code reading and/or scanning station 414; (3) a container weighing station 416; (4) a container pick-up station 418; and (5) a system transfer station 420 for transfer of the container to another instrument. The locator device may further comprise a rotatable turntable device 406, for rotating a container to facilitate bar code reading and/or container scanning, and/or a scale or weighing device 408, for weighing a container.

[0197] As previously described, in operation, the container management device or locator device 400, operates to move or otherwise locate a given specimen container 500 to a given work-flow station 404. In one embodiment, these work-flow stations 404 are included within the housing 102 of the detection system 100. For example, as shown in FIGS. 13-15 and 18, an automated loading mechanism can deposit or place a specimen container 500 into a locator well 402, as described elsewhere herein. The container management means or locating device 400 can then rotate to move or locate the specimen container among various work-flow stations within the system, such as for example, a bar code reading station, a container scanning stations, a container imaging station, a container weighing station, container pick-up station, and/or a container transfer station.

Transfer Means or Mechanism

[0198] As shown, for example in FIGS. 5-9B and 17-21, the automated detection system 100 may further comprise an automated transfer means or mechanism operable for the transfer of a specimen container 500, and/or for container management, within the system. As already described, the entrance location or port 110 receives containers from, for example, a conveyor system 206 shown best in FIGS. 1-3. As the containers accumulate in the entrance location or port 110, the containers are moved within the detection system 100 whereby a transfer mechanism (e.g., a robotic transfer arm with a container gripping means) can pick-up, or otherwise receive, an individual specimen container 500 and transfer and place that container into a holding structure or rack 600 within the detection system 100, as described in more detail herein. As known in the art, the transfer mechanism may use a vision system (e.g., camera), pre-programmed dimensional coordinates and/or

precision motion controlling to transfer a specimen container to, and load the specimen container into, the holding structure or rack 600.

[0199] As shown in FIGS. 1-3 and 13-15, specimen containers 500 are loaded into, and/or transported within, the detection system 100 using an automated loading mechanism 200 (FIGS. 1-3) or 300 (FIGS. 13-15). As shown, the containers 500 are typically loaded into the detection system 100 in a vertical orientation (i.e., such that the top or cap portion 502 of the container 500 is up-right). In accordance with one embodiment, the containers 500 are placed or held in a plurality of holding structures or racks 600, and optionally agitated to enhance microorganism growth therein. As shown for example in FIGS. 5A and 5B, the receiving structures or wells 602 of the holding structures or racks 600 can be orientated in a horizontal axis. Accordingly, in accordance with this embodiment, an automated transfer mechanism (see, e.g., FIG. 5B, 650) must re-orientate the container 500, from a vertical orientation to a horizontal orientation, during the transfer of the container 500 from the automated loading mechanism 200, 300 to the receiving structures or wells 602.

[0200] In operation, the automated transfer mechanism (e.g., FIG. 5B, 650 or FIG. 20, 700) can operate to transfer or otherwise move, or relocate, a specimen container 500 within the interior chamber 620 of the detection system 100. For example, in one embodiment, the transfer mechanism can transfer a specimen container 500 from an entrance location or port 110 to one of a plurality of holding structures or racks 600. In another embodiment, the transfer mechanism can pick-up a specimen container 500 from a well 402 of the container locator device 400 and transfer the container to a holding structure or well 602 of the holding structure or rack 600. The transfer mechanism can operate to place the container 500 in one of a plurality of container receiving structures or wells 602 that are located in one of a plurality of holding structures or racks 600. In another embodiment, the transfer mechanism can operate to remove or unload “positive” and “negative” containers from the holding structures or racks 600. This automated unloading mechanism can operate to ensure that once a “positive” or “negative” reading has been made for each specimen container 500, the container 500 is removed from the container receiving structures or well 602, making room for another container to be loaded into the detection system 100, thereby increasing system through-put.

[0201] In one embodiment, the transfer mechanism can be a robotic transfer arm. In general, any type of robotic transfer arm known in the art can be used. For

example, the robotic transfer arm can be a multi-axis robotic arm (for example, a 2-, 3-, 4-, 5-, or 6-axis robotic arm). The robotic transfer arm can operate to pick-up and transfer a specimen container 500 (e.g., a blood culture bottle) from an entrance location or port 110 to one of a plurality of container receiving structures or wells 602 located in one of a plurality of holding structures or racks 600 (optionally having an agitation assembly). Furthermore, to facilitate the necessary movements of the transfer mechanism or robotic transfer arm, the interior chamber 620 of the detection system 100, may include one or more supports for the robotic transfer arm. For example, one or more vertical supports and/or one or more horizontal supports may be provided. The transfer mechanism or robotic transfer arm will slide up and down and across the supports as necessary to access any of the receiving structures or wells 602 of the holding structures or racks 600. As previously described, the robotic transfer arm can operate to change the orientation of a specimen container from a vertical orientation (i.e., up-right orientation such that the top 502 of the container 500 is up) to a horizontal orientation (i.e., such that the container 500 is laying on its side), for example, to facilitate in container transfer from a loading station or location, and placement within a holding structure and/or agitation assembly.

[0202] In one embodiment, the robotic transfer arm is a 2-, or 3-axis robotic arm and will be capable of transferring the container 500 in one or more horizontal axes (for example, the x- and/or z-axes) and optionally a vertical axis (y-axis) to a specific location, such as the container receiving structures or wells 602 described herein. In accordance with this embodiment, a 2-axis robotic arm will allow movement in 2-axes (for example, the x-, and z-axes), whereas a 3-axis robotic arm will allow movement in 3-axes (for example, the x-, y-, and z-axes).

[0203] In another embodiment, the 2-, or 3-axis, robotic arm may further employ one or more rotational movements, capable of transferring or moving the specimen container 500 rotationally about one or more axes. This rotational movement may allow the robotic transfer arm to transfer a specimen container 500 from a vertical loading orientation to a horizontal orientation. For example, the robotic transfer arm may employ a rotational movement to move the specimen container rotationally about or around a horizontal axis. This type of robotic transfer arm would be defined as a 3-, or 4- axis robotic arm. For example, a robotic arm that allows movement in one horizontal axis (the x-axis), one vertical axis (e.g., the y-axis) and one rotational axis would be considered a 3-axis robotic arm. Whereas, a

robotic arm that allows movement in two horizontal axes (e.g., the x-, and z-, axes), a vertical axis (the y-axis) and one rotational axis would be considered a 4-axis robotic arm. Similarly, a robotic arm that allows movement in a single horizontal axis (e.g., the x-axis), a vertical axis (the y-axis) and two rotational axes would also be considered a 4-axis robotic arm. In yet another embodiment, the robotic transfer arm 700 can be a 4-, 5-, or 6-axis robotic arm, thereby allowing movement in the x-, y-, and z-axes, as well as rotational movement about, or around, one-axis (i.e., a 5-axis robot), two axes (i.e., a 5-axis robotic arm), or all three horizontal (x-, and z-axes) and vertical axes (y-axes) (i.e., a 6-axis robotic arm).

[0204] In yet another embodiment, the robotic transfer arm may include one or more devices for obtaining measurements, scans and/or readings of a specimen container 500. For example, the robotic transfer arm may include one or more video cameras, sensors, scanners, and/or bar code readers. In accordance with this embodiment, the video camera, sensor, scanner and/or bar code reader may aid in container location, reading of container labels (e.g., bar codes), container scanning, remote field servicing of the system, and/or detecting for any possible container leaks within the system. In yet another design possibility, the robotic transfer arm may include a UV light source to aid in automated decontamination, if necessary.

[0205] One design possibility of the transfer mechanism is shown in FIGS. 6-8C. As shown in FIG. 6, the transfer mechanism comprises a robotic transfer arm 650, which comprises an upper horizontal support rail 652A, a lower horizontal support rail 652B, a single vertical support rail 654 and a robotic head 656 that will include a gripping mechanism (not shown) for picking-up, gripping or otherwise holding a specimen container 500. The transfer mechanism shown in FIGS. 6-8C is shown schematically and the parts not to scale, for example, the horizontal supports 652A, 652B, vertical support and robotic head 656 shown are not to scale. As one of skill in the art would readily appreciate, the horizontal supports 652A, 652B, and vertical support can be increased or decreased in length as needed. As shown, the robotic head 656 is supported by the vertical support rail 654, which in turn is supported by, coupled to, and/or attached to the horizontal support rails 652A and 652B. Also as shown in FIG. 6, the transfer mechanism may comprise one or more mounting supports 696 that can be used to mount the transfer mechanism in the detection system.

[0206] In operation, the vertical support rail 654 can be moved along the horizontal support rails 652A and 652B, thereby moving the vertical support rail 654 and the robotic head 656 along a horizontal axis (e.g., the x-axis). In general, any known means in the art can be used to move the vertical support rail 654 along the horizontal support rails 652A and 652B. As shown in FIG. 6, the upper and lower support rails 652A and 652B, can comprise upper and lower threaded shafts (not shown) operable to drive upper and lower horizontal slide blocks 659A and 659B, respectively. Also, as shown in FIG. 6, the upper and lower shafts 652A and 652B can include hollow, elongate reinforcing sleeves 653A, 653B that extends the length of the upper and lower support rails 652A, 652B, and thereby surrounds the upper and lower threaded screws (see, e.g., U.S. Patent No. 6,467,362). The sleeves 653A, 653B will each further comprise a slot (see, e.g., 653C) in the sleeve 653A, 653B that extends the length of the upper and lower support rails 652A, 652B. Threaded tongues (not shown) are provided that extend through the slot (see, e.g., 653C) and have threads engageable with the threaded shafts (not shown) which are encased in the reinforcing sleeves 653A, 653B. As the threaded shafts (not shown) of the upper and lower support rails 652A, 652B are turned by a first motor 657, the threaded tongues (not shown) moves horizontal slide blocks 659A, 659B along the longitudinal length of the upper and lower support rails 652A, 652B, thereby moving the robotic head 656 along a horizontal axis (e.g., the x-axis) (again, see, e.g., U.S. Patent No. 6,467,362). A first motor 657 can operate to turn the upper and lower threaded shafts (not shown) and thereby drive upper and lower horizontal slide blocks 659A and 659B (each having internal threads that engage the threaded shafts, respectively) in a horizontal direction along the upper and lower threaded shafts. In one design possibility, the first motor 657 can be used to turn both the upper and lower threaded shafts by including a drive belt 660 and set of pulleys 662 to turn one of the threaded shafts (e.g., the lower threaded shaft) in parallel with the first threaded shaft, as the first threaded shaft is turned by the motor 657.

[0207] As shown in FIG. 6, the vertical support rail 654 may further comprise vertical threaded drive shaft (not shown) operable to drive a vertical slide block 655 and thereby move the robotic head 656 along a vertical axis (e.g., the y-axis). In operation, a second motor 658 can operate to turn a vertical threaded shaft (not shown) and thereby drive vertical slide block 655 in a vertical direction along the vertical threaded shaft. In another embodiment, as shown in FIGS. 6-7B, and as

described hereinabove, the vertical threaded shaft may further comprise a hollow, elongate reinforcing sleeve 654A that extends the length of the vertical support rail 654, and thereby surrounds the vertical threaded shaft (not shown). The sleeve 654A will further comprise a slot 654B that extends the length of the vertical support rail 654. A threaded tongue (not shown) is provided that extends through the slot (not shown) and has threads engageable with the threaded shaft (not shown). As the threaded shaft (not shown) is turned by motor 658, the threaded tongue (not shown) moves a vertical slide block 655, thereby moving the robotic head 656 along a vertical axis (e.g., the y-axis) (again, see, e.g., U.S. Patent No. 6,467,362). The vertical slide block 655 may be directly attached to the robotic head 656, or as shown in FIG. 6, may be attached to a first rotational mechanism 664. The vertical slide block 655 has internal threads (not shown) that engage the threaded vertical shaft and operated to drive the vertical slide block, and thus the robotic head 656, in a vertical direction, along the threaded vertical shaft.

[0208] The transfer mechanism 650 may further comprise one or more rotational mechanisms operable to provide rotational movement about or around one or more axes. For example, as shown in FIG. 6, the robotic head may comprise a first rotational mechanism 664 for providing rotational movement about or around the y-axis and a second rotational mechanism 665 for providing rotational movement about or around the x-axis. The first rotational mechanism 664 comprises a first rotational plate 667 that can be attached to the robotic head 656. The first rotational mechanism 664 further comprises a first rotational motor 668, and a first pinion gear 670 and a first opposable ring gear 672, which operate to rotate the first rotational plate 667, and thus the robotic head 656, about a vertical axis (e.g., about the y-axis). In one embodiment, as is well known in the art, the first pinion gear 670 and first ring gear 672 may be provided with gripping teeth (not shown) or other gripping feature (not shown). The first rotational plate 667 may be directly attached to the robotic head 656, or as shown in FIG. 6, may be attached to a second rotational mechanism 665. Also as shown in FIG. 6, the first rotational plate 667 may comprise a bent plate to facilitate attachment to the second rotational mechanism 665. The second rotational mechanism 665, like the first rotational mechanism 664, comprises a second rotational plate 674. As shown in FIG. 6, the second rotational plate 674 is attached to the robotic head 656. The second rotational mechanism 665 further comprises a second rotational motor 678, a second pinion gear 680 and a second opposable ring

gear 682, which operate to rotate the second rotational plate 674, and thus the robotic head 656, about a horizontal axis (e.g., the x-axis). In one embodiment, as is well known in the art, the second pinion gear 680 and second ring gear 682 may be provided with gripping teeth (not shown) or other gripping feature (not shown).

5 **[0209]** The robotic head 656, best shown in FIG. 7B, comprises a housing 684 enclosing a holding chamber 685 for holding a single specimen container 500 therein. The robotic head further comprises a gripping mechanism 686 and a drive mechanism 688 to move the gripping mechanism 686, and thereby a single specimen container 500, into and out of the housing 684 and holding chamber 685. The gripper
10 mechanism 686, as shown in 7B, may comprise a spring clip 687 operable to snap over the lip of a specimen container 500. After transferring the specimen container 500 to a holding structure 600, as described elsewhere herein, the robotic head 656, and thus the gripping mechanism 686, can be raised or lowered relative to the holding structure 600 to release the specimen container 500. The drive mechanism 688
15 further comprises a motor 690, a guide rail 692, a threaded gripper shaft 694 and a gripper drive block 696, as shown in FIG. 7B. In operation, the motor 690 turns the threaded gripping shaft 694, thereby moving the gripping drive block 696, and thus the gripping mechanism 686 along the guide rail 692.

[0210] Another design possibility of the transfer mechanism is shown in
20 FIGS. 9A-9B. As shown in FIGS. 9A-9B an automated transfer mechanism 820 is incorporated into the detection system 100 shown in FIGS. 9A-9B in order to grasp or pick-up a container 500 from the entrance location or port 110, and move or transfer a container 500 to a give receiving structure or well 802, of an upper or lower drum holding structure 800 (described elsewhere herein). The automated transfer
25 mechanism 820 in this embodiment is also operable to move a negative container 500 to a waste location and subsequently dropping or otherwise depositing the container 500 into a waste bin 146, or operable to move a positive container to a positive container location (see, e.g., 130 in FIG. 1). To provide such movement, the transfer mechanism 820 includes a robotic head 824 which may include a gripping mechanism
30 826 for picking-up and holding a container 500, and a rotatable support rod 828 that extends across the interior chamber 850 of the system 100. As shown, the robotic head 824 is supported by, coupled to, and/or attached to the rotatable support rod 828. In general, the gripping mechanism can be any known gripping mechanism in the art. In one embodiment, the gripping mechanism may be the gripping mechanism and

drive mechanism described hereinabove in conjunction with FIGS. 6-8C. The robotic head 824 is moveable to any position along the rotatable support rod 828. In operation, the support rod 828 can be rotated about its longitudinal axis, so as to orient the robotic head 824 towards either the upper or lower cylinder or drum holding structures 800A, 800B.

[0211] In one embodiment, the robotic head 820 is operable to pick-up a container 500 from the entrance location or port 110 and load the container 500 head-first (i.e., top portion 502 first) into the receiving structures or wells 802 of the drum holding structures 800A, 800B. This orientation exposes the bottom or base 506 of the container 500 to a detection unit 810 which can read the sensor 514 located at the bottom of the container 500 to detect microbial or microorganism growth within the container.

[0212] Yet another design possibility for the transfer mechanism is shown in FIGS. 17-21B. As shown in FIGS. 17-21B, the robotic transfer arm 700 will include one or more horizontal support structures 702, one or more vertical support structures 704, and a robotic head 710 that will include one or more features or devices (e.g., a gripping mechanism) to pick-up, grip and/or hold a specimen container 500. The robotic head 710 can be supported by, coupled to, and/or attached to one of the horizontal supports and/or vertical supports. For example, in one embodiment, as shown in FIGS. 17-21B, the robotic transfer arm 700 comprises a lower horizontal support structure 702B and a single vertical support structure 704. Although, not shown, as one of skill in the art would appreciate an upper horizontal support structure (not shown), or other similar means can be used to further support or guide the vertical support structure. In general, any known means in the art can be used to move the robotic head 710 up and down the vertical support rail 704 (as represented by arrow 726 (see FIG. 18)), and move the vertical support rail 704 back-and-forth along the horizontal support structure(s) 702B (as represented by arrow 736 (see FIG. 20)). For example, as shown in FIG. 20, the robotic transfer arm 700 may further comprises a vertical drive motor 720 and vertical drive belt 722 that will operate to transfer or move the robotic head 710 up and down (arrow 726) the vertical support rail 704 to transfer or move a container 500 along (i.e., up and down) a vertical axis (i.e., the y-axis). The vertical support structure 704 may further comprise a vertical guide rail 728 and a robotic head support block 708, as shown in FIG. 20. Accordingly, the vertical support structure 704, vertical guide rail 728, vertical drive

motor 720 and vertical drive belt 722 allow the robotic transfer arm 700 to move or transfer the robotic head support block 708, and thus, the robotic head 710 and a specimen container 500 along the y-axis. Likewise, also as shown in FIG. 20, the robotic transfer arm 700 may further comprise a first horizontal drive motor 730, first horizontal drive belt 732 and horizontal guide rail 738 that will operate to move the vertical support structure 704 back-and-forth (i.e., from left-to-right and/or from right-to-left) along the horizontal guide rail 738, and thus, along a first horizontal axis (i.e., the x-axis) within the housing 102 of the detection system 100 (see arrow 736)). Accordingly, the horizontal support structure(s) 702B, first horizontal drive motor 730, first horizontal drive belt 732 and horizontal guide rail 738 allow the robotic transfer arm 700 to move or transfer a specimen container 500 along the x-axis. Applicants have found that by including a vertical support that is movable along a horizontal axis allows for an increased capacity within the detection system, as the robotic transfer arm is movable over an increased area within the instrument. Furthermore, Applicants believe a robotic transfer arm having a movable vertical support may provide a more reliable robot transfer arm.

[0213] As shown best in FIG. 17-21B, the automated transfer mechanism or robotic transfer arm 700 may further comprise a linear or horizontal slide 706 and pivot plate 750. As shown, for example in FIGS. 17- 20, the linear or horizontal slide 706 supports the robotic head 710 and gripper mechanism 712. The linear or horizontal slide 706 and robotic head 710 may be supported by, coupled to, and/or attached to, a robotic head support block 708 and vertical guide rail 728 (previously described). In accordance with this embodiment, the linear or horizontal slide 706 can be moved up and down (see FIG. 18, arrow 726) along a vertical axis (i.e., the y-axis), via the a robotic head support block 708 and vertical guide rail 728, to move or transfer the robotic head 710 and/or specimen container 500 up and down within the housing 102 of the detection system 100 (i.e., along the vertical axis (y-axis)). As shown in FIGS. 21A-21B, the linear or horizontal slide 706 may further comprises a pivot plate 750 comprising a pivot plate guide rail 752, a pivot slot 754 and pivot slot cam follower 756 operable to allow the robotic head 710 to slide or moved along the linear or horizontal slide 706, from front-to-back or from back-to-front (see FIG. 18, arrow 746), to transfer or move a container 500 along a second horizontal axis (i.e., the z-axis). In accordance with this embodiment, a second horizontal drive motor or horizontal slide motor 760 and a slide belt (not shown) can be used to move the

robotic head 710 along the z-axis. Accordingly, the linear or horizontal slide 706, the horizontal slide motor and slide belt, allows the robotic head 710 to move or transfer a specimen container 500 along the z-axis. As known in the art, one or more sensors (see, e.g., 764 in FIG. 21A) can be used to indicate the position of the robotic head

5 710 on the linear or horizontal slide 706.

[0214] As shown in FIGS. 21A-21B, as the robotic head 710 is moved along the linear or horizontal slide 706, pivot plate 750, and pivot plate guide rail 752 the pivot slot 754 and pivot slot cam follower 756 rotate the pivot carriage 758 about or around a horizontal axis (i.e., the z-axis), and thus, rotates the robotic head 710 from a horizontal orientation (as shown in FIG. 21A) to a vertical orientation (as shown in FIG. 21B), or vice versa. As described elsewhere herein, the transfer of a container 500 from a vertical entry orientation to a horizontal orientation may be necessary for depositing or placing the container in a horizontally orientated receiving structure or well 602 of the holding structure or rack 600. Accordingly, the pivot plate 750, pivot slot 754 and pivot carriage 758 allow the robotic head 710 to re-orientate a specimen container 500 from a vertical orientation, as loaded (see, e.g., FIG. 18) to a horizontal orientation (as seen, e.g., in FIG. 21A), thereby allowing a specimen container 500 to be transferred from an automated loading mechanism (see, e.g., 200 in FIG. 18) to a well in a holding structure (e.g., 602 and 600 in FIG. 18). As shown in FIG. 20 the automated transfer mechanism may also comprise one or more cable management chains 782, for cable management within the detection system 100, and a circuit board 784 for controlling the robotic transfer mechanism. In yet another embodiment, the robotic transfer arm 700 may further comprise a break mechanism 786 that can operate to break the vertical drive belt 722, thereby preventing it from falling to the bottom of the instrument (e.g., due to a power outage).

[0215] The robotic transfer arm 700 may further comprise a gripping mechanism 712 to pick-up, grip or otherwise hold a specimen container 500. As shown, for example in FIGS. 21A and 21B, the gripping mechanism may comprise two or more gripping fingers 714. Furthermore, the gripping mechanism 712 may further comprise a linear actuator 716 and a linear actuator motor 718 which can operate to move the linear actuator to open and close the gripper fingers 714. In operation, as is well known in the art, the actuator motor 718 can be used to move the linear actuator 716 of the gripper mechanism 712 thereby moving the gripper fingers 714. For example, the linear actuator can be moved in a first direction (e.g., toward

the motor) to close the fingers and grip the container 500. Conversely, the linear actuator can be moved in a second direction (e.g., away from the motor) to open the gripper fingers and release the container 500. Applicants have unexpectedly found that the use of one or more gripping fingers 714 allows the gripping mechanism 712 to accommodate (i.e., pick-up and/or hold) a large variety of different specimen containers 500. Moreover, Applicants have found that by using gripper fingers 714 that extend from about one-quarter (1/4) to about one-half (1/2) the length of the specimen container 500, the gripper fingers will accommodate (i.e., pick-up and/or hold) a number of well known containers (e.g., long neck blood culture bottles) in the art.

[0216] As described further herein, the automated transfer mechanism or robotic transfer arm 700 can be placed under the control of a system controller (not shown) and programmed for specimen container 500 management (e.g., pick-up, transfer, placement and/or container removal) within the detection system 100.

[0217] In yet another embodiment, as discussed further hereinbelow, the transfer mechanism 700 can be used for automated unloading of “positive” and “negative” specimen containers 500.

Holding Means or Structure with Optional Agitation Means

[0218] The holding means or structure of the detection system 100 can take a variety of physical configurations for handling a plurality of individual specimen containers 500 so that a large number of containers (e.g., 200 or 400 containers, depending on the specific holding structures used) can be processed simultaneously. The holding means or structure can be used for storage, agitation and/or incubation of the specimen containers 500. One possible configuration is shown in FIGS. 5A-5B, and another possible configuration is shown in FIGS. 9A and 9B. These configurations are provided by way of illustration and not limitation. As one of skill in the art will appreciate, other designs are possible and contemplated.

[0219] As shown in FIGS. 5A-5B and FIGS. 17-20, one possible configuration uses a plurality of vertically stacked container holding structures or racks 600 each having a multitude of specimen container receiving structures or wells 602 each for holding individual specimen containers 500. In accordance with this embodiment, two or more vertically stacked holding structures or racks 600 can be used. For example, from about 2 to about 40, from about 2 to about 30, from about 2

to about 20, or from about 2 to about 15 vertically stacked holding structures or racks can be used. Referring to FIGS. 5A- 5B and 17-20, in this configuration the detection system 100 includes a climate controlled interior chamber 620, comprising an upper interior chamber 622 and a lower interior chamber 624, and a plurality of vertically disposed holding structures or racks 600 (e.g., as shown in FIGS. 5A-5B, 15 vertically stacked holding structures or racks 600) each having a plurality of individual container receiving structures or wells 602 therein. Each individual holding structure or rack 600 can comprise two or more container receiving structures or wells 602. For example, each holding structure or rack 600 can comprise from about 2 to about 40, from about 2 to about 30, or from about 2 to about 20 receiving structures or wells 602 therein. In one embodiment, as shown in FIGS. 5A-5B, the receiving structures or wells 602 can comprise 2 rows of vertically aligned receiving structures or wells 602. In an alternative embodiment, the receiving structures or wells 602 can be staggered, thus reducing the vertical height of each individual holding structure or rack 600 (see, e.g., FIG. 20), and thereby allowing for an increased number of total holding structures or racks 600 in a given vertical distance within the incubation chamber 620. As shown, for example in FIGS. 5A-5B, the detection system comprises 15 holding structures or racks 600 each comprising two rows of 10 individual container receiving structures or wells 602, thereby giving the system exemplified in FIGS. 5A-5B a total container capacity of 300. In another possible design configuration, the detection apparatus may comprise 16 vertically stacked racks, each containing 25 receiving structures or wells, thereby giving a total container capacity of 400.

[0220] Furthermore, each of the individual container receiving structures or wells 602 has a specific X and Y coordinate position or address, where X is the horizontal location and Y is the vertical location of each container receiving structure or well 602. The individual wells 602 are accessed by a transfer mechanism, such as a robotic transfer arm, for example, as described hereinabove in conjunction with FIGS. 17-21). As shown in FIGS. 17-21, the automated transfer mechanism 700 can operate to move the robotic head 710, and thus, the specimen container 500, to a specific of the X, Y positions in the rack 600 and deposit the container 500 therein. In operation, the automated transfer mechanism 700 can operate to pick-up a specimen container 500 at the entrance station 110 or the pick-up station 418 of the container locator device 400, move a container 500 determined positive for microbial growth

therein to a positive container or exit location 130, and/or to move a container 500 determined negative for microbial growth to a negative container location or waste bin 146.

[0221] In one embodiment, the entire holding structure or rack 600 can be agitated by an agitation assembly (not shown) to promote or enhance microorganism growth. The agitation assembly can be any known means or mechanism for providing agitation (e.g., a back-and-forth rocking motion) to the holding structures or racks 600. In another embodiment, the holding structures or racks 600 can be rocked in a back-and-forth motion for agitation of the fluid contained within the containers. For example, the holding structures or racks 600 can be rocked back-and-forth from a substantially vertical position to a substantially horizontal position, and repeated to provide agitation of the fluid contained within the container. In yet another embodiment, the holding structures or racks 600 can be rocked back-and-forth from a substantially horizontal position to a vertical position 10 degrees, 15 degrees, 30 degrees, 45 degrees or 60 degrees from horizontal, and repeated to provide fluid agitation within the containers. In one embodiment, a racking motion from a substantially horizontal position to a vertical position from about 10 degrees to about 15 degrees from horizontal may be preferred. In still another embodiment, the holding structure or racks 600 can be rocked back-and-forth in a linear or horizontal motion to provide agitation of the fluid contained within the containers. In this embodiment, the holding structures or racks 600 and receiving structures or wells 602 can be orientated in a vertical, or alternatively in a horizontal position. Applicants have found that a linear or horizontal agitation motion, with the holding structures 600, and thus the receiving structures or wells 602 and specimen containers 500, in a horizontal orientation can provide substantial agitation with a relatively minimum energy input. Accordingly, in some embodiments, a horizontal holding structure or rack 600 orientation and a linear or horizontal agitation motion, may be preferred. Other means of agitating the holding structures or racks 600, and thus, the fluid within specimen containers 500 are contemplated and would be well understood by one skilled in the art. These back-and-forth, liner and/or horizontal rocking motions can be repeated as desired (e.g., at various cycles and/or speeds) to provide agitation of the fluid within the containers.

[0222] One possible design for the agitation assembly is shown in conjunction with FIG. 26. As shown in FIG. 26, the agitation assembly 626

comprises one or more holding structures 600 comprising a plurality of holding wells 602 for holding a plurality of specimen containers 500. The agitation assembly 626 further comprises an agitation motor 628, an eccentric coupling 630, a first rotation arm 632, a second rotation arm or linkage arm 634 and a rack agitation bearing assembly 636. In operation, the agitation motor 628 rotates the eccentric coupling 630 in an off-center motion thereby moving a first rotation arm 632 in an off-center circular or off-center rotational motion. The off-center rotational movement of the first rotation arm 632 moves a second rotation arm or linkage arm 634 in a linear motion (as represented by arrow 635). The linear motion of the second rotation arm or linkage arm 634 rocks the rack agitation bearing assembly 636 in a back-and-forth rocking motion, thereby providing a back-and-forth rocking agitation motion (represented by arrow 638 of FIG. 26) to the holding structures 600.

[0223] In another possible design configuration, as shown in FIGS. 9A and 9B, the detection system 100 may include upper and lower holding structures 800A and 800B in the form of cylindrical or drum structures containing a multitude of individual specimen container receiving structures or wells 802 for receiving one of the containers 500. In this embodiment, the cylindrical or drum holding structures 800A, 800B each rotate about a horizontal axis to thereby provide agitation of the containers 500. In accordance with this embodiment, each drum holding structure can comprise from about 8 to about 20 rows (e.g., from about 8 to about 20, from about 8 to about 18, or from about 10 to about 6 rows), each comprising from about 8 to about 20 container receiving structures or wells 802 (e.g., from about 8 to about 20, from about 8 to about 18, or from about 10 to about 16 receiving structures or wells 802).

[0224] As described hereinabove, an automated transfer mechanism 820 is incorporated into the detection system 100 of FIGS. 9A-9B in order to grasp or pick-up a container 500 from the entrance location or port 110, and move or transfer the container 500 to a given receiving structure or well 802, of either the upper or lower drum holding structure 800, and deposit the container 500 therein. The automated transfer mechanism 820 in this embodiment can further operate to move a negative container 500 to a waste bin 146, or can operate to move a positive container to the positive container location 130, shown for example, in FIG. 1. Also, as previously described, the robotic head 820 of FIGS. 9A-9B can pick-up a container 500 from the entrance location or port 110 and load the container 500 head-first (i.e., top portion

502 first) into the receiving structures or wells 802 of the drum holding structures 800A, 800B. This orientation exposes the bottom or base 806 of the container 500 to a detection unit 810 which can read the sensor 514 located at the bottom of the container 500 to detect microbial or microorganism growth within the container.

5 [0225] As described elsewhere herein, positive and negative containers can be retrieved by the robotic transfer arm and transferred to other locations within the system. For example, a container determined “positive” for microbial growth can be retrieved and transferred via the transfer mechanism to a positive container location or port where a user or technician can easily remove the positive container. Similarly, a
10 container determined “negative” for microbial growth after a designated time has passed can be transferred via the transfer mechanism to a negative container location or waste bin for disposal.

 [0226] In one embodiment, the holding structure or rack 600 may further comprise a retention feature operable to hold or otherwise retain a specimen container
15 500 in the receiving structures or wells 602 of the rack 600. As shown in FIGS. 27A-27C, the retention device 860 comprises a canted coiled spring 864 and a v-shaped holding plate 862. In accordance with this embodiment, by using a canted coiled spring 868, multiple points of the coiled spring contact the container surface to retain the bottle in the rack well 602. The coils of the canted spring 864 are set at an angle
20 relative to the vertical axis of the container, as shown in FIG. 27C, which shows exaggerated coils to demonstrate the coil angle relative to the vertical axis of the container. However, typically the canted spring 864 is a tightly coiled spring. For example the canted spring 864 can be at an angel of about 10 degrees to about 50 degrees, from about 20 degrees to about 40 degrees, or about 30 degree (as shown in
25 FIG. 27C), relative to the vertical axis of the container. The v-shaped holding plate 862 is capable of holding and/or retaining said canted coiled spring 864 relative to, or adjacent to the holding structure 600. As shown, the holding plate 862 comprising a v-grooved retainer plate for retaining the canted coiled spring 864. The v-groove retainer plate 864 prevents any movement of the spring 864 relative to the container
30 500 and/or holding structure 600. Accordingly, unlike a traditional extension spring, which would typically contact a container at a single point (e.g., a flat leaf spring), the canted coiled spring 864 can be rigidly retained by the v-shaped groove 862 while the coils will deflect under pressure. The use of a canted spring 864 allows the load to be spread out, thereby providing uniform deflection.

[0227] As shown, e.g., in FIGS. 27A and 27C, the receiving structures or wells 602 further comprise one or more ribs 868. In one design possibility, as shown in FIG. 27C, two of these ribs 868 are located directly opposite the canted coiled spring 864. These two ribs 868 form a groove that functions to self-center the container 500 within the well 602 along a vertical centerline (not shown). In operation, the canted coiled spring 864 applies force to the container 500 wall, thereby holding or retaining the container securely within the well 602 of the rack 600. In one embodiment, the two ribs 868 located opposite the coiled spring 864 can be spaced from 30 degrees to about 90 degrees apart, or from about 40 degrees to about 80 degrees apart. In another embodiment, the two ribs 868 located opposite the canted coiled spring 864 can be spaced about 60 degrees apart. Also, as shown in FIG. 27C, the holding structure may comprise a first row and a second row of parallel holding wells, the parallel holding rows being capable of, or operable for, holding a plurality of containers therein, and wherein the holding structure further comprises a first canted coiled spring located adjacent to the first row and a second canted coiled spring adjacent to the second row, wherein each of the canted coiled spring are operable for retaining the plurality of containers in said holding wells.

[0228] Using the canted coiled spring 864, v-groove retainer 862 and two ribs 868 located opposite said canted coiled spring 864, the bottle will always be held securely in the same location within the well 602, regardless of any side loads applied through agitation or during rack cell insertion. The canted coiled spring 864 and v-groove retainer 862 also allow for the use of a shorter depth holding well 602 and holding structure 600. The shorter holding well 602 depth will allow for multiple container designs and container lengths to be retained equally well, as well as allow more of the container surface to be exposed to the incubation air flow within the system.

[0229] As one of skill in the art would appreciate other possible designs or configurations for the holding structure or structures 600 and/or agitation assembly are possible and are considered part of present invention.

Detection Unit

[0230] The various possible design configurations of the detection system for detecting microbial agent growth within a specimen container, as shown in FIGS. 1-6, 9A-9B, 21A-21B and 27, can include the use of similar detection means.

In general, any known means in the art for monitoring and/or interrogating a specimen container for the detection of microbial growth can be used. As previously mentioned, the specimen containers 500 can be monitored continuously, or periodically, during incubation of the containers 500 in the detection system 100, for the positive detection of microbial growth. For example, in one embodiment, a detection unit (e.g., 810 of Figure 9B) reads the sensor 514 incorporated into the bottom or base 506 of the container 500. A variety of sensor technologies are available in the art and may suitable. In one possible embodiment, the detection unit takes colorimetric measurements as described in the U.S. patents 4,945,060; 5,094,955; 5,162,229; 5,164,796; 5,217,876; 5,795,773; and 5,856,175, which are incorporated herein. A positive container is indicated depending upon these colorimetric measurements, as explained in these patents. Alternatively, detection could also be accomplished using intrinsic fluorescence of the microorganism, and/or detection of changes in the optical scattering of the media (as disclosed, for example, in co-pending U.S. patent application serial no. 12/460,607, filed July 22, 2009 and entitled, "Method and System for Detection and/or Characterization of a Biological Particle in a Sample.")). In yet another embodiment, detection can be accomplished by detecting or sensing the generation of volatile organic compounds in the media or headspace of the container. Various design configurations for the detection unit can be employed within the detection system. For example, one detection unit could be provided for an entire rack or tray, or multiple detection units could be provided per rack or per tray.

Climate-Controlled Interior Chamber

[0231] As previously described, the detection system 100 may include a climate-controlled interior chamber (or incubation chamber), for maintaining an environment to promote and/or enhance growth of any microbial agents (e.g., microorganisms) that may be present in the specimen container 500. In accordance with this embodiment, the detection system 100 may include a heating element or hot air blower to maintain a constant temperature within said interior chamber. For example, in one embodiment, the heating element or hot air blower will provide and/or maintain the interior chamber at an elevated temperature (i.e., a temperature elevated above room temperature). In another embodiment, the detection system 100 may include a cooling element or cold air blower (not shown) to maintain the interior

chamber at a temperature below room temperature. In accordance with this embodiment, the interior chamber or incubation chamber will be at a temperature of from about 18° to about 45° C. In one embodiment, the interior chamber can be an incubation chamber and can be maintained at a temperature from about 35° C. to about 40° C., and preferably at about 37° C. In another embodiment, the interior chamber may be maintained at a temperature below room temperature, for example from about 18° C. to about 25° C., and preferably at about 22.5° C. A particular advantage provided is the ability to provide a more constant temperature environment for promoting and/or enhancing microbial growth within a specimen container 500.

The detection system 100 accomplishes this by providing a closed system, in which automated loading, transfer and unloading of specimen containers 500 occurs without the need to open any access panels that would otherwise disrupt the incubation temperature (from about 30° to 40° C., preferably from about 37° C.) of the interior chamber 620.

[0232] In general, the detection system 100 can employ any known means in the art for maintaining a climate-controlled chamber for promoting or enhancing microbial growth. For example, to maintain a temperature controlled chamber, one or more heating element or hot air blower, baffles and/or other suitable equipment known in the art, can be used to maintain the interior of the detection system 100 at the appropriate temperature for incubating the container and promoting and/or enhancing microbial growth.

[0233] Typically, one or more heating element or hot air blower under control of the system controller are used to maintain a constant temperature within the interior chamber 620 of the detection system 100. As known in the art, the heating element or hot air blower can be employed in a number of locations within the interior chamber. For example, as shown in FIGS. 5 and 6 one or more heating elements or hot air blowers 740 can be positioned at the base of the holding structures or racks 600, for directing warm air across the plurality of holding structures or racks 600. A similar arrangement can be provided in the embodiments of FIGS. 9A and 9B (see, e.g., 840).

The details of the incubation features are not particularly pertinent, and are known in the art, therefore a detailed description is omitted.

Controller and User Interface

[0234] The detection system 100 will include a system controller (e.g., a computer control system) (not shown) and firmware for controlling the various operations and mechanisms of the system. Typically, the system controller and firmware for controlling the operation of the various mechanisms of the system can be any known conventional controller and firmware known to those of skill in the art. In one embodiment, the controller and firmware will performs all operations necessary for controlling the various mechanisms of the system, including: automated loading, automated transfer, automated detection and/or automated unloading of specimen containers within the system. The controller and firmware will also provide for identification and tracking of specimen containers within the system.

[0235] The detection system 100 may also include a user interface 150 and associated computer control system for operating the loading mechanism, transfer mechanism, racks, agitation equipment, incubation apparatus, and receiving measurements from the detection units. These details are not particularly important and can vary widely. When a container is detected as being positive, the user can be alerted via the user interface 150 and/or by the positive indicator 190 (see, e.g., Figure 1) becoming active (i.e., an indicator light turning on). As described herein, upon a positive determination, the positive container can be automatically moved to a positive container location 130, shown for example in FIGS. 1-3, 10-11 and 22-24 for retrieval by a user.

[0236] The user interface 150 may also provide an operator or laboratory technician with status information regarding containers loaded into the detection system. The user interface may includes one or more of the following features: (1) Touch screen display; (2) Keyboard on touch screen; (3) System status; (4) Positives alert; (5) Communications to other systems (DMS, LIS, BCES & other detection or identification Instruments); (6) Container or bottle status; (7) Retrieve containers or bottles; (8) Visual and audible Positive Indicator; (9) USB access (back ups and external system access); and (10) Remote Notification of Positives, System Status and Error Messages. In another embodiment, as shown in FIGS. 22-23, a status update screen 152 can also be used. The status update screen 152 can be used to provide status information regarding containers loaded into the detection system, such as, for example: (1) container location within the system; (2) container information, such as, patient information, sample type, input time, etc.; (3) positive or negative container

alerts; (4) interior chamber temperature; and (5) an indication that the waste bin is full and needs to be emptied.

[0237] The particular appearance or layout of the detection system and user interface 150, and/or status update screen 152, is not particularly important, and can vary widely. Figures 1-2 show one possible embodiment, which is provided by way of illustration and not limitation. Figures 22-23 show another possible embodiment, which is also provided by way of illustration and not limitation.

Automated Unloading

[0238] The detection system 100 may also provide for automated transfer or automated unloading of "positive" and "negative" specimen containers 500. As previously described, containers in which a microbial agent is present are termed "positive" containers, and containers in which no microorganism growth is detected after a given time period are termed "negative" containers.

[0239] Once a container is detected as positive, the detection system will notify the operator of the results through an indicator (e.g. visual prompt 190) and/or through notification at the user interface 150. Referring now to FIGS. 1-3 and 5A-5B, positive bottles can be automatically retrieved via the transfer mechanism 650 (e.g., robotic transfer arm) and placed in a designated positive container area, such as a positive container location or exit port 130. This positive container area will be located outside of the instrument housing for easy user access to the container. In one embodiment, the container will be placed in a vertical orientation within the positive container area. In one design configuration, the automated unloading of a positive container will employ the use of a transfer tube (not shown) through which a positive container (e.g., a positive blood culture bottle) can travel to be relocated to a designated positive container location or exit port 130. In accordance with this design feature, the transfer mechanism (e.g., the robotic transfer arm) will drop or otherwise deposit the positive specimen container into a top end of the transfer tube, and the container will travel through the transfer tube via gravity to the positive container location or port 130. In one embodiment, the transfer tube (not shown) can hold one or more "positive" specimen containers therein. For example, the transfer tube (not shown) can hold from about 1 to about 5, from about 1 to about 4, or from about 1 to about 3 "positive" specimen containers. In another embodiment, for example as shown in FIGS. 22-24, the positive container location or exit port 130 may comprise

holding wells for one or more “positive” specimen containers, for example, two holding wells for separately holding two “positive” specimen containers.

[0240] In another embodiment of the detection system 100, negative containers can be transferred by the transfer mechanism 700 (e.g., robotic transfer arm) from the holding structure or rack 600 to a negative container location, such as a waste bin 146. Typically, the containers will be released from the robotic transfer arm and dropped into the waste bin 146, however other embodiments are contemplated and should be apparent to one of skill in the art. In one design configuration, the automated unloading of a negative container will employ the use of a transfer tube (not shown) through which a negative container (e.g., a negative blood culture bottle) can travel to be relocated to a designated negative container location, such as a waste bin 146. In accordance with this design feature, the transfer mechanism (e.g., the robotic transfer arm) will drop or otherwise deposit the negative specimen container into a top end of the transfer tube, and the container will travel through the transfer tube via gravity to the negative container location or waste bin 146. The detection system 100 may also include an access door 140 or drawer 142 that opens to provide user access to the negative container location, such as a negative container waste bin 146. In another embodiment, the waste bin 146 may include a scale to weigh the waste bin 146. As one of skill in the art would appreciate, by monitoring the weight of the waste bin 146, the system controller (not shown) can determine how full the waste bin 146 is, and can optionally provide a signal (e.g., at the user interface 150) indicating to the user or technician that the waste bin 146 is full, and thus, needs to be emptied.

Automated Laboratory System

[0241] As noted above, the detection system 100 of this disclosure can take on a variety of different possible configurations. One such configuration, particularly suited for high volume implementations, is shown in FIG. 24. As shown in FIG. 24, the detection system 100A can be employed in an automated microbiology laboratory system. For example, the detection instrument 100 can be included as one component of an automated laboratory system. In this embodiment, the detection instrument 100A can be linked or “daisy chained” to one or more additional other analytical modules or instruments for additional testing. For example, as shown in FIG. 24, the detection instrument 100A can be linked or “daisy chained” to a second detection unit

100B. However, in other embodiments, the detection instrument can be “daisy chained” or otherwise linked to one or more other systems or modules. These other systems or modules can include, for example, identification testing systems such as the VITEK or VIDAS systems of the assignee bioMérieux, Inc., a gram stainer, a mass spectrometry unit, a molecular diagnostic test system, a plate streaker, an automated characterization and/or identification system (as disclosed in co-pending US patent application no. 60/216,339, entitled “System for Rapid Non-invasive Detection of a Microbial Agent in a Biological Sample and Identifying and/or Characterizing the Microbial Agent”, which was filed May 15, 2009) or other analytical systems.

[0242] Referring now to FIG. 24, an automated laboratory system can comprise a first detection system 100A, and a second detection system 100B. In other embodiments, the automated laboratory system can comprise a first detection system 100A, a second detection system 100B, and an automated characterization/identification system (not shown). In accordance with this embodiment, positive containers can be moved or transferred from the first detection system 100A to the second detection system 100B, and/or subsequently to the automated characterization/identification system, using a system transfer device 440. In other embodiments, the first detection system 100A can be coupled to a microorganism identification module or an antimicrobial susceptibility module (not shown).

[0243] As shown in FIGS. 24-25C two detection systems 100A and 100B are “daisy chained” together by system transfer device 440. This allows containers to be transferred from one detection system to another in case the first one is full. A similar system transfer device may also be provided for subsequent transfer of the specimen container 500 from the second detection system 100B to a subsequent systems or modules, as described elsewhere herein. The system transfer mechanism 441 comprises a first container locator device 400A having a transfer station 420 for transferring a container to a second or downstream instrument. The system transfer mechanism 441 also comprises a pusher arm 444 operable controlled by a pusher motor 442 and a transfer bridge 446, as shown in FIG. 24-25C. As shown, the pusher arm 444 may comprise a pair of parallel arms. In operation, when a container to be transferred is moved by the transfer station 420 of the first container locator device 400A, a pusher arm 444 is activated to push or move the container from the transfer

station 420, across a transfer bridge 446, to the down-stream detection system 100B. As shown, the pusher arm 444 is connected to a pusher motor 442 via a pusher arm support structure 445. Figures 25A-C show the transfer of a container from the transfer station 420 of the first detection system 100A to the conveyor belt 206B (see FIG. 24) of the second detection system 100B, and show the container in: (1) a first position (FIG. 25A) as the pusher arm 444 begins to push the container across the transfer bridge 446; (2) a second or intermediate position (FIG. 25B) as the container crosses the transfer bridge 446; and (3) a final position (FIG. 25C) as the container arrives at the conveyor belt (not shown) of the down-stream detection system 100B. Furthermore, as shown in FIGS. 25A-25C, the system transfer device 440 may further comprise one or more locator device guide rails 450 attached to a base plate of the locator device 404 via one or more guide rail supports 452, and/or bridge guide rails 446, 448, to guide the container from the first locator device 400A and across the bridge 446 to the conveyor belt 206B (see FIG. 24) of the automated loading mechanism 200B of the down-stream detection system 100B. As would be well known in the art, the transfer of a container from the first detection system 100A to the second or down-stream detection system 100B, via the operation of the first container locator device 400A and pusher arm 444, can be controlled by the system controller. Typically, as shown in FIG. 24, only the first detection system 100A needs to include a user interface 150. The first 100A and second 100B detection systems may further comprise status screens 152A, 152B, positive container ports 130A, 130B, lower access panels 140A, 140B, automated loading mechanisms 200A, 200B and conveyor belts 206A, 206B.

[0244] Further, in accordance with this embodiment, positive containers can be transferred to other systems in the automated laboratory system. For example, as shown in FIG. 24, a container 500 determined positive in the first detection system 100A can be transferred to the second detection system 100B and/or subsequently to an automated characterization/identification system 104 described in Figures 31, 57, 77) for automated characterization and/or identification of the microbial agent therein.

[0245] As one of skill in the art would appreciate other possible designs or configurations for the automated laboratory system are possible and are considered part of this invention.

Method of Operation

[0246] In one embodiment, a method for detection of microorganism growth in an automated detection system is described herein; the method comprising: (a) providing a specimen container comprising a culture medium for promoting and/or enhancing growth of said microorganism; (b) inoculating said specimen container with a test sample to be tested for the presence of a microorganism; (c) loading said inoculated specimen container into said detection system using an automated loading mechanism; (d) transferring said specimen container to a holding structure located within said detection system using an automated transfer mechanism, said holding structure comprising a plurality of wells for holding one or more of said specimen containers; and said holding structure optionally providing agitation of said specimen containers to promote and/or enhance microorganism growth therein; (e) providing a detection unit for detecting microbial growth in said specimen container by detecting one or more by products of microorganism growth within said container; and (f) detecting growth of a microorganism using said detection unit and thereby determining said container positive for microorganism growth.

[0247] The method of operation of the detection system 100 will now be described with reference to FIG. 30. After inoculation of a specimen container 500 with a sample to be tested (e.g., by a laboratory technician or doctor) the specimen container 500 is delivered to the automated loading mechanism 200, for automated loading of the specimen container 500 into the detection system 100.

[0248] At step 540, the specimen container 500 is loaded into the detection system 100, e.g., by placing the container onto a loading station or area 202 of a transport mechanism 204, as shown for example in Figure 1. The specimen container 500 is then moved by the transport mechanism 204 (e.g., a conveyor belt) to an entrance location or port 110, and subsequently through said entrance location or port 110 and into the detection system 100, thereby automatically loading the specimen container 500 into the detection system 100.

[0249] At step 550, an automated transfer mechanism 700, such as a robotic transfer arm, as shown for example in FIGS. 5A-5B, can then be used to transfer the container 500 to, and deposit the container in, a holding structure or rack 600 contained within the interior chamber 620 of the detection system 100.

[0250] At step 560, the specimen container 500 is incubated within the detection system 100. The detection system 100 optionally provides for agitation (e.g., using an agitation assembly) of the holding structures or racks 600, and/or one

or more warm air blowers (see, e.g., 740 in FIGS. 5A-5B) to provide a temperature controlled environment, to promote and/or enhance microbial growth within the specimen container 500.

5 [0251] At step 570, the specimen container 500 is read by a detection unit (see, e.g., 810 in FIGS. 9A and 9B) to determine if the specimen container 500 is positive for microbial growth.

10 [0252] At step 580, the reading of the specimen container is analyzed to determine if the container is positive for the growth of a microbial agent (e.g., a microorganism) therein. If not, the processing proceeds along the NO branch 582 and a check is made if a timer has expired (step 584). If the timer has expired, the container is deemed negative and the container is transferred to the waste container 146 (see for example FIG. 1) at step 586. Otherwise, the incubation continues and the reading of the specimen container 500 (step- 580) continues periodically.

15 [0253] If at step 580, if the specimen container 500 is determined to be positive, the processing proceeds to the YES branch 590. In one embodiment, the specimen container 500 is moved or transferred using the automated transfer mechanism (e.g., the container is automatically unloading, as described elsewhere herein) to the positive container location or port 130 (see for example FIG. 1) at step 594 for user access to the container and/or further processing. In another
20 embodiment, the specimen container can be transferred using a system transfer device to another detection instrument and/or another analytical system (e.g., to an automated characterization and/or identification system 104 of Figures 31, 57, 77) for further processing.

25 [0254] Part 2 Automated Identification and/or Characterization of a Microbial Agent

(Figures 31-108)

I. Overview

30 [0255] An automated instrument is described herein that provides a new architecture and method for automated identification and/or characterization of a microbial agent in a specimen sample, e.g., biological sample. The identification and/or characterization instrument 104 is shown in block diagram form in Figure 31. Two embodiments are described herein in great detail, a first embodiment described

in conjunction with Figures 32-56 and a second embodiment described in conjunction with Figures 57-76. The embodiments of the instrument 104 operate on a specimen container 500 (Figure 31) containing a sample. In one example, the specimen container 500 is a standard culture bottle, e.g., a blood culture bottle, for containing a specimen sample therein, e.g., a blood sample. The bottle may have been just tested positive by the detection instrument 100 of the previous section when it is delivered the identification and/or characterization instrument 104.

[0256] In general, any type of sample that may contain a microbial agent, e.g., bacterium, fungi or yeast species, can be tested in the instrument 104 such as for example biological samples. For example, the specimen sample can be a clinical or non-clinical sample suspected of containing one or more microbial agents. Clinical samples, such as a bodily fluid, include, but not limited to, blood, serum, plasma, blood fractions, joint fluid, urine, semen, saliva, feces, cerebrospinal fluid, gastric contents, vaginal secretions, tissue homogenates, bone marrow aspirates, bone homogenates, sputum, aspirates, swabs and swab rinsates, other body fluids, and the like. Non-clinical samples that may be tested include, but not limited to, foodstuffs, beverages, pharmaceuticals, cosmetics, water (e.g., drinking water, non-potable water, and waste water), seawater ballasts, air, soil, sewage, plant material (e.g., seeds, leaves, stems, roots, flowers, fruit), blood products (e.g., platelets, serum, plasma, white blood cell fractions, *etc.*), donor organ or tissue samples, biowarfare samples, and the like.

[0257] One possible configuration for the instrument 104 of this disclosure is in a combined system which integrates detection of a microbial agent in a specimen container (instrument 100 of the previous discussion) with automated identification and/or characterization of the microbial agent. This combined approach is also described in conjunction with the embodiment of Figure 27.

[0258] In this configuration, a specimen container 500 (Figure 1) is inoculated with a specimen sample (e.g., clinical or non-clinical sample) and loaded/unloaded into/out of an automated detection instrument 102 (e.g. Figure 77). After a sufficient time interval to allow natural amplification of microorganism (this time interval varies from species to species), the specimen container is tested within the detection instrument 102 for the presence of a microorganism. The testing occurs on a periodic basis so that as soon as a specimen container is tested positive it can be

transferred to the identification and/or characterization instrument 104 for further analysis of the specimen sample.

[0259] Detection can be accomplished using a variety of technologies such as the colorimetric sensor described in the patent literature (see U.S. Patents 4,945,060; 5,094,955; 5,162,229; 5,164,796; 5,217,876; 5,795,773; and 5,856,175). Detection could also be accomplished using intrinsic fluorescence of the microorganism, detection of changes in the optical scattering of the media, or detection in the generation of volatile organics in the media or headspace. These techniques are known in the art and described in previously cited patent literature in the Background section of this document.

[0260] Once a specimen container 500 is detected as positive in the automated detection instrument 102 (see Figure 77), the detection instrument 102 will notify the operator through an indicator (e.g., visual prompt), or via a notification at the user interface display, or by other means. The system may be set up to automatically analyze a positive specimen container or require end user acknowledgement prior to sample analysis in the identification/characterization instrument 104 described below. With automatic characterization, it would be possible to notify the physician immediately via electronic means of the results from the identification/characterization system.

[0261] Once a specimen container is determined to be positive in the detection instrument 102, the positive specimen container is handed off or transferred to the identification and/or characterization instrument 104 described below. See Figure 77. The manner in which this is accomplished can vary widely depending on the physical configuration of the detection and identification/characterization instruments 102 and 104. One example of how this can be accomplished is described below in conjunction with Figure 57.

[0262] Referring now in particular to Figure 31, a specimen container or bottle 500 is received in the identification and/or characterization instrument 104, either in an automated or manual fashion. The manner in which this occurs is not particularly important and can vary widely depending on the configuration of the instrument 104. The specimen container 500 is placed in a suitable holding structure or rack 1906 in the identification and/or characterization instrument 104. Figures 32, 35, 57 and 58 show several possible configuration for the holding structure 1906. The holding structure 1906 is typically adapted for holding a multitude of specimen

containers 500. The holding structure 1906 has a facility for rotating the specimen containers to inclined positions above and below horizontal to facilitate venting and sample removal, as described below and optionally agitation of the sample and thereby promoting microbial growth. In an alternative configuration, the positively
5 declared specimen container could remain in the racks within the detection instrument 102 and the sampling could occur directly from the detection instrument.

[0263] The identification and/or characterization instrument 104 includes a sample removal apparatus 1912 which holds or grasps a disposable sampling device 1902. Together, they operate to remove a test sample (i.e., a portion of the specimen
10 sample in the positive specimen container 500) and subsequently add the portion to a separation device 1904 (see Figures 36-41). The separation device 1904 can take several forms, and one configuration is described herein in which the separation device includes a reservoir (Figure 38, item 2602) for receiving the sample and a capillary tube 2604 connected to the reservoir 2602. The
15 identification/characterization instrument 104 further includes a separation and/or concentration station 1916, optionally in the form of a centrifuge, which operates on the separation device 1904 so as to separate the microbial agent from other components in the test sample and concentrate the microbial agent within the separation device 1904. In one example, the microbial agent is concentrated in the
20 form of a pellet or pellet-like mass in the bottom of the capillary tube 2604 of the separation device 1904. The identification/characterization instrument further includes a identification and/or characterization module or read station (Figure 31, 1918) which interrogates the concentrated microbial agent to identify and/or characterize the microbial agent.

[0264] The instrument 104 receives a cassette 1900 of disposables. The disposables are of two types: (1) sampling devices 1902 for venting and removing a test sample from the specimen container 500, and (2) separation devices 1904 which receive a portion of the sample from the container 500 via the sampling device 1902 and in which the microbial agent in the test sample is concentrated. In alternative
25 configuration of the instrument the functions of the sampling device 1902 and the separation device 1904 are combined into a single disposable device as shown in Figures 60-78 in which case the cassette 1900 will only include a multitude of the combined sampling and separation devices.

[0265] The instrument 104 further includes a robotic transfer mechanism 1910 which operates to access the disposables 1902 and 1904, positive specimen containers 500 held in the holder or rack 1906, a waste container 1908, the separation and concentration device 1916, and the identification module 1918. The robotic transfer mechanism 1910 may also operate to receive a positive specimen container from a separate detection instrument, and load the positive specimen container into the holding structure or rack 1906. The robotic transfer mechanism 1910 accesses the waste container, separation and concentration station 1916, identification module 1918 and other modules or components in the instrument 104 as necessary to perform the functions described below. The manner of construction of the transfer mechanism 1910 can vary widely depending on the configuration of the instrument 104.

[0266] The sample removal apparatus 1912 is preferably incorporated into, or coupled to, the robotic transfer mechanism 1910 as indicated by the dashed lines 1913. The apparatus 1912 further includes robot gripping and handling mechanisms to grasp one of the venting and sampling devices 1902, the separation device 1904 and/or the specimen container 500. The sample removal apparatus 1912 is connected to a pneumatic system 1914 which enables robotic gripping functions. The pneumatic system 1914 may include a vacuum pump, as described in the second embodiment below. The vacuum pump operates to provide vacuum to the venting and sampling device 1902 to draw a sample from the specimen container 500 and provide positive pressure to the sampling device 1902 to inject the sample from the sampling device 1902 into the separation device 1904. These aspects of the identification instrument 104 will all be described in greater detail below.

[0267] In one embodiment, the identification module 1918 includes a light source (e.g., an excitation light source) which illuminates the concentrated microbial agent in the separation device 1904. In response to the illumination, the concentrated microbial agent emits a detectable fluorescence signal, i.e., intrinsic fluorescence, as described below. In addition, the illumination of the concentrated microbial agent by the light source will generate a reflectance signal or Rayleigh scattering signal; this signal is of the same wavelength of the excitation light and provides additional information about the absorption of the microbial agent. The reflectance signal may also provide the basis of normalization of the fluorescence data. The configuration of the identification module 1918 includes a means for spatially dispersing the reflectance/fluorescence spectrum, which may take the form of a spectrometer. These

fluorescence and reflectance signals (spectrum) are captured by a sensor array 1920 which generates signals supplied to a computer 1924. The computer executes algorithms to process the reflectance/fluorescence signals and responsively identifies and/or characterizes the microbial agent. In one embodiment, a report containing the identification or characterization result is sent to an output device 1926 (e.g., display or associated computer workstation, pager, cell phone or e-mail server). The results can include clinical gram type, direct identification of the microbial agent (e.g., to the genus or species level in a taxonomic hierarchy), or other clinical information regarding the microbial agent in the sample.

First Embodiment (Figures 31-56)

[0268] Sample removal apparatus and sampling from the specimen container (e.g., blood culture bottle 500) (Figures 31-35, 45-46, items 1910 and 1912)

[0269] A sample removal apparatus, in the form of a sample head 1912, retrieves a sampling device 1902 (disposable) from a cassette 1900 of such devices (Figures 31, 35). This sampling device 1902 (Figure 44, see also Figures 62, 63) may take the form of a sterile sheathed needle or other means to pierce a stopper or other closure member in the specimen container 500 and vent the specimen container (if necessary) so as to equilibrate the bottle pressure with atmospheric pressure. The sampling device (Figure 44, 62, 1902) includes a sampling container or chamber 3204 to hold the withdrawn test sample. The test sample will include a portion of the specimen sample and any culture media present. Another possible embodiment is that the sampling device contains the sterile sheathed needle and is directly connected to or incorporated into the separation device (i.e., a combined sampling and separation device, see Figures 90-108). The sample removal apparatus 1912 may optionally include features to decontaminate the surface of the bottle prior to sampling (if necessary).

[0270] The robotic transfer mechanism 1910 (Figure 35) can be moved in three mutually orthogonal translation axes in addition to one rotational axis around one of the orthogonal translation axes so as to be able to position the sample removal apparatus 1912 opposite the access point (e.g. stopper, or septum) of each specimen

container/bottle 500 while the bottle is held in the racks 2310 of the specimen container holder 1906. Alignment of a sheathed needle 3202 of the sampling device 1902 to the bottle access point can either be accomplished by a docking feature built-in to the container 500, a vision system (e.g., camera) or using pre-programmed
5 dimensional coordinates and precision motion controlling of the robot transfer mechanism 1910. The bottle 500 is preferably first tilted upward so that the space below the access point or stopper contains the headspace gases and not liquid media. The rationale for this step is that the container should first be vented so that the pressure in the bottle is close to atmospheric pressure. This would prevent venting of
10 aerosols from the bottle and excess fluid transfer and overflow and possible spillage in the case of a bottle over-pressure situation.

[0271] Similarly, if the culture has not produced significant by-products (e.g. headspace gases) or the microorganism is not a “gas producer”, there will be an under-pressure condition or the pressure inside the bottle will be below atmospheric
15 pressure which would make sampling difficult. The aseptic venting will equilibrate the pressure so that a fluid sample can be removed from the bottle.

[0272] After proper venting, the bottle 500 is tilted so that the access port of the bottle is oriented downwards and a liquid sample can be transferred to the sampling device 1902. The sampling device withdraws for example a 0.5 ml, 1.0, or
20 2.0 ml sample of blood/media from the specimen container. Alternatively, a positive displacement syringe like device could be developed to provide sampling of specimen containers over a wide range of vacuum or pressure conditions.

Optional lysis of components in the test sample

[0273] After the test sample has been withdrawn from the specimen container
25 500, any cellular components contained therein (e.g., blood cells) may need to be lysed so that they do not interfere with separation and identification/characterization processes described below. The optional lysis step can be performed using a lysis buffer (which is a pH balanced surfactant solution) or can be accomplished using
30 sonication. Both approaches cause disruption of the blood cell walls. The lysis operation can be performed by adding the lysis buffer to the disposable sampling device 1902 either off-line or within the identification and/or characterization instrument 104. Alternatively, the lysis buffer can be mixed with the blood/media sample during the loading of the sample into the separation device 1904. After the

lysis buffer and blood/media sample are combined, some amount of agitation or mixing needs to be performed to ensure the lysis buffer contacts the blood cells and cell wall rupture occurs. In one possible embodiment, the robotic transfer mechanism may move up and down or otherwise to accomplish this mixing. In another
5 embodiment, a mixing station (e.g., a vortexer as described in the second embodiment below) can be included in the instrument 104 for accomplishing this mixing.

[0274] As an alternative, the separation device 1904 could have two compartments separated by a thermoresponsive gel or other separation material that would allow the lysis buffer and the blood/media mixture to be combined, then pass
10 through into the microorganism separation device.

[0275] Another approach could incorporate a filter to collect the microorganisms on a surface and then resuspend the microorganisms into an inoculum for testing.

[0276] It is envisioned the multiple separation devices 1904 could be
15 provided in a format such as a cartridge, cassette, disk or strip to facilitate ease of user loading the system.

[0277] Separation and/or concentration station (Figures 31, 51, item
1916) and separation device (Figure 31, 36-41, 51, item 1904)

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[0278] After withdrawal of the specimen from the specimen container, and after optional lysing of the cellular components (e.g., blood cells) in the sampling device 1902, the sample is then injected or otherwise introduced into one of the separation devices 1904. A microbial agent present in the sample is separated from
25 other components and concentrated into a pellet or pellet-like mass within the separation device 1904.

[0279] The details of the separation and/or concentration of the microorganism in the separation device (1904) are described in related patent applications incorporated by reference into this application hereinabove, but the basic
30 method will be described below. The separation is accomplished using a density solution or density cushion filtration. In one embodiment, the separation device 1904 is preloaded with the density cushion. Separation and concentration occurs by means of centrifugation of the separation device 1904.

[0280] The separation device 1904 (Figures 36-41) itself can take the form of a capillary tube design with a 1 – 2 mm diameter internal cross section capillary tube filled with the density solution. Above this capillary tube region is a fluted structure that opens up (i.e., opens to a larger cross sectional area) to provide a reservoir for the blood/media sample and the density solution. The bottom surface of the separation device is made of a material that has very good ultraviolet and visible light transmission. The top of the structure has a lid that is applied before centrifugation. In alternative configurations the separation device could be illuminated from the side in which case the lower portion of the separation device is made from a material that has very good ultraviolet and visible light transmission; the cross-sectional shape of the capillary tube may be circular or square.

[0281] The mixed or lysed sample contents (lysis buffer and test sample) are loaded into the separation device 1904 (see Figures 50A-C and the description below) by means of injecting the sample from the sampling device 1902 into the separation device 1904. The density solution is either loaded into the separation device 1904 on-line in the identification and/or characterization instrument 104 or, more preferably the separation device 1904 is shipped pre-filled with the density solution. After the mixed or lysed sample is loaded into the separation device 1904 and the device 1904 capped, the separation device 1904 is loaded into a centrifuge 1916. Alternatively, this lid is configured with a septum. The sample can be added to the device 1904 by piercing the septum, preventing the need for lid removal and replacement. The centrifuge is activated and spun, e.g. for several minutes at high rpm. This action causes the microbial agent (which is not lysed) to pass through the density solution and concentrate at the base of the capillary tube in the separation device 1904 into a pellet or pellet-like mass in the very bottom of the tube (see Figure 40, concentrated microbial agent pellet 2804). In one embodiment, the device 1904 loaded with density cushion is centrifuged prior to loading of the test sample to remove any air bubbles or the like that may otherwise interfere with the separation and/or concentration step.

[0282] Identification/characterization module (read station) for microbiological identification and/or characterization (Figure 31, 51, 52 item 1918)

[0283] After the separation device 1904 has been centrifuged as described above, the centrifuge 1916 can be rotated so that the separation device 1904 is in a reading position wherein a identification and/or characterization module (read station) 1918 can interrogate the separated and/or concentrated microbial agent (Figure 40, pellet 2804). Alternatively, the separation device 1904 can be removed from the centrifuge by the robotic transfer mechanism 1910 and placed in a read station in a separate location.

[0284] In one form, the read station 1918 includes an optical reader assembly for interrogating the concentrated microbial agent (pellet) within the separation device 1904. Since the microorganism/microbial agent in the blood/media sample is forced to the bottom surface of the capillary tube in the separation device 1904 (see Figures 40 and 41), the microbial agent will be in contact with the bottom surface. In one possible implementation, the optical reader assembly observes the fluorescence signal (e.g., intrinsic fluorescence signal) emitted from the concentrated microbial agent due to illumination from an excitation lights source.

[0285] The fluorescence signal (e.g., intrinsic fluorescence) results from excitation by a UV, visible spectrum or IR light source (see Figure 41). The light sources could be continuum lamps such as a deuterium or xenon lamp for UV and/or a tungsten halogen lamp for visible/IR excitation. Since these light sources have a broad range of emission, the excitation band can be reduced using optical bandpass filters. Other methods for emission wavelength spectral width that may be utilized include an acousto-optic tunable filter, liquid crystal tunable filter, an array of optical interference filters, prism spectrograph, and still others. Alternatively, lasers are available in discrete wavelengths from the ultraviolet to the near infra-red; additionally many multiplexing methods are known to those skilled in the art,

[0286] Alternatively, light emitting diodes can be used as narrowband excitation light sources. LED's are available from a peak wavelength of 240 nm to in excess of 700 nm with a spectral width of 20-40 nm. The same methods for the reduction of spectral width can be incorporated with the LED's to improve discrimination between excitation and emission spectra.

[0287] The emission from the sample may be measured by any suitable means of spectral discrimination, most preferably employing a spectrometer. The spectrometer may be a scanning monochromator that detects specific emission wavelengths whereby the output from the monochromator is detected by a

photomultiplier tube and/or the spectrometer may be configured as an imaging spectrograph whereby the output is detected by an imaging detector array such as a charge-coupled device (CCD) detector array. In one embodiment, a discriminator allows the observation of the fluorescence and/or scattering signal by a photodetection means (such as a photomultiplier tube, avalanche photodiode, CCD detector array, a complementary metal oxide semiconductor (CMOS) area sensor array and/or electron multiplying charge coupled device (EMCCD) detector array (Figure 1, item 1920). An optical lens system (2904 in Figure 41) in front of the sensor array will magnify the 0.78 -2.0 mm² area forming the bottom of the capillary tube 2604 so that it fills the frame of the sensor array. Alternatively, coupling between the disposable separation device 1902 and the optical fiber is direct optical fiber coupling with no lens system; the optical fiber probe is a six around one configuration at the distal end, with the proximal end having a linear configuration for emission fibers to couple into the entry slit of a spectrometer. Fluorescence signal strength at several different wavelengths are acquired and saved in a computer memory.

[0288] An alternative configuration is to reduce the capillary tube 2604 to less than 1 mm in diameter to account for low biomass samples. Furthermore, the geometry of the capillary area may take other shapes, such as a rectangular-shaped internal cross-section. Another optional embodiment is to configure the reading of the capillary tube from the side instead of from the bottom. There are two possible benefits to doing so: (1) avoid debris or fibers that sediment to the base of the capillary tube and (2) provide the opportunity to optically identify the presence of polymicrobial agents. A rectangular shaped capillary tube may be preferred for this side read application.

[0289] The identification and/or characterization module 1918 includes a computer (Figure 31, item 1924) that operates on the fluorescence signal strength measurements which are stored in memory. The measurements are compared to experimentally-determined fluorescence spectra measurements for different types of microorganisms (i.e. Gram positive, Gram negative, yeast, etc.) that are also stored in memory. The computer executes a classification algorithm and generates a classification result for the microbial agent, e.g., gram classification, gram family, and species. In one configuration, further analysis of the spectra of the captured intrinsic fluorescence signal is accomplished so that species identification and/or characterization or at least the top three probabilities for species identification is

achieved. Details on the methods executed by the computer for identification and/or characterization are explained below.

[0290] The identification of Gram type, gram family and species could also be accomplished using a micro-Raman evaluation. Raman spectroscopy is a non-
5 contact technique where the sample is illuminated by laser radiation. The scattered light is either elastically or inelastically scattered by interaction with the molecules which comprise the microbial agent. The elastically scattered light is referred to as Rayleigh scattering and the inelastically scattered light is Raman scattering. Raman spectroscopy has been shown to be a potentially viable method of microorganism
10 identification and/or characterization by examination of the vibrational spectra of the microorganism.

[0291] The laser illumination and scattering collection optics are designed to focus the beam to a near-diffraction limited spot size. This size ensures adequate laser signal on the microbe since Raman scattering is very inefficient. The collection
15 optics are designed to efficiently capture scattered light and couple it into an optical spectrometer for analysis. The Raman signal can be acquired at one or more locations and the subsequent signal averaged.

[0292] Once the Raman spectra are obtained, it is analyzed for location and strength of key peaks in the spectra. This is compared to a stored reference data set of
20 known microorganisms so that Gram type, morphological information and species identification can be obtained. The reference data set from known microorganisms can be obtained in the same instrument using the same methods and reading instrumentation.

[0293] The methods used for identification are described in greater detail in
25 the co-pending applications filed on October 2009 cited and incorporated by reference herein at the beginning of this document, and the reader is directed to such patent applications for further details. The methods using intrinsic fluorescence and a taxonomic hierarchical classification method are also explained in detail below.

[0294] Disposal of sampling device 1902 and separation device 1904
30 (Figures 31, 53, item 1908)

[0295] After the test sample is injected from the sampling device 1902 into the separation device 1904, the sampling device 1902 is discarded into a biowaste container 1908 within the identification and/or characterization instrument 104. After the reading of the separation device 1904, the separation device 1904 is also discarded

in the biowaste container 1908. The biowaste container is periodically removed from the identification/characterization instrument and emptied, and then replaced into the identification/characterization instrument.

5 User Interface

[0296] The identification instrument 104 preferably includes a user interface (not shown) which provides an operator with status information regarding specimen containers loaded into the identification instrument. The user interface may include some or all of the following features:

- 10 • Touch screen display
- Keyboard on touch screen.
- System status
- Positives alert
- Communications to other systems (DMS, LIS, BCES & other detection or
- 15 identification Instruments).
- Specimen Container status
- Retrieve specimen containers
- Visual and audible Positive Indicator
- USB access (back ups and external system access).
- 20 • Remote Notification of Identification and/or Characterization Results, System Status and Error Messages

[0297] The particular appearance or layout of the user interface is not particularly important.

[0298] The results are sent to an output device 1926 (Figure 31), which may

25 be a computer memory, instrument display, printer, pager, cell phone, personal digital assistant, e-mail server, or other device. The results will typically include one or more of the following: clinical gram type of the microbial agent, identification and/or characterization of the species of the microbial agent, or other clinical information.

30 Specimen Container 500

[0299] The specimen container 500 shown in Figure 31 is designed to hold and contain a sample and may take the form of a standard culture bottle, e.g., blood culture bottle. Preferred embodiments of the bottle incorporate a bar code (Figure 31) for automated reading of the bottle 500 within the identification/characterization

instrument 104 or off-line equipment. The bottle 500 includes a stopper (not shown) sealing the container from the environment having a pierceable septum. Optionally, where the bottle is used for both detection and automated identification, the bottle includes a colorimetric sensor formed or placed in the bottom of the bottle for purposes of colorimetric detection of the presence of microbial growth in the bottle 500. Specimen containers of the type shown in Figure 1 are well known in the art and described in the patent literature cited in the Background section of this document, therefore a further description is unnecessary.

[0300] The configuration of the bottle is not particular important and the inventive system and methods can be adapted to a variety of containers for containing a sample. Thus, the present description of blood culture specimen containers is offered by way of example and not limitation.

II. Detailed Description of First Embodiment (Figures 31-56)

[0301] Figure 32 shows one possible configuration of the identification/characterization instrument 104, including the cassette of disposables 1900, a rack or holder 1906 for positive specimen containers, a waste container 1908, a robotic transfer mechanism 1910, a sample removal apparatus 1912 which is attached or otherwise coupled to the robotic transfer mechanism 1910, a separation and concentration station 1916, and the identification and/or characterization module 1918. Figure 33 is a top plan view of the arrangement of Figure 32. The holder 1906 includes three racks that are oriented in one position for incubation and receiving new positive specimen containers, e.g., from a remote detection instrument or a manual loading door. In Figure 34, the racks are moved to a position for sample removal from the specimen containers, and loading of the sample into the separation device 1904.

[0302] Figure 35 is a perspective view of the identification/characterization instrument in the position of Figure 34, showing the identification/characterization instrument 104 in further detail. The holder 1906 includes three separate racks 2310, each holding twenty specimen containers 500. The racks 2310 are rotatable as a unit about the horizontal axis to tilt the specimen containers into upward orientation (shown in Figure 35) for purposes of venting the specimen containers and to a downward orientation (see Figure 45) for sample removal.

[0303] The robotic transfer mechanism 1910 includes vertical guide rails 2320 and a horizontal guide rail 2324. The sample removal apparatus 1912 is moved from left to right and up and down by means of collars connected to the guide rails and a motor and belt driving subassembly (not shown, but conventional). Thus, the sample removal apparatus 1912 can move to any of the bottle positions in the three racks 2310, when the specimen containers are in either the upward or downward orientation. The sample removal apparatus 1912 can further move fore and aft by sliding along the guides 2322.

[0304] Figure 35 also indicates that the instrument 104 includes electronics 2300, which includes a computer 1924 for processing fluorescence measurements, a memory 2302 storing results of the analysis and a further memory or processing units 2304 for storing program code for operation of the identification/characterization instrument 104. The electronics 2300 are preferably located behind suitable panels, which are not shown.

Cassette of disposables

[0305] Figure 35 shows a cassette 1900 of disposable devices which is loaded into the identification/characterization instrument 104. The cassette 1900 includes a multitude of sampling devices 1902 and separation devices 1904.

[0306] The separation device 1904 is shown in Figures 36-41. Referring to these Figures, the separation device consists of a body 2402 that defines a reservoir 2602 and a capillary tube 2604 which is connected to the reservoir 2602. The body 2402 defines an axis 2608 and the capillary tube 2604 is oriented along the axis 2608. A first end of the capillary tube 2610 is connected to the reservoir 2602 and the second end 2612 of the capillary tube communicates with a tube portion 2702 of an end piece 2502. The reservoir is accessed via a removable cap 2404 that threads onto threads 2502 formed at the top portion of the body 2402. The lower portion of the body 2402 is closed off by an end piece 2502 which is affixed to the body by means of a ridge 2704 fitting into a corresponding recess 2606 in the body and welding or use of an adhesive. The bottom wall 2506 of the end piece 2502 is of reduced thickness as indicated in Figure 38. The end piece incorporates a capillary tube 2702 which is aligned with the capillary tube 2604 of the body 2402. The body 2402 proximate to the second end of the capillary tube is made from an optically transparent material; in the embodiment of Figure 37 the end piece 2502 is optically

transparent for facilitating optical interrogation of the concentrated microbial agent 2804 located at the bottom of the capillary tube 2604. The separation device 1904 is loaded with a density solution or “cushion” 2802 (Figure 40), either preloaded with the material or less preferably the material is added to the separation device within the identification/characterization instrument .

[0307] Figures 42 and 43 show an embodiment of the separation device 1904 in which the body of the separation device 1904 is a one-piece construction. Walls 3002 provide support for the lower portion of the capillary tube 2602. The body proximate to the lower portion of the capillary tube 2604 is made from an optically transparent material.

[0308] Figure 41 shows the operation of interrogation of concentrated microbial agent 2804 within the separation device 1904, an operation performed by the identification module 1918 of Figures 31 and 35. Light from a light source passes along an optical fiber 2902 and is directed by lens system 2904 to the base of the separation device 1904. The light stimulates the production of fluorescence from the microbial agent 2804 and the fluorescence is directed via the optical fiber 2906 through the lens 2904 and fiber 2902 to a spectral dispersion system in the identification module 1918 (Figure 31) to the sensor array (1920, Figure 31).

[0309] The sampling device 1902 is shown schematically and parts not to scale in Figure 44. The device 1902 can take the form of a syringe-like device having a body 3200 defining a chamber 3204 and a sheathed needle 3202. The chamber 3204 may be pre-loaded with a selective lysis buffer 3206. The top of the chamber 3204 is sealed. The chamber may have a port 3208 which allows the sampling device to be connected to a vacuum or pneumatic unit to facilitate venting or sampling of a sample from the bottle 500. The lysis buffer 3206 can be pre-loaded into the sampling device 1902, or it may be loaded into the device 1902 in the instrument at the time of use.

[0310] In one embodiment, the lysis buffer loaded into the sampling device 1902 may be tailored to the specie(s) expected to be found. In one possible configuration, several reservoirs of selective lysis buffers are present in the instrument 104 and one of the lysis buffers is loaded into the sampling device at the time of use which is selected to be most optimal for the sample contained in a given specimen container. Additionally, the sampling can be repeated with different sampling devices each containing a different selective lysis buffer.

Sample Removal Apparatus (Sampling Head) 1912

[0311] The sample removal apparatus 1912 of Figures 31 and 35 operates to remove a portion of the biological sample in the positive detection container 500 from the detection container 500 and add the portion to a separation device 1904 obtained from the supply of separation devices 1900. The physical configuration of the sample removal apparatus 1912 can take a variety of forms, depending on the configuration of the specimen containers, the sampling device, and the separation device. In the illustrated embodiment the sample removal apparatus 1912 takes the form of articulating fingers that open and close so as to grasp the sampling device 1902 and the separation device 1904. The sample removal apparatus 1912 is moved to the required position for sampling and loading into the separation device by means of operation of the robotic transfer mechanism 1910.

Venting and sampling

[0312] With reference to Figure 45, the sample removal apparatus 1912 is moved to a position where it is placed directly over one of the sampling devices 1902 in the cassette 1900. The fingers of the sample removal apparatus 1912 grip the sampling device 1902 and the apparatus 1912 is raised upwards, removing the sampling device 1902 from the cassette 1900. As shown in Figure 46, the specimen containers 500 are tilted upwards. The stopper at the top of the bottle is sterilized using UV light or a disinfecting agent (e.g., bleach or alcohol). As shown in Figure 47, the bottle is vented by introducing the needle 3202 (Figure 44) of the sampling device through a pierceable septum in the stopper of the bottle 500, equalizing the pressure within the interior of the bottle to that of ambient conditions. The port 3208 of the sampling device may be connected to the pneumatic system (1914, Figure 31) during this process, e.g., a rolling diaphragm pump 1710 as shown in the second embodiment below.

[0313] As shown in Figures 18 and 19, the racks 2310 are then rotated to the downward orientation. The sample removal apparatus 1912, in conjunction the pneumatic system, withdraws a test sample (i.e., a portion of the specimen sample) from the bottle 500 into the sampling device 1902.

Lysis

[0314] The sampling device 1902 is optionally loaded with approximately 1 ml of a lysis buffer 3206 (Figure 44). In this embodiment, an approximately 2 ml test sample is removed from the bottle 500 and mixed with the lysis buffer in the sampling device 1902, e.g., by agitation of the device 1902 after loading of the test sample into the sampling device 1902. The lysis operation is selective to non-microorganism components, e.g., blood cells, i.e., the microbial agent cells are not lysed.

[0315] The lysis buffer 3206 selectively lyses undesired cells (i.e., non-microorganism cells) that may be present in the sample, e.g., blood cells and/or tissue cells. The selective lysis of non-microorganism cells permits separation of microorganisms from other components that may be present in the sample. Accordingly, the lysis solution is one that is capable of selectively lysing cells, e.g., non-microorganism cells (e.g., by solubilizing eukaryotic cell membranes). The lysis solution may comprise one or more detergents, one or more enzymes, or a combination of one or more detergents and one or more enzymes.

[0316] Useful detergent may include one or more non-denaturing lytic detergent, such as Triton[®] X-100 Triton[®] X-100-R, Triton[®] X-114, NP-40, Genapol[®] C-100, Genapol[®] X-100, Igepal[®] CA 630, Arlasolve[™] 200, Brij[®] 96/97, CHAPS, octyl β -D-glucopyranoside, saponin, and nonaethylene glycol monododecyl ether (C12E9, polidocanol). Optionally, denaturing lytic detergents can be included, such as sodium dodecyl sulfate, N-laurylsarcosine, sodium deoxycholate, bile salts, hexadecyltrimethylammonium bromide, SB3-10, SB3-12, amidosulfobetaine-14, and C7BzO. Optionally, solubilizers can also be included, such as Brij[®] 98, Brij[®] 58, Brij[®] 35, Tween[®] 80, Tween[®] 20, Pluronic[®] L64, Pluronic[®] P84, non-detergent sulfobetaines (NDSB 201), amphipols (PMAL-C8), and methyl- β -cyclodextrin. In one embodiment, polyoxyethylene detergent detergents may be preferred. The polyoxyethylene detergent can comprise the structure C₁₂₋₁₈/E₉₋₁₀, wherein C₁₂₋₁₈ denotes a carbon chain length of from 12 to 18 carbon atoms and E₉₋₁₀ denotes from 9 to 10 oxyethylene hydrophilic head groups. For example, the polyoxyethylene detergent can be selected from the group consisting of Brij[®] 97, Brij[®] 96V, Genapol[®] C-100, Genapol[®] X-100, nonaethylene glycol monododecyl ether (polidocanol), or a combination thereof. ethylenediaminetetraacetic acid (EDTA)

[0317] The lysis solution may also comprise one or more enzymes. Enzymes that can be used in the lysis solutions include, without limitation, enzymes that digest

nucleic acids and other membrane-fouling materials (*e.g.*, proteinase XXIII, DNase, neuraminidase, polysaccharidase, Glucanex[®], and Pectinex[®]).

[0318] In another embodiment, one or more additional agents can be used, including for example, reducing agents such as 2-mercaptoethanol (2-Me) or
5 dithiothreitol (DTT), stabilizing agents such as magnesium, pyruvate, and humectants, and/or chelating agents such as ethylenediaminetetraacetic acid (EDTA). The lysis solution can be buffered at any pH that is suitable to lyse the desired cells, and will depend on multiple factors, including without limitation, the type of sample, the cells to be lysed, and the detergent used. In some embodiments, the pH can be in a range
10 from about 2 to about 13, *e.g.*, about 6 to about 13, *e.g.*, about 8 to about 13, *e.g.*, about 10 to about 13. Suitable pH buffers include any buffer capable of maintaining a pH in the desired range, *e.g.*, about 0.05 M to about 1.0 M CAPS.

Dispense into separation device 1904 and separation/concentration

15 [0319] As shown in Figure 20A and 20B, the sample removal apparatus 1912 carries the sampling device 1902 (loaded with a mixed lysis buffer and sample solution) to the position of one of the separation devices 1902 in the cassette 1900. The sample removal apparatus pierces the cap of the separation device 1904 with the
20 needle 3202 of the sampling device 1902 and injects 0.5 to 1.0 ml of the test sample + lysis buffer mixture into the reservoir of the separation device 1904. The dispensing could also be performed after uncapping the separation device 1904 and recapping the separation device 1904 after recapping. The sample removal apparatus then transfers the sampling device 1902 to the waste container 1908 as shown in Figure 50C and deposits it into the waste container.

25 [0320] In one embodiment, the separation is carried out by a centrifugation step in which the sample (*e.g.*, a lysed sample) is placed on top of an approximately 1 ml liquid phase density cushion 2802 (Figure 40) previously loaded in the separation device 1904 and the device 1904 is centrifuged under conditions (*e.g.*, 10,000 *g*) which allow the microorganisms to be isolated and concentrated (*e.g.*, the
30 microorganisms form a pellet or pellet-like mass at the bottom and/or sides of the separation device 1904). "Density cushion" refers to a solution having a homogenous density throughout. The density of the cushion is selected such that the microorganisms in the sample pass through the cushion while other components of the

sample (*e.g.*, blood culture broth, cell debris) remain on top of the cushion or do not pass all of the way through the density cushion.

[0321] The material for the density cushion 2802 can be any material that has the appropriate density range for the methods of this invention. In general, the density of the cushion is in the range of about 1.025 to about 1.120 g/ml. In one embodiment, the material is colloidal silica. The colloidal silica may be uncoated (*e.g.*, Ludox[®] (W.R. Grace, CT)) or coated, *e.g.*, with silane (*e.g.*, PureSperm[®] (Nidacn Int'l, Sweden) or Isolate[®] (Irvine Scientific, Santa Ana, CA)) or polyvinylpyrrolidone (*e.g.*, Percoll[™], Percoll[™] Plus (Sigma-Aldrich, St. Louis, MO)). The colloidal silica may be diluted in any suitable medium to form the proper density, *e.g.*, balanced salt solutions, physiological saline, and/or 0.25 M sucrose. Suitable densities can be obtained with colloidal silica at a concentration of about 15% to about 80% v/v, *e.g.*, about 20% to about 65% v/v. Another suitable material for density cushions is an iodinated contrast agent, *e.g.*, iohexol (Omnipaque[™] NycoPrep[™], or Nycodenz[®]) and iodixanol (Visipaque[™] or OptiPrep[™]). Suitable densities can be obtained with iohexol or iodixanol at a concentration of about 10% to about 25% w/v. Sucrose can be used as a density cushion at a concentration of about 10% to about 30% w/v *e.g.*, about 15% to about 20% w/v, for blood culture samples. Other suitable materials that can be used to prepare the density cushion include low viscosity, high density oils, such as microscope immersion oil (*e.g.*, Type DF; Cargille Labs, New York), mineral oil (*e.g.*, Drakeol[®] 5, Draketex 50, Penetec[®]; Penreco Co., Pennsylvania), silicone oil (polydimethylsiloxane), fluorosilicone oil, silicone gel, metrizoate-Ficoll[®] (LymphoPrep[™]), *e.g.*, at a concentration of about 75% to about 100% for blood culture samples, diatrizoate-dextran (PolymorphaPrep[™]), *e.g.*, at a concentration of about 25% to about 50% for blood culture samples, carboxymethyl cellulose, hydroxypropylmethyl cellulose, polyethylene oxide (high molecular weight), Pluronic[®] F127, Pluronic[®] F68, mixtures of Pluronic[®] compounds, polyacrylic acid, cross-linked polyvinyl alcohol, cross-linked polyvinyl pyrrolidine, PEG methyl ether methacrylate, pectin, agarose, xanthan, gellan, Phytigel[®], sorbitol, Ficoll[®] (*e.g.*, Ficoll[®] 400 at a concentration of about 10% to about 15% for blood culture samples), glycerol, dextran (*e.g.*, at a concentration of about 10% to about 15% for blood culture samples), glycogen, cesium chloride (*e.g.*, at a concentration of about 15% to about 25% for blood culture samples), perfluorocarbon fluids (*e.g.*, perfluoro-n-octane), hydrofluorocarbon fluids (*e.g.*, Vertrel XF), and the like as are well known in the art.

In one embodiment, the density cushion is selected from one or more of colloidal silica, iodixanol, iohexol, cesium chloride, metrizoate-Ficoll[®], diatrizoate-dextran, sucrose, Ficoll[®] 400, and/or dextran in any combination. The density cushion can also be made up of a combination of materials, *e.g.*, a combination of colloidal silica and oil.

Transfer to Separation and concentration station (Centrifuge)

[0322] As shown in Figure 51, after loading of the separation device 1904 with the mixed or lysed test sample, the sample removal apparatus 1912 retrieves the loaded separation device 1904, lifts it out of the cassette 1900, and moves the separation device 1904 to the centrifuge 1916. The separator 1904 is then placed into a holder or loading position of the centrifuge 1916.

[0323] A separation and concentration of the microbial agent in the sample occurs within the separation device 1904 using the centrifuge 1916.

[0324] The separation step can be carried out to separate the microorganisms from other components of the sample (*e.g.*, non-microorganisms or components thereof) and to concentrate the microorganisms into a pellet that can be interrogated for identification and characterization purposes. The separation does not have to be complete, *i.e.*, it is not required that 100% separation occur. All that is required is that the separation of the microorganisms from other components of the sample be sufficient to permit interrogation of the microorganisms without substantial interference from the other components.

[0325] The centrifuge spins the separation device 1904 at high speed in order to concentrate the microbial agent into the bottom of the capillary tube within the separation device 1904. The combination of the action of the lysis buffer on the non-microorganism cells (*e.g.*, blood cells), the presence of the density solution within the separation device 1904, and the centrifugation, results in the separation of microbial agent from the lysed blood/broth mixture and the concentration of the microbial agent into a pellet or pellet-like mass in the bottom of the capillary tube, as shown in Figure 40 and 41.

[0326] In one embodiment, the separation device 1904 is centrifuged in station 1916 using a swing out rotor so that the microorganisms form a pellet directly on the bottom of the separation device 1904 (in the bottom of the capillary tube shown in Figures 8, 10 and 13). The separation device 1904 is centrifuged at a

sufficient acceleration and for a sufficient time for the microorganisms to be separated (e.g., a pellet formed) from other components of the sample. The centrifugation acceleration can be about 1,000 x g to about 20,000 x g, e.g., about 2,500 x g to about 15,000 x g, e.g., about 7,500 x g to about 12,500 x g, etc. The centrifugation time can
5 be about 30 seconds to about 30 minutes, e.g., about 1 minute to about 15 minutes, e.g., about 1 minute to about 5 minutes.

Reading

[0327] The identification and/or characterization module (read station 1918),
10 which is shown positioned adjacent to the centrifuge then interrogates the concentrated microbial agent using fluorescence spectroscopy (e.g., intrinsic fluorescence and/or diffuse reflectance), Raman spectroscopy or other optical technique. In other embodiments, the microorganisms in the pellet can be interrogated using mass spectrometry techniques, such as MALDI-TOF mass
15 spectrometry, desorption electrospray ionization (DESI) mass spectrometry, GC mass spectrometry, LC mass spectrometry, electrospray ionization (ESI) mass spectrometry and Selected Ion Flow Tube (SIFT) spectrometry. As shown in Figure 22, the identification and/or characterization module 1918 may be physically located proximate to the centrifuge 1916, in which case the separation device 1904 does not
20 need to be moved further by the robotic transfer mechanism. Alternatively, the identification and/or characterization module 1918 could be located in a different location within the identification/characterization instrument and the robotic transfer mechanism operates to move the separation device to the location of the identification and/or characterization module 1918.

25

Transfer to Waste

[0328] After reading, as shown in Figure 23, the robotic transfer mechanism 1910 and sample removal apparatus 1912 operates to lift the separation device 1904 from the centrifuge 1916, transfers the separation device 1904 laterally and places it
30 in the waste container 1908. Figure 24 shows the waste container 1904 containing a multitude of the sampling devices 1902 and the separation devices 1908. When the waste container 1908 is full it is removed from the instrument and then replaced with an empty waste container. Prior to disposal of the separation device, a photographic image of the lower region of the separation device may be taken with a camera (not

shown) to verify the separation process and provide valuable information on the identity of the isolate, such as pellet size, shape, color and density.

External processing of concentrated microbial agent

5 [0329] While in the above embodiment the concentrated microbial agent is interrogated while it is still located within the separation device 1904, it is possible to remove the concentrated microbial agent from the separation device and test it directly to identify and/or characterize the microbial agent.

10 [0330] In this variation, referring to Figure 55, the separation device 1904 is transferred to a removal device or station 4302. At the station 4302, the cap 2404 of the separation device 1904 is removed and the concentrated microbial agent 2804 is removed from the separation device 1904. The microbial agent is then subject to one or more additional tests. In one possible configuration, the microbial agent is supplied to a molecular diagnostic test unit 4310 which may include a disposable test
15 strip or the like and processing instrument for identification of the agent. Alternatively, a sample of the microbial agent could be applied to a MALDI mass spectrometry plate 4314 and the plate inserted into a mass spectrometry unit 4312. Alternatively, the microbial agent could be delivered to a microbial identification and/or characterization test device 4318 (e.g., test card) and the card incubated and
20 tested in a processing instrument 4316.

III. Method of Operation

A. Flow chart (Figures 56A, B, C)

25 [0331] The method of operation of the identification/characterization instrument 104 in an embodiment in which the specimen container 500 is subject to both detection and identification steps will now be described with reference to Figures 56A -56C.

30 [0332] The process starts at step 4402 with the loading of a sample into one of the containers 500 and delivery of the loaded container 500 to a detection instrument (as described above in conjunction with Figures 1-30). See Figure 77, instrument 102.

 [0333] At step 4404, the container 500 is loaded into the detection instrument 102, e.g., by placing the detection container on conveyer which delivers the container

to the detection instrument or by manually loading the container. (See Figures 77 and 78 and the description of those figures below)

[0334] At step 4406, the container 500 is incubated within the detection instrument 102.

5 [0335] At step 4408 the detection container is read (by a detection unit in the instrument 102).

[0336] At step 4410, the reading of the detection container is analyzed to determine if the container is positive. If no, the processing branches along NO branch 4411 and a check is made if a timer has expired (step 4412). If the timer has
10 expired, the bottle is deemed negative and the bottle is transferred to the waste container at step 4414. Otherwise, the incubation continues and steps 4406, 4408 and 4410 continue periodically.

[0337] If at step 4410 the detection container is positive, the processing proceeds to the YES branch 4416. The detection container is moved to the exit
15 location in the detection instrument at step 4418. At step 4420 the detection container is transferred to the identification/characterization instrument 104, e.g., by moving the detection container 500 onto a conveyor and moving it into the entrance location of the identification/characterization instrument (see Figure 77). The transfer could occur by some other manner, the details of which can vary widely.

20 [0338] At step 4422 (Figure 56B), the detection container is placed into one of the racks 2310 of the identification/characterization instrument 104. The robotic transfer mechanism 1910 may be used in this process.

[0339] At step 4424, the detection container is aseptically vented. This step may occur prior to picking up of the sampling device or may occur after picking up
25 the sampling device, see Figures 15 and 16.

[0340] At step 4426, one of the sampling devices 1902 is picked up from the cassette 1900. The sampling device 1902 is pre-loaded with a selective lysis buffer as shown in Figure 15; alternatively the lysis buffer is added to the sampling device at this time.

30 [0341] At step 4428, a protective cap (not shown), if fitted, covering the needle 3202 of the sampling device is removed.

[0342] At step 4430, the needle 3202 is inserted into a upright vented container 500 (see Figures 16 and 17).

[0343] At step 4432, the detection container is inverted (see Figures 48 and 49) and a small sample (e.g., a 2.0 ml sample) is removed from the container 500.

[0344] At step 4434, the container 500 is rotated to an upright orientation and the needle 3202 of the sampling device 1902 is removed.

5 [0345] At step 4436, a small volume (e.g., 0.5 ml sample) of air is introduced into the sampling device. This could be accomplished automatically using the pneumatic system 1914 connected to the sampling device.

[0346] At step 4438, a protective cap for the needle 3202, if fitted, is replaced.

10 [0347] At step 4440, the sampling device 1902 is inverted and agitated to thoroughly mix the test sample with the selective lysis buffer.

[0348] At step 4442, the protective cap for the needle 3202, if fitted, is again removed. (Note: a station fitted with appropriate gripping or grasping features could be provided for automatically removing and replacing the cap of the needle or
15 alternatively the cap could remain on the needle as described in the second embodiment)

[0349] At step 4444, a small portion of the positive broth/lysis buffer mix is discarded into a waste container.

[0350] At step 4446, the sample removal apparatus moves the sampling
20 device 1902 to the position above one of the separation devices 1904 (see Figure 76) and pierces the cap with the needle of the sampling device. The separation device 1904 is pre-loaded with the density cushion in this embodiment.

[0351] In one possible variation, the lysis buffer is also loaded into the separation device 1904 with the density cushion, and the mixing of the sample and the
25 lysis buffer takes place within the separation device 1904.

[0352] At step 4448, the sample removal apparatus 1912 gently adds 0.5 to 1.0 ml of the sample/lysis buffer mixture (i.e., lysed sample) on top of the density cushion already present in the reservoir of the separation device 1904. See Figure 50A and 50B.

30 [0353] At step 4450, the sample removal apparatus 1912 is moved to the position of the waste container 1908 and the sampling device 1902 is discarded. See Figure 50C.

[0354] At step 4452, the sample removal apparatus returns to the separation device 1904 and picks it up out of the cassette 1900 and moves it to the location of the

separation and concentration station 1916, and places the separation device 1904 into the centrifuge. See Figure 51.

[0355] At step 4454, the centrifuge cycle is started.

5 [0356] At step 4456, after completion of the centrifugation process, the separation device is moved to the identification and/or characterization module 1918 (reading station). Where the reading station is proximate to the centrifuge, the centrifuge is rotated to a reading position wherein the separation device 1904 is positioned for reading as shown in Figure 11.

10 [0357] At step 4458, the optical scan of the separation device 1904 in the identification and/or characterization module is started (See Figure 51, 52).

[0358] At step 4460, after completion of the reading operation, the separation device 1904 is placed into the waste container 1908 (see Figures 53, 54).

15 B. [0359] Interrogation step 4458, and the Identification and/or characterization of microorganisms in identification module 1918

[0360] Once the microorganisms present in the sample have been isolated and/or pelleted in the separation device 1904, the isolated sample or pellet can be interrogated (e.g., spectroscopically) to characterize and/or identify the microorganisms in the sample or pellet in step 4458. The interrogation can take place
20 in a non-invasive manner, that is, the pellet can be interrogated while it remains in the separation device 1904. The ability to identify the microorganisms in a non-invasive manner, optionally coupled with keeping the container sealed (e.g., hermetically sealed) throughout the separation and characterization/identification process and automating the procedure avoids the constant handling of contaminated and/or
25 infectious samples and greatly increases the safety of the entire process. Furthermore, the ability to characterize and/or identify microorganisms by direct interrogation without further processing of the sample or pellet (e.g., resuspension, plating, and growth of colonies), greatly increases the speed with which identification/characterization can be made.

30 [0361] In one embodiment, optical spectroscopic methods can be used to analyze one or more intrinsic properties of the microorganisms, e.g., a property present within the microorganism in the absence of additional agents, such as stains, dyes, binding agents, *etc.* In other embodiments, the optical spectroscopic methods can be used to analyze one or more extrinsic properties of the microorganisms, e.g., a

property that can only be detected with the aid of additional agents. The interrogation can be carried out using, for example, fluorescence spectroscopy, diffuse reflectance spectroscopy, infrared spectroscopy, terahertz spectroscopy, transmission and absorbance spectroscopy, Raman spectroscopy, including Surface Enhanced Raman Spectroscopy (SERS), Spatially Offset Raman spectroscopy (SORS), transmission Raman spectroscopy, and/or resonance Raman spectroscopy or combination thereof.

[0362] The spectroscopic interrogation can be carried out by any technique known to those of skill in the art to be effective for detecting and/or identifying one or more intrinsic or extrinsic properties of microorganisms. For example, front face fluorescence (where the exciting and emitted light enters and leaves the same optical surface, and if the sample is generally optically thick, the excitation light penetrates a very short distance into the sample (*see, e.g.,* Eisinger, J., and J. Flores, "Front-face fluorometry of liquid samples," *Anal. Biochem.* 94:15 (1983)) can be used for identification of microorganisms in pellets. Other forms of measurement, such as epifluorescence, reflectance, absorbance, and/or scatter measurements, can also be employed in step 4458.

[0363] Typically, the light source, or excitation source, results in the excitation of the sample, followed by measurement of the emission of fluorescence of the sample at predetermined time points or continuously. Similarly, the reflected light from interaction of the excitation source with the sample may be measured to provide pertinent data for identification and/or characterization. The emission from the sample may be measured by any suitable means of spectral discrimination, most preferably employing a spectrometer.

[0364] In a presently preferred embodiment, control measurements (e.g., fluorescence spectra) are taken for known microorganisms, thus allowing for correlation of measured test data with characterization of the microorganisms of interest using various mathematical methods known to those skilled in the art. The measured test data from known microorganisms is stored in machine-readable memory, e.g., within the instrument 104 itself or within an associated data processing device, such as connected workstation. For example, the data from samples being tested by the instrument 104 may be compared with the baseline or control measurements utilizing software routines known to or within the ability of persons skilled in the art to develop. More particularly, the data may be analyzed by a number of multivariate analysis methods, such as, for example, General Discriminant

Analysis (GDA), Partial Least Squares Discriminant Analysis (PLSDA), Partial Least Squares regression, Principal Component Analysis (PCA), Parallel Factor Analysis (PARAFAC), Neural Network Analysis (NNA) and/or Support Vector Machine (SVM). These methods may be used to classify unknown microorganisms of interest
5 in the sample being tested into relevant groups (e.g., species) based on existing nomenclature, and/or into naturally occurring groups based on the organism's metabolism, pathogenicity and/or virulence in designing the system for monitoring, detecting and/or characterizing the organism as described previously.

[0365] To enhance Raman (SERS) and fluorescence signals, microorganisms
10 could either be coated with gold and/or silver nanoparticles prior to centrifugation, and/or the inner optical surface could be pre-coated with metal colloids of particular size and shape (refs: Lakowicz, *Anal. Biochem.* 337:171 (2005) for fluorescence; Efrima *et al.*, *J. Phys. Chem. B. (Letter)* 102:5947 (1998) for SERS). In another embodiment, the nanoparticles are present in the density cushion prior to
15 centrifugation and associate with microorganisms as the microorganisms pass through the density cushion.

[0366] The sample illumination source (See Figure 41), or excitation source, may be selected from any number of suitable light sources as known to those skilled in the art. Any portion of the electromagnetic spectrum that produces usable data can
20 be used. Light sources capable of emission in the ultraviolet, visible and/or near-infrared spectra, as well as other portions of the electromagnetic spectrum, can be utilized and are known to those skilled in the art. For example, light sources may be continuum lamps such as a deuterium or xenon arc lamp for generation of ultraviolet light and/or a tungsten halogen lamp for generation of visible/near-infrared excitation.
25 These light sources provide a broad emission range and the spectral bandwidth for specific excitation wavelengths may be reduced using optical interference filters, prisms and/or optical gratings, as are well known in the art.

[0367] Alternatively, a plurality of narrowband light sources, such as light emitting diodes and/or lasers, may be spatially and/or temporally multiplexed to
30 provide a multi-wavelength excitation source. For example, light emitting diodes are available from 240 nm to in excess of 900 nm and the sources have a spectral bandwidth of 20-40 nm (full width at half maximum). Lasers are available in discrete

wavelengths from the ultraviolet to the near-infrared and can be employed using multiplexing methods well known to those skilled in the art.

[0368] The spectral selectivity of any of the light sources may be improved by using spectral discrimination means such as a scanning monochromator. Other methods of discrimination may be utilized, as known to those of skill in the art, such as an acousto-optic tunable filter, liquid crystal tunable filter, an array of optical interference filters, prism spectrograph, *etc.*, and in any combination. A consideration in selecting the spectral discriminator takes into the account the range of tunability as well as the level of selectivity. By way of illustration, for example, a discriminator might utilize the wavelength range of 300 – 800 nm with a selectivity of 10 nm. These parameters generally determine the optimum technology necessary to achieve the tunability range as well as the selectivity.

[0369] Typically, the light source results in the excitation of the sample, followed by measurement of the emission of fluorescence of the sample at predetermined time points or continuously. Similarly, the reflected light from interaction of the excitation source with the sample may be measured to provide pertinent data for detection and/or characterization.

[0370] The emission from the sample may be measured by any suitable means of spectral discrimination, most preferably employing a spectrometer. The spectrometer may be a scanning monochromator that detects specific emission wavelengths whereby the output from the monochromator is detected by a photomultiplier tube and/or the spectrometer may be configured as an imaging spectrograph whereby the output is detected by an imaging detector array such as a charge-coupled device (CCD) detector array. In one embodiment, a discriminator allows the observation of the fluorescence and/or scattering signal by a photodetection means (such as a photomultiplier tube, avalanche photodiode, CCD detector array, and/or electron multiplying charge coupled device (EMCCD) detector array).

[0371] The spectroscopic technique is used to obtain measurements that are preferably provided as Excitation-Emission Matrix (EEM) measurements. As used herein, EEM is defined as the luminescent spectral emission intensity of fluorescent substances as a function of both excitation and emission wavelength, and includes a full spectrum or a subset thereof, where a subset may contain a single or multiple excitation/emission pairs(s). Additionally, a cross section of the EEM with a fixed excitation wavelength may be used to show the emission spectra for a specific

excitation wavelength, and a cross section of the EEM with a fixed emission wavelength may be used to show the excitation spectra for a sample. In one embodiment, multiple EEMs are measured at more than one specific excitation-emission wavelength pair, *e.g.*, at least at 2, 3, 4, 5, 6, 7, 8, 9, 10, or more specific
5 excitation-emission wavelength pairs.

[0372] It has been found that a front-face fluorescence spectroscopy provides an advantage in measuring the fluorescence and/or reflectance properties of highly scattering and highly quenching samples. In one embodiment, the front-face method may be particularly useful. For example, front-face fluorescence may be particularly
10 useful in highly absorbent samples because the excitation and emission beam does not need to travel through the bulk of the sample, and thus, may be less affected by the interfering components that may be contained therein (*e.g.*, blood cells and microbiological culture media). The optical surface of the separation device 1904 may be illuminated at such an angle as to provide acceptable results as known to those
15 skilled in the art, (*e.g.*, Eisinger, J., and J. Flores, "Front-face fluorometry of liquid samples," *Anal. Biochem.* 94:15-21 (1983)). In one embodiment, the system is designed such that the spectroscopic system measures diffuse reflected light at a minimum of one fixed angle in addition to measuring emitted fluorescence at a minimum of one fixed angle.

[0373] In yet another embodiment, non-spectroscopic measurements from the
20 detection system that detected the specimen container as "positive" (item 102 in Figure 77), such as detection times and growth rates can be used to assist in the characterization and/or identification of microorganisms from the isolated sample or pellet. Additionally, measurements taken from a photographic image of the lower
25 region of the separation device can provide valuable information on the identity of the isolate, such as pellet size, shape, color and density.

[0374] In some embodiments, characterization and/or identification of the microorganisms in the isolated sample or pellet need not involve identification of an exact species. Characterization encompasses the broad categorization or classification
30 of biological particles as well as the actual identification of a single species. Classification of microorganism from an isolated sample or pellet may comprise determination of phenotypic and/or morphologic characteristics for the microorganism. For example, characterization of the biological particles may be accomplished based on observable differences, such as, composition, shape, size,

clustering and/or metabolism. In some embodiments, classification of the biological particles of interest may require no prior knowledge of the characteristics of a given biological particle but only requires consistent correlations with empiric measurements thus making this method more general and readily adaptable than methods based on specific binding events or metabolic reactions. As used herein “identification” means determining to which family, genus, species, and/or strain a previously unknown microorganism belongs to. For example, identifying a previously unknown microorganism to the family, genus, species, and/or strain level.

[0375] In some instances, characterization encompasses classification models which provide sufficient useful information for action to be taken. As used herein, the preferred classification models comprise grouping into one or more of the following: (1) Gram Groups; (2) Clinical Gram Groups; (3) Therapeutic Groups; (4) Functional Groups; and (5) Natural Intrinsic Fluorescence Groups.

[0376] (1) Gram Groups: Within the Gram Groups classification, microorganisms may be placed into one of three broad classification categories based on their Gram staining reaction and overall size, said groups selected from one or more of the following: (a) Gram positive microorganisms that stain dark blue with Gram staining; (b) Gram negative microorganisms that stain red with Gram staining; and (c) yeast cells that stain dark blue with Gram staining, but are very large rounded cells that are distinguished from bacteria by their morphological characteristics and size.

[0377] (2) Clinical Gram Groups: The Gram Groups may be further divided into several sub-categories representing distinguishing morphological features. These sub-categories comprise all the relevant clinical information reported by an experienced laboratory technologist, and thus provide a higher level of identification than a positive or negative Gram reaction. This particular classification is very helpful because it eliminates concerns about relying on the quality of a Gram stain and/or the skill level of the technician reading the smear by providing the equivalent clinically relevant information with an automated system. More specifically, subcategories of microorganisms based on this classification model may be selected from one or more of the following: (a) cocci, which are small rounded cells; (b) diplococci, which are two small rounded cells joined together; (c) rods, which are rectangular shape; and (d) bacilli, which are rod shaped. Examples of these sub-categories that can be ascertained by additional morphological information include: (i)

Gram positive cocci; (ii) Gram positive cocci in chains; (iii) Gram positive cocci in clusters (*i.e.*, “grape-like” clusters); (iv) Gram positive diplococci; (v) Gram positive rods; (vi) Gram positive rods with endospores; (vii) Gram negative rods; (viii) Gram negative coccobacilli; (ix) Gram negative diplococci; (x) yeast; and (xi) filamentous fungi.

[0378] (3) Therapeutic Groups: The therapeutic groups comprise multiple microbial species that, when isolated from particular specimen types, are treated with the same class of antibiotics or mixture of antibiotics (*e.g.*, as described in “*Sanford Guide to Antimicrobial Therapy 2008*”). In many cases, identity to the species level is not required by the clinician to enable a change from initial empiric therapy to a more targeted therapy because more than one species can be treated with the same choice of antibiotic(s). This classification level correctly places these “same-treatment” microorganisms into single therapeutic categories. Examples of this characterization level include the ability to distinguish highly resistant Enterobacteriaceae (EB) species from sensitive EB species (*Enterobacter* spp. from *E. coli*), or fluconazole-resistant *Candida* species (*C. glabrata* and *C. kruzei*) from sensitive *Candida* species (*C. albicans* and *C. parapsilosis*), and so on.

[0379] (4) Functional Groups: According to the invention, microorganisms may also be placed into several groups based upon a mixture of metabolic, virulence and/or phenotypic characteristics. Non-fermentative organisms may be clearly distinguished from fermentative ones. Furthermore, microorganism species that produce hemolysins may be grouped separately from non-hemolytic species. In some cases, these groups represent broader categories than genus level (*e.g.*, coliforms, Gram negative non-fermentative rods), some at the genus level (*e.g.*, *Enterococcus*, *Candida*), and some with closer to species-level discrimination (*e.g.*, coagulase-negative staphylococci, alpha-hemolytic streptococci, beta-hemolytic streptococci, coagulase-positive staphylococci, *i.e.*, *S. aureus*).

[0380] (5) Natural Intrinsic Fluorescence (“IF”) Groups: Microorganisms may also be placed into categories based on their natural tendency to group together by their innate and/or intrinsic fluorescence characteristics. Some of these groups may be common to Therapeutic and Functional Group categories. These groupings may comprise individual species, such as *E. faecalis*, *S. pyogenes*, or *P. aeruginosa* that have characteristic IF signatures and/or may contain small groups of organisms

with relatively conserved IF signatures such as the *K. pneumoniae*-*K. oxytoca* or *E. aerogenes*-*E. cloacae* groups.

[0381] In addition to measuring intrinsic properties of microorganisms (such as intrinsic fluorescence) for identification purposes, the methods may use additional identifier agents to aid in the separation and/or identification process. Agents that bind to specific microorganisms, such as affinity ligands, can be used to separate microorganisms, to identify a class or species of microorganism (*e.g.*, through binding to a unique surface protein or receptor) and/or to identify a characteristic of the microorganism (*e.g.*, antibiotic resistance). Useful identifier agents include, without limitation, monoclonal and polyclonal antibodies and fragments thereof (*e.g.*, anti-Eap for *S. aureus* identification), nucleic acid probes, antibiotics (*e.g.*, penicillin, vancomycin, polymyxin B), aptamers, peptide mimetics, phage-derived binding proteins, lectins, host innate immunity biomarkers (acute phase proteins, LPS-binding protein, CD14, mannose binding lectin, Toll-like receptors), host defense peptides (*e.g.*, defensins, cathelicidins, proteoglycans, magainins), bacteriocins (*e.g.*, lantibiotics, such as nisin, mersacidin, epidermin, gallidermin, and plantaricin C, and class II peptides), bacteriophages, and dyes selective for nucleic acids, lipids, carbohydrates, polysaccharides, capsules/slime or proteins, or any combination thereof. If the agent does not itself give out a detectable signal, the agent can be labeled to provide a detectable signal, such as by conjugating the agent to a marker (*e.g.*, visible or fluorescent). Markers include, without limitation, fluorescent, luminescent, phosphorescent, radioactive, and/or colorimetric compounds. The agent can be added to the microorganisms at any step in the methods of the invention, *e.g.*, when the sample is obtained, during lysis, and/or during separation. In some embodiments, the presence of the agent in the pellet can be determined during interrogation of the pellet. Other useful identifier agents include substrates for microbial enzymes, chelating agents, photosensitizing agent, quenching agent, reducing agent, oxidizing agent, buffer, acid, base, solvent, fixative, detergents, surfactants, disinfectants (*eg.* alcohols, bleach, hydrogen peroxide) and toxic compounds (*eg.* sodium azide, potassium cyanide) and metabolic inhibitors such as cyclohexamide, etc. Similarly, many fluorescent compounds for measuring microbial cell viability, metabolism and/or membrane potential may be used as an identifier agent in the present invention. As would be readily appreciated by one of skill in the art, the sensitivity of a particular microorganism to any compound affecting its

physical state or metabolism, such as an antibiotic, could be rapidly ascertained by adding the compound to the sample, lysis buffer, density cushion or any mixture thereof.

[0382] An embodiment of a method for performing identification and/or characterization of microbial agents in samples using intrinsic fluorescence will now be described in conjunction with Figures 81-89. Basically, the method can be embodied as a sequence of processing instructions stored in memory and executed using a conventional data processor or computer. The method executes an algorithm shown in Figures 81A-81C which is designed to provide the identification of a blood culture isolate (concentrated pellet) given an intrinsic fluorescence (IF) scan of the isolate from a predefined set of emission wavelengths.

[0383] In preferred embodiments, the method is encoded as software instructions implementing a multi-level identification algorithm, the different levels corresponding to different levels of a taxonomic hierarchy. Traditional classification algorithms that take input data and determine the identification of a microorganism use a single classification model. Given data from an intrinsic fluorescence scan at a predefined set of wavelengths of an unknown organism, the multi-leveled identification algorithm classifies the organism following the branches of a taxonomic hierarchy – Gram class, family, and species. A unique feature is the use of separate classification models at each identification step from highest, Gram class, to lowest, species. Additionally, the approach incorporates the use of parallel classification models to evaluate consistency between results. Thus, the probability of accurate identification and/or characterization is maximized, and generation of incorrect identification or characterization results is minimized. The multi-level taxonomic hierarchical classification method is applicable to other data sets besides intrinsic fluorescence data (e.g. it could be used to Raman spectral data or mass spectral data).

[0384] The identification method includes a set of data pre-processing steps (shown as blocks 5102, 5104 and 5106 of Figure 81A, and a set of analysis steps (the remaining blocks 5108, 5110, etc. in Figures 81B, 81C). The method determines the identification of the organism at multiple levels of the taxonomic hierarchy. The pre-processing steps are designed to acquire IF scan data and perform data transformations that minimize variation between different strains of a microbial agent within a given organism group or species. The data analysis steps implement a multi-

level identification using parallel classification models, as will be understood from the following discussion.

- [0385] As noted above, preferred embodiments provide an organism identification at the Gram, family, and species levels. Organisms commonly found in blood cultures that can be identified by the algorithm include, but not necessarily limited to, those listed in Table 1. Obviously, for different applications (e.g., food, water, environmental samples, etc.) the organisms may differ from those listed in Table 1, however the methodology is the same.

10 **Table 1: Intrinsic Fluorescence Algorithm Identification Organism List**

Gram Class	Family	Species
Gram-negative	Enterobacteriaceae	C. freundii
		E. aerogenes
		E. cloacae Complex
		E. coli
		K. oxytoca
		K. pneumoniae
		M. morganii
		P. mirabilis
		P. stuartii
		P. vulgaris
		S. enteritidis
		S. marcescens
	Moraxellaceae	A. baumannii
	Neisseriaceae	N. meningitidis
	Pasteurellaceae	H. influenzae
Gram-positive	Pseudonomadaceae	P. aeruginosa
	Xanthomonadaceae	S. maltophilia
	Enterococcaceae	E. faecalis
		E. faecium
	Listeriaceae	L.monocytogenes
	Staphylococcaceae	S. aureus
		S. capitis
		S. epidermidis
		S. hominis
		S. lugdunensis
		S. warneri
	Streptococcaceae	S. agalactiae
		S. bovis
		S. mitis / S. oralis

		S. pneumoniae
		S. pyogenes
Yeast	Ascomycetes	C. albicans
		C. glabrata
		C. krusei
		C. parapsilosis
		C. tropicalis

[0386] The processing steps or modules shown in Figures 81A-C will now be described in detail.

5

Pre-processing

[0387] Step 5102: Obtain a fluorescence value, n_{ij} , for each excitation value, $i = 1, 2, \dots, x$, and each emission, $j = 1, 2, \dots, y$, combination. The ratio, emission value/excitation value, must fall within the interval (1.05, 1.95).

10 [0388] Step 5104: For each fluorescence value, n_{ij} , calculate the natural logarithm value, $\ln(n_{ij})$.

[0389] Step 5106: Calculate the 1st derivative of the natural log transform (from step 5104) for each emission value, $j = 2, 3, \dots, y-1$, across a given excitation wavelength, i .

15 [0390] It is advantageous to transform the raw fluorescence data to minimize strain-to-strain variation within each organism group, using both steps 5104 and 5106. Additionally, the transformation process tends to create similar variance across organism groups. Figures 82, 83 and 84 illustrate by way of example the effects of performing the described pre-processing for multiple strains of *Staphylococcus aureus*

20 evaluated across the emission range at excitation 315. In Figure 82, each line represents the fluorescence signal from a single strain. The line 5202 indicates the mean fluorescence signal at each emission value. Figure 83 shows the strain-to-strain variation in the fluorescence signal after application of the natural logarithm transformation (step 5104); note that the curve for all of the strains are close together.

25 Figure 84 shows the strain-to-strain variation at excitation of 315 nm after calculation of the first derivative of the natural logarithm transform (step 5106). Again, note that the curve for all the strains are very close together, particularly at the emission range of 400-610 nm.

[0391] As another example, Figure 85 shows the strain-to-strain variation in the fluorescence signal at excitation of 415 nm for *Candida parapsilosis*, prior to performing the transformation steps. Note the wide variation in emission in the range of 400-650 nm. Strain-to-strain variation for this organism at excitation of 415 nm after performing the natural logarithm transformation is shown in Figure 86. Strain-to-strain variation after performing the first derivative transformation is shown in Figure 87. Note that in Figure 87 the strain-to-strain variation is much reduced.

Analysis

[0392] Step 5108: The first level of classification in the analysis after performing the pre-processing steps is gram classification 5108. At this step, the processing includes two branches, one represented by steps 5110 and 5112 and another represented by steps 5114 and 5116. Figure 81A is not meant to imply that the branches could not be performed sequentially; the branches could be performed either sequentially or in parallel.

[0393] Step 5110: Gram Classification Distance Calculation. Using the 1st derivative transforms for a predefined set of excitation / emission pairs, calculate the distance,

$$d_a = [(m - m_a)^t \Sigma^{-1} (m - m_a)]^{1/2}$$

for each Gram class defined in the model

where

- $a = 1, 2, 3$, represents the Gram classes defined in the model
- m represent the vector of calculated values of the 1st derivative, m_{ij} , for each excitation / emission pair i, j
- m_a represent the vector of mean values $m_{a(ij)}$ from a distribution for each class a at excitation / emission pair i, j
- t represent the transpose of the vector
- $(m - m_a)$ represent the vector of differences $m_{ij} - m_{a(ij)}$ for each excitation / emission pair i, j
- Σ^{-1} represents the inverse of the covariance matrix for the predefined set of excitation / emission pair. The set of excitation and emission pairs are experimentally determined from fluorescence measurements (with preprocessing

performed) of known microorganisms (see Figures 88 and 89 and the discussion below).

[0394] The term “model” is used to refer to a set of known microbial agents for which IF measurements (including transforms) at the predetermined excitation wavelengths have been previously obtained and for which a specimen is a candidate for classification, e.g., the agents listed in Table 1.

[0395] Step 5112: Gram Classification Interpretation.

- Let u_g represent the maximum distance threshold
- 10 - If all distances, d_1 , d_2 , and d_3 , are greater than u_g , the classification result is *Unknown*
- Else, determine the value of $d_{\min}$, the minimum value of d_1 , d_2 , and d_3
- Let w_g represent the low discrimination threshold factor
- If more than one distance, d_1 , d_2 , and d_3 , is less than $(d_{\min} * w_q)$, the classification
- 15 result is Low Discrimination between the Gram classes having distances less than $(d_{\min} * w_q)$
- If only one distance, d_1 , d_2 , and d_3 , is less than $(d_{\min} * w_q)$, the classification result is the corresponding Gram class.

[0396] Step 5114: All Families Classification Distance Calculation

- 20 Using the 1st derivative transforms for a predefined set of excitation / emission pairs, calculate the distance,

$$d_a = [(m - m_a)^t \Sigma^{-1} (m - m_a)]^{1/2}$$

for each organism family defined in the model

25 where

- $a = 1, 2, \dots, k$, represents all of the organism families defined in the model
- Σ^{-1} represents the inverse of the covariance matrix for the predefined set of excitation / emission pairs (same remark as above, the set of excitation and emission pairs are experimentally determined)
- 30 - m represent the vector of calculated values of the 1st derivative, m_{ij} , for each excitation / emission pair i, j
- m_a represent the vector of mean values $m_{a(ij)}$ from a distribution for each class a at excitation / emission pair i, j
- t represent the transpose of the vector

- $(m - m_a)$ represent the vector of differences $m_{ij} - m_{a(ij)}$ for each excitation / emission pair i, j

Note: The predefined set of excitation / emission pairs can be unique for the gram classification versus all species classification.

5 **[0397]** Step 5116: All Families Classification Interpretation

- Let u_f represent the maximum distance threshold
- If all distances, $d_1, d_2, \dots, d_a$, are greater than u_f , the classification result is *Unknown*
- Else, determine the value of $d_{\min}$, the minimum value of $d_1, d_2, \dots, d_a$
- 10 - Let w_f represent the low discrimination threshold factor
- If more than one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_f)$, the classification result is Low Discrimination between the organism families having distances less than $(d_{\min} * w_f)$
- If only one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_q)$, the classification result
- 15 is the corresponding family.

[0398] Step 5118: Pooling gram and all families classification interpretations for final gram classification result.

- [0399]** If the Gram classification is a single choice and the all families classification is a single choice, the pooled classification result is the indicated Gram
- 20 class if the family classification falls under the taxonomic hierarchy of the Gram class.

[0400] If the Gram classification is a single choice and the all families classification is a single choice, the pooled classification result is *Unknown* if the family classification does not fall under the taxonomic hierarchy of the Gram class.

- 25 **[0401]** If the Gram classification is a single choice and the all families classification is a low discrimination, the pooled classification is the indicated Gram class if the family associated with the shortest distance falls under the taxonomic hierarchy of the Gram class.

- [0402]** If the Gram classification is a single choice and the all families classification is a low discrimination, the pooled classification is *Unknown* if the family associated with the shortest distance does not fall under the taxonomic hierarchy of the Gram class.
- 30

[0403] If the Gram classification is a low discrimination and the all families classification is a single choice, the pooled classification result is the Gram class that

corresponds to the Gram class under which the family resides on the taxonomic hierarchy.

[0404] If the Gram classification is a low discrimination and the all families classification is a single choice, the pooled classification result is *Unknown* if none of the Gram classes correspond to the Gram class under which the family resides on the taxonomic hierarchy.

[0405] If the Gram classification and the all families classification are both *Unknown*, the pooled classification result is *Unknown*.

[0406] The processing then proceeds to step 5120, Gram Family Classification, a second level of classification. This step consists of sub-steps 5122, 5124 and 5126.

[0407] Step 5122: Gram family classification distance calculation.

[0408] Using the 1st derivative estimates for a predefined set of excitation / emission pair that are specific to the Gram classification result, calculate the distance,

$$d_a = [(m - m_a)^t \Sigma^{-1} (m - m_a)]^{1/2}$$

for each organism family defined in the model,

where

- $a = 1, 2, \dots, k$, represents the number of organism families defined in the model
- Σ^{-1} represents the inverse of the covariance matrix for the predefined set of excitation / emission pairs (same remark as before regarding the pairs)
- m represents the vector of calculated values of the 1st derivative, m_{ij} , for each excitation / emission pair i, j
- m_a represent the vector of mean values $m_{a(ij)}$ from a distribution for each class a at excitation / emission pair i, j
- t represent the transpose of the vector
- $(m - m_a)$ represent the vector of differences $m_{ij} - m_{a(ij)}$ for each excitation / emission pair i, j

[0409] Step 5124: Gram Family Classification Interpretation

Let u_t represent the maximum distance threshold

If all distances, $d_1, d_2, \dots, d_a$, are greater than u_t , the classification result is *Unknown*

Else, determine the value of $d_{\min}$, the minimum value of $d_1, d_2, \dots, d_a$

Let w_t represent the low discrimination threshold factor

If more than one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_t)$, the classification result is Low Discrimination between the organism families having distances less than $(d_{\min} * w_t)$

5 If only one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_t)$, the classification result is the corresponding family.

[0410] Step 5126 Gram Family Classification Result.

[0411] If the Gram family classification result is *Unknown*, the test organism classification is finalized at the Gram level.

10 [0412] If the Gram family classification result is Low Discrimination, the test organism classification is finalized as the Gram and families included in the low discrimination.

[0413] If the Gram family classification result a single family, the IF data from the test organism are further analyzed to determine if a species level identification can be determined.

15 [0414] Step 5128 Gram family Species Classification. The processing instructions proceed to a gram family species classification level, consisting of sub-steps 5130, 5132, and 5134.

[0415] Step 5130 Gram family species classification distance calculation.

20 [0416] Using the 1st derivative estimates for a predefined set of excitation / emission pair that are specific to the Gram family classification result, calculate the distance,

$$d_a = [(m - m_a)^t \Sigma^{-1} (m - m_a)]^{1/2}$$

25 for each organism species defined in the model,

where

- $a = 1, 2, \dots, k$, represents the number of organism species defined in the model
- Σ^{-1} represents the inverse of the covariance matrix for the predefined set of
- 30 excitation / emission pairs (same remark as before)
- m represents the vector of calculated values of the 1st derivative, m_{ij} , for each excitation / emission pair i, j
- m_a represent the vector of mean values $m_{a(ij)}$ from a distribution for each class a at excitation / emission pair i, j
- 35 - t represent the transpose of the vector

- $(m - m_a)$ represent the vector of differences $m_{ij} - m_{a(ij)}$ for each excitation / emission pair i, j

[0417] Step 5132 Gram family species classification interpretation.

- 5 - Let u_s represent the maximum distance threshold.
- If all distances, $d_1, d_2, \dots, d_a$, are greater than u_t , the classification result is *Unknown*.
- Else, determine the value of $d_{\min}$, the minimum value of $d_1, d_2, \dots, d_a$.
- Let w_s represent the low discrimination threshold factor.
- 10 - If more than one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_s)$, the classification result is Low Discrimination between the organism species having distances less than $(d_{\min} * w_s)$
- If only one distance, $d_1, d_2, \dots, d_a$, is less than $(d_{\min} * w_t)$, the classification result is the corresponding species.

15 [0418] Step 5134 Gram family species classification result.

 [0419] If the Gram family species classification result is *Unknown*, the test organism classification is finalized at the Gram and family level.

 [0420] If the Gram family species classification result is Low Discrimination, the test organism classification is finalized as the Gram, family, and species included
20 in the low discrimination.

 [0421] If the Gram family species classification result a single species, the test organism classification is finalized at the Gram, family, and species level.

 [0422] At step 5136, the results determined at steps 5134, 5118, and 5126 are returned and reported to the user, e.g., on a user interface for the identification
25 instrument, transmitted to an attached workstation, returned to another software module, or otherwise generated for the user.

 [0423] In regards to organism identification (steps 5134, 5118 and 5126), discrimination between species is possible only if the values of the first derivative (of the natural logarithm transform of the emission value) are unique for each species in
30 the model at some portion of the emission range for at least one excitation wavelength. Figures 88 and 59 illustrate the discrimination potential between a subset of species for excitation wavelengths 315 nm (Figure 88) and 415 nm (Figure 89). Referring to Figure 88, it is apparent that several of the species can be discriminated from the others based on the first derivative at excitation wavelength 315. The

mathematical model uses the first derivative values for emissions where visual differences exist as inputs to discriminate between species. Using selected sections of values across the emission range the following species can be clearly discriminated from the others: *E. coli*, *H. influenzae*, *P. aeruginosa*, and *S. pneumoniae*. In addition, *S. aureus* and *S. epidermidis* can be discriminated from other species but not each other. The sections of values across the emission range at a given excitation wavelength are the predefined pairs in the inverse matrices Σ^{-1} in the distance calculations in the processing steps described above. These pairs may for example be excitation at 315 nm and the range of emission values indicated by the circles shown in Figure 88, i.e., (315/300-450), (315, 485-500), (315/570-580).

[0424] Referring to Figure 89, it is apparent that the emissions at excitation wavelength 415 nm has the ability to discriminate between species. Using selected sections of values across the emission range *C. parasilopsis* and *P. aeruginosa* can be clearly discriminated from the other species. It is also of interest to note the difference between first derivative values for *S. aureus* and *S. epidermidis* that occurs around emission 450 nm. When the information from the selected sections of values across the emission range for wavelengths 315 and 415 (Figures 88 and 89) is combined, all of the species in the model can be discriminated from each other at a high rate (> 97% reliability).

IV. Second Embodiment (Figures 57-76)

[0425] A second embodiment of the identification system 104 will be described in conjunction with Figures 57-76. This embodiment is similar to the first embodiment of Figures 31-56 in terms of overall function and operation; the main differences are (1) a different construction of the robotic transfer mechanism 1910; (2) a provision for vortexing of the sample and lysis buffer in the sampling device 1902, and (3) inclusion of optional detection features in the rack holding the specimen containers 500 (see Figure 58) for detecting microbial growth within the container 500 so that the identification system is intimately combined with a detection system for detecting whether a specimen container is positive for presence of a microbial agent. A few other points of differentiation in the details of the configuration of the second embodiment will also be noted in the following description.

[0426] However, the second embodiment, like the first embodiment of Figures 31-56, shares the same overall goals and design objectives. That is, the second embodiment of Figures 57-76 automates the removal of a test sample from a specimen container (preferably soon after a positive determination has been made),
5 automates lysing of non-microorganism cells in the test sample, automates loading of the lysed sample into a disposable separation device, automates separation and concentration of the microbial agent present in the lysed sample in the separation device, and automates interrogation of the microbial agent to identify and/or characterize the microbial agent.

10 [0427] The culture bottles/specimen containers 500 are loaded into racks or holding structures of the identification instrument 104 either manually or automatically. In an optional configuration, the specimen containers 500 are tested for the presence or microorganisms by a detection subsystem which is incorporated into the racks. In a manual, prior art method, without automation, a technician would
15 remove a bottle from a separate detection instrument after the bottle is deemed “positive”. This could be several hours after the diagnostic determination, especially if the determination is made in the middle of the night or when the lab is understaffed. However, with the automated identification instrument in this embodiment, the steps of automated identification and/or characterization of the microbial agent can proceed
20 immediately, and automatically, after the specimen container is deemed “positive” .

[0428] In the case of lytic centrifugation and intrinsic fluorescence measurement, features of both of the illustrated embodiments, it may be desirable that the sample be processed for purposes of identification and/or characterization shortly after a positive call by an associated detection instrument. As the bottle is called
25 positive the microorganisms are in an exponential stage of growth. This growth phase is distinguished from the lag phase and death phase which are both before and after, respectively, the exponential phase. Microorganisms in this exponential phase have different physical and genetic expression characteristics than the lag and death phase.

[0429] By automating this process of identification and/or characterization,
30 the technician is removed from the system. Identification and/or characterization of the microbial agent can occur much more rapidly in the present embodiments as compared to prior approaches.

A. System layout

[0430] The identification instrument 104 in accordance with a second embodiment is shown in Figure 57. The instrument 104 includes a first cassette 1900A containing a plurality of disposable sampling devices 1902 and a second cassette 1900B containing a plurality of disposable separation devices 1904. A rack or holding structure 1906 includes receptacles for holding a multitude of containers 500 containing samples for identification testing. The rack 1906 is shown contained within an insulated incubation enclosure 1812. The enclosure 1812 includes a door 1810 that is opened to expose the bottles 500 and allow venting of the bottles and removal of a test sample via a robotic transfer mechanism 1910, sample removal apparatus 1912, and the sampling device 1902.

[0431] The robot transfer mechanism 1910 includes a rotating base and movable joints and segments between the joints so as to allow the robotic transfer mechanism 1910 and in particular gripping structures included in the sample removal apparatus or sampling head 1912 to access the various components in the instrument 104. These components include a separation and concentration device (centrifuge 1916), the cassettes 1900A and 1900B, a vortexer 1814 for mixing a lysis buffer and test sample within the sampling device 1902, a read station 1918, and various containers 1802, 1804, 1806 containing different lysis buffers and/or density cushions in the situation where the lysis buffers and density cushions are added to the sampling device or separation device at the time of use. The robotic transfer mechanism 1910 is able to access each of the bottles 500 and optionally grip and hold the bottles 500. Thus, the robotic transfer mechanism 1910 may optionally be the device to automatically load the bottles 500 into the holding structure or rack 1906. Alternatively, the bottles 500 could be loaded into the rack manually via an access door positioned on the opposite side of the enclosure 1812 from the door 1810. See Figure 79, door 4902.

[0432] In the configuration of Figure 57, a centrifuge cup holder 1800 holds a small cup-like holder 1801 into which the separation device 1904 is placed (see Figure 76A); the combination of the separation device 1904 and cup-like holder 1801 are placed into the centrifuge 1916 for separation and concentration of the microbial agent in the separation device 1904. After centrifugation, the cup-like device 1801 is returned to the cup holder 1800. The sample removal apparatus 1912 grips the separation device and the robotic transfer mechanism places it into the reading/identification module 1918. The concentrated microbial agent in the

separation device 1904 is interrogated by the reading/identification module 1918. The reading step may include the features described above, such as measuring intrinsic fluorescence spectra of the sample and, with the aid of a computer, comparison of the measured spectra to a data set containing spectra from known microbial agents and classification using a classification algorithm. After reading, the separation device 1904 is placed into a waste container (see Figures 31, 45, item 1908).

[0433] Figure 58 is an illustration of an alternative arrangement for the identification instrument 104. In this embodiment, the walls or panels from the incubation enclosure are removed to show one embodiment of the racks 1906 that hold the bottles 500. The racks 1906 are incorporated into a rotating turret which rotates about a vertical axis. Detection instrumentation for noninvasively detecting whether a bottle is positive is incorporated in the racks 1906. These aspects are described in more detail in co-pending application serial no. _____, attorney docket no. 01145/MBHB 09-271-E, filed on the same date as this application, the content of which is incorporated by reference herein. Accordingly, in this embodiment the racks 1906 and associated detection instrumentation function as an automated detection system 102 for determining the presence of a microbial agent in a specimen container.

[0434] Figure 59 is another perspective view of the embodiment of Figure 57, showing the centrifuge 1916, vortexer 1814 and sample removal apparatus 1912 included in the robotic transfer mechanism 1910.

[0435] Figure 60 shows the cassettes 1900A and 1900B of disposables in more detail. While each cassette is shown holding twenty-five disposable sampling devices 1902 or separation devices 1904, the number or arrangement of the devices 1902 and 1904 within a replaceable cassette 1900 is not important.

B. Robot Transfer Mechanism 1910 and sampling removal apparatus 1912

[0436] Figure 61 is a perspective view of the robotic transfer mechanism 1910. The transfer mechanism 1910 is shown in a form of a six-axis robot. The robot includes six rotational joints 1700 indicated by the arrows and segments 1702 between the robot joints that expand or contract linearly in order to extend or contract or otherwise move the position of the sample removal apparatus 1912 placed at the tooling end of the robot arm in three-dimensional space. A rolling diaphragm pump

assembly 1710 is fitted to the robot transfer mechanism 1910 to apply vacuum or positive pressure to the sampling device 1902 via a connecting tube 3402 to facilitate venting and sampling the containers 500 as described below. The pneumatic system 1914 (Figure 31) provides pneumatic controls for the gripping tooling forming the sample removal apparatus 1912.

[0437] A six axes robot is chosen to allow for flexibility especially the ability to vent and sample the bottle. A pneumatic gripper 1954, see Figures 64 and 65, is placed at the tooling end of the robot on the last rotary joint. Plates attached to the gripper 1954 hold three end effectors; that is, there are three separate gripping components: one 1956 for the sampling device 1902, one 1958 for the separation device 1904 and specimen containers 500, and one (not shown) for a vacuum tube which may be used in future configurations. A connector 1952 is used to attach the free end of the tube 3402 to the fitting 3208 (Figure 33) on the proximal end of the sampling device 1902. A pneumatically operated linear slide 1950 is moved forward to advance the connector 1952 to engage with the sampling device 1902 and backward to disengage from the sampling device when the device 1902 is deposited in the waste container.

[0438] The gripper 1954 and linear slide 1950 are pneumatic driven, with the gripper and linear slide controlled from the same air line (3602 Figure 66). Flow control valves (not shown) control the rate movement on the gripper and linear slide. When the sampling device 1902 is to be picked up, the gripping component 1956 is positioned around the sampling device 1902 with the component 1956 open and the linear slide 1950 retracted. An air valve (not shown) is activated to close the gripper 1954 and close the gripping component 1956 and advance the linear slide 1950. Through flow controls, the gripper closes first, grabbing the sampling device 1902. Shortly after, the linear slide 1950 advances and engages the connector (1952 in Figure 64) with the sampling device 1902. Tubing 3402 connects the pump assembly 1710 with the connector 1952 in Figure 64 and sampling device 1902, thereby establishing a connection from the sampling device 1902 to the pump 1710 (Figure 66) via the tubing 3402.

C. Sampling device 1902

[0439] The sampling device 1902 in this embodiment is shown in Figures 62 and 63. The operation of the device 1902 for venting and sampling a specimen

container 500 is described below in conjunction with Figures 66 and 67. The operation of the device 1902 for injecting the sample into the separation device 1904 is described below in conjunction with Figures 73-76.

[0440] Referring now to Figures 62 and 63, the sampling device 1902 includes a 18-gauge needle 3202 having a rubber sheath 3214. A luer fitting 3212 connects the needle 3202 to a 5 ml syringe body or tube 3200. A 0.2 μ m hydrophobic filter 3218 is coupled to the syringe body 3200 with an elastomeric fitting 3210. A port or fitting 3208 receives the tube 3402 (Figure 34) which is connected to the rolling diaphragm pump 1710 fitted on the robot transfer mechanism 1910 of Figure 61.

[0441] The sheath 3214 has four functions: 1) sheath the needle 3202 to avoid needle stick injuries, 2) keep needle 3202 sterile, 3) prevent leaking of components out of tube 3200, and 4) act as spring to push back on components during sampling from the specimen container 500 and the injection of the separation device 1904 (see Figures 74 and 45). The sheath prevents the needle 3202 from sticking or binding to a septum or stopper fitted on the end of the specimen container 500. As the needle is withdrawn from the septum the rubber sheath pushes against the septum preventing the binding of the needle and septum. Similarly, during injection of the separation device the spring-like compression of the sheath 3214 pushes against the screw cap of the separation device 1904 and prevents the needle for sticking or binding to the cap.

[0442] The hydrophobic filter 3218 (Figure 63) prevents microbes from contaminating the pumping system and tubing. Since this filter is hydrophobic, liquid is prevented from passing to the pump 1710 of Figure 61. Another function of the filter besides preventing contamination, is repeatable fluid withdrawal. Once liquid touches the filter 3218 no more air can be evacuated from the tube 3200 since the water blocks the flow of air. Thus, the pump 1710 can continue pumping but the volume of liquid extracted will be a function of the tubing volume and not the precision of the pump.

D. Vacuum pump assembly 1710

[0443] The vacuum pump assembly 1710 of Figure 31 is shown isolated in perspective view in Figure 36. The pump 1710 contains a rolling diaphragm 1712 connected to a linear actuator 1714. Solenoid valves 1716 and 1718 switch the pump from input to output. During the venting step, in which the bottles 500 are vented to

atmosphere using the sampling device 1902, the solenoid valves 1716 and 1718 are actuated to let positive pressure vent through the system (input). Also, during sampling the pump draws in fluid from the bottle 500 into the sampling device 1902. The fluid is ejected (output) to the separator device 1904 (Figures 73-74). For both
5 input and output modes, the linear actuator 1714 continues to operate, moving the rolling diaphragm 1712 back and forth. Check valves (not shown in Figure 36) take part in controlling the direction of the fluid.

E. Venting and Sampling

10 [0444] The venting and sampling steps are shown in Figures 67 and 68. The robot 1910 first picks up one of the sampling devices 1902 from the cassette 1900A. The robot 1910 moves into the position shown in Figure 67. The door 1810 is opened. The racks 1816 of Figure 67 are rotated to an upwards pointing position and venting occurs by means of inserting the needle of the sampling device 1902 into the
15 specimen containers 500. The racks 1816 are then rotated to a downward pointing position shown in Figure 67 and the pump 1710 operated to draw a small a test sample (e.g., 0.5 to 1.0 ml) from the specimen container 500 into the sampling device 1902. The sampling device 1902 is either pre-loaded with lytic agent or alternatively the lytic agent is added to the sampling device 1902 prior to the venting and sampling
20 steps. In the case where the sampling device is loaded with lytic agent in-situ, the robotic transfer mechanism grasps one of the sampling devices, accesses lytic agent solutions stored in containers 1802, 1804, 1806 etc., see Figure 57, and withdraws 1.0 to 2.0 ml of lytic agent into the sampling device 1902 and then proceeds with the venting and sampling steps.

25

F. Mixing of Lytic agent and Sample in Sampling Device 1902

[0445] As noted previously, the embodiment of Figures 27-46 includes features for agitating the sampling device 1902 in order to mix the test sample withdrawn from the specimen container with the lytic agent present in the sampling
30 device 1902, e.g., by means of vortexing.

[0446] The vortexing will now be described in conjunction with Figures 59, and 69-72, showing the vortexer 1814. A unique feature of this system is a vortex cup 3900 which holds the sampling device 1902. The robotic transfer mechanism 1910 first places the sampling device 1902 in the vortex cup 3900 (see Figure 69), releases

the sampling device 1902, and then moves upward into the position shown in Figure 70. The robot gripper fingers 3450 then closes loosely around the above the hydrophobic filter 3218 (Figures 62, 63) of the sampling device 1902. The sampling device 1902 is held loosely in the vortexer cup 3900 so that the vortexer 1814 can be
5 free to agitate the sampling device 1902. If it is held tightly the vortexer 1814 is not free to agitate the sample/lysis buffer mixture which is present in the sampling device 1902. The bottom surface 1957 of the gripper tooling 1956 retains the sampling device 1902 in the vortexer 1814 during vortexing.

[0447] The vortexer 1814 includes a base 3902 that the cup or holder 3900 is
10 mounted to as via fasteners extending through holes 4202 in the flange 4204 of the holder 3900 as shown in Figures 71-72. The interior channel 4200 of the holder 3900 is sized to fit the sampling device 1902 as shown in Figures 70 and 71. The vortexing sufficiently mixes the sample and the lysis buffer with a 5 second cycle at 3000 rpm.

[0448] In one optional configuration, the vortex cup 3900 include heating
15 elements to maintain the sample in the sampling device 1902 at 37 degrees C. The heating may take the form of a coil resistive heater 3910 shown in Figure 69A. The agitation frequency, duration and temperature of the vortex process may change for particular samples and buffers.

20 G. Injection of mixed sample into separation device 1904

[0449] It may be desirable to first load the separation device into the centrifuge to pre-spin the lytic buffer and insure no trapped air is present in the capillary tube of the separation device. Also, if the optics system is configured in the centrifuge a quality check (e.g., a pre-read of the separation device before adding
25 lysed sample) can be performed. Quality control checks could include inspection for debris or fibers that may be present in the separation device, scratches to the optic surfaces, or other optic defects. After the vortexer 1814 completes the mixing of the sample and lysis buffer in the sampling device 1902, an approximately 1 ml portion of the mixed sample and lysis solution (lysed sample) is then injected into the disposable
30 separation device 1904. This operation may occur while the separation device 1904 is still contained within the cassette 1900B of Figures 56 and 67. The mixed sample and lysis buffer is shown as mixture 4302 in Figure 43A. (Figure 73D shows the rubber sheath 3214 shown partially removed from the needle 3202 but this is only to better

illustrate the sheath and the needle; the needle is covered by the sheath during use as shown in Figures 73B and 73C).

[0450] To accomplish the injection of the sample into the separation device 1902, the robotic transfer mechanism positions the (loaded) sampling device 1902 over one of the separation devices 1904 as shown in Figure 73A and then proceeds to lower the sampling device 1902 so that the needle 3202 is forced through both the rubber sheath 3214 and the septum 4300 provided in the cap 2404 of the separation device 1904 so as to place the tip of the needle 3202 into the interior chamber 2602 of the separation device. See Figures 74, 75 and 76. This action compresses the rubber sheath 3214 as shown in Figures 74, 75 and 76, with the sheath 3214 acting on a spring and applying force to the cap 2404. As shown in Figure 76, the roller diaphragm pump 1710 operates to pump air into the sampling device 1902, creating positive pressure in the interior of the sampling device and thereby forcing the test sample/lysis buffer mixture 4302 to be injected via the needle into the separation device 1904 as shown in Figure 76. The mixture 4302 is dispensed on top of the 1.0 ml density cushion 2802 already present in the separation device 1904.

H. Transfer of loaded separation device 1904 into centrifuge 1916

[0451] After loading of the separation device 3202 in this manner, the robotic transfer mechanism 1910 proceeds to transfer the sampling device 1902 to a waste container, and then pick up the loaded separation device 1904 and place it in the cup 1801 held by the cup holder 1800 (Figure 58, Figure 76A-76C). Then, the combination of the cup 1801 and separation device 1904 is picked up and lifted off the holder 1800 (Figure 76A) by the robotic transfer mechanism 1910 and placed in the centrifuge 1916 (Figure 58) for separation and concentration of the sample in the separation device 1904.

[0452] In one possible embodiment, the centrifuge 1916 is not an indexed centrifuge, i.e., it does not come to the exact same position after spinning. The centrifuge lid is open and closed by a pneumatic cylinder. The position of the centrifuge is found by a camera (not shown) on the robot transfer mechanism 1910. A picture of the centrifuge is taken and machine vision software determines the position of the centrifuge so that the separation device 1902 can be correctly placed in the centrifuge. In particular, the camera looks for a fiduciary mark on the rotor and the

robot moves to the appropriate position in the centrifuge rotor. The separation device 1904 is inserted into the proper location to maintain balance in the centrifuge 1916.

[0453] The centrifuge could be configured to just spin one separation device at a time (as in the case of the first embodiment), or multiple devices at a time as shown in Figures 57-59.

[0454] The machine vision component (camera) could be eliminated by using an indexed centrifuge rotor. In this configuration, the centrifuge rotor would stop at the same position after centrifugation. This could be accomplished by using a mechanical clutch to engage the rotor and moving it past an optical sensor to the correct position. This method could eliminate complexities (e.g. lighting, complex software algorithms) and costs associated with machine vision, and thus for some implementations may be preferred.

I. Separation and concentration of microbial agent in Separation device 1904

[0455] The centrifuge operates to spin the separation device 1902 at high revolutions per minute for sufficient time to concentrate the microbiological specimen within the separation device into a pellet or pellet-like mass, as described in conjunction with the first embodiment, e.g., 10,000 g for 2 minutes. During centrifugation the lysed red blood cells separate to the top of the density cushion and the intact microbes form a pellet at the bottom of the 1 mm capillary tube 2604 in the separation device 1902 (see Figure 73A). The centrifuge lid is opened using a pneumatic cylinder and the robot removes the separation device 1902 and cup 1801. The position of the capillary tube and holder is determined by machine vision as in the placement step above. The separation device 1902 and cup 1801 are removed as a unit from the centrifuge 1918 and placed on the cup holder 1800 (using pin 1805 as a locating mechanism, see Figure 76C), and then the robot 1910 picks up the separation device 1902 and moves it to the reading unit 1918.

J. Reading of concentrated microbial agent in separation device 1904

[0456] The reading unit 1918 interrogates the concentrated microbial agent forming the pellet within the separation device 1902 in the manner described at length above. The results (characterization and/or identification information for the microbial agent) are output to the user interface of the instrument, a connected workstation, a printer, or other output device depending on the configuration of the instrument.

K. Sterilization of specimen container 500 stopper

[0457] In some biological applications for the present instrument 104, the specimen container 500 is inoculated with a specimen sample such as human body fluids or other normally-sterile body fluids. This is accomplished by injecting the specimen sample via a needle through a stopper formed at the top of the container 500. There is a chance that the sample may contain biohazardous material. Often a small drop of the specimen sample, such as blood, may be left on the surface of the stopper. It is desirable to sterilize this surface before sampling and processing to avoid contamination of the container 500 with airborne or surface microbes.

[0458] Several methods could be developed to sterilize this surface in an automated manner. These include:

[0459] 1) UV sterilization of the stopper surface. Ultraviolet light is a standard method of sterilizing surfaces. Automation could be accomplished by attaching a UV light source to a second robot or automation mechanism provided in the instrument that would move to the stopper surface for sterilization before venting the bottle or removing a test sample.

[0460] 2) Misting the surface with a disinfectant such as isopropyl alcohol or other chemical and then wiping the surface clear. Presently this is the most common manual method of sterilizing inoculation sites. Normally, swabs are soaked in a disinfectant and a technician wipes the surface before inoculating the bottle or removing a sample. Mechanical wiping is necessary in the case of dried blood spots on the surface since a chemical mist may not penetrate through the blood. The misting of the surface can be automated by pressurizing a disinfectant reservoir with air and spraying this onto the surface of the stopper. The mechanical wipe can be accomplished by picking up a swab or fabric wipe and wiping the stopper surface. Other mechanical methods of wiping the surface include a rolling fabric soaked in the disinfectant. Again, these methods could be accomplished by means of a separate robotic mechanism in the instrument 104, or by providing the existing robot transfer mechanism 1910 with additional gripping/wiping/misting/UV sterilization components as the case may be.

L. Other configurations for robotic transfer mechanism 1910

[0461] While the second embodiment shown in Figures 57-59 uses a six-axes robot for the automation robot transfer mechanism 1910 to accomplish transfer and positioning of components or materials in the instrument, it is but one of a variety of choices that could be made and the scope of the present disclosure is intended to encompass other robotic transfer mechanisms. A multi-axis robot arm was chosen because it is flexible. New automation steps can be easily programmed without requiring major mechanical tooling redesigns. Once the process is established, the robot could be replaced by a simpler and more compact robot, with fewer axes, or a Cartesian (x,y,z) automation system. The Cartesian system would be more inexpensive than the six-axes robot. A Cartesian system is used for example in the first embodiment (see Figure 35).

M. Electric actuators

[0462] A few of the actuators of the second embodiment (and in particular the gripper and slide aspects of the sample removal apparatus 1912) are operated by pneumatics (compressed air). Pneumatic mechanisms are simple to program and design, however they are not amenable to clinical or some laboratory settings where compressed air is not available. These actuators can be replaced by electrical/mechanical systems such as linear drives, stepper and servo motor connected to linear drives and solenoids.

N. Alternative mixing methods

[0463] In the second embodiment, a vortexer 1814 is used to vigorously mix the sample and lytic buffer. A different mixing method such as sonication or reciprocal mixing could be used in place of vortexing.

O. Other applications for identification system

[0464] We have described in detail a method and instrument for automatically vent and sample a specimen container, e.g., blood culture bottle. The sample is lysed and centrifuged to process the microbial agent present in the sample for further analysis. The features of the instrument can be applicable to other diagnostic systems and other types of culture bottles. These systems could include molecular biology tests or automated culture bottles for industrial samples. Industrial samples could include sterility testing of drugs or food.

[0465] In the case of molecular biology tests it may be very important to perform a microbial test during exponential growth of a microorganism. During the exponential growth phase the genetic expression of microbes is different than during the lag phase. In the lag phase, which is prior to the exponential growth phase, microbes are converting their genetic machinery to express proteins to consume the media nutrients which may be different from their previous environment. As the microbes enter exponential phase the genetic expression has become set.

[0466] An automated detection instrument (102), such as that described here and in our prior provisional application or the BacT/ALERT system, can determine when the microbes begin exponential phase and the automated identification method above can process a sample soon after exponential phase begins. In a manual culture method it would be difficult to determine when exactly the microbes enter into exponential phase since this would entail checking the bottles frequently for turbidity. Should the beginning of the exponential phase be missed by the technician, there is a risk that microbes would pass into death phase as the limited nutrients are consumed. Hence, in preferred embodiments the present identification instrument automatically processes the positive specimen containers soon or immediately after the container is deemed "positive."

[0467] In some other non-clinical embodiments of the identification system, the lysis step is optional or not preformed. Hence, the provision of a lytic buffer in the sampling device and vortexing the sampling device are not required in every possible configuration of the present inventive instrument.

P. Re-Sampling of Specimen Containers

[0468] The process of venting, sampling, separation and interrogation described above can be repeated on the same specimen container 500 as needed. In one possible variation, a given specimen container 500 is sampled successively using sampling devices 1902 loaded with different lytic buffers (e.g., loaded in situ from the supply of lytic buffers in the instrument) and loaded into different separation devices 1904 which are then subject to separation and concentration steps and then reading.

[0469] The instrument 104 may also perform identification and/or characterization testing without first performing the detection step; possibly shortening the time to identification. This mode of operation could be employed when other clinical data are available that are predictive of infection. Patient

condition, biomarkers (e.g., PCT) etc. are examples of data that could be predictive of infection. In this mode, specimen containers are loaded into the identification instrument 104 (e.g., using the rack designs of either embodiment), the bottles are incubated in racks provided in the identification instrument, and every bottle is periodically sampled and subject to the separation and concentration step and the interrogation step. If a given sample is not able to be identified or characterized at the first iteration, the specimen container can be re-sampled, e.g., every 30 minutes, until sufficient microbial agent growth has occurred within the specimen container such that the reading step for that subsequent iteration returns an identification and/or characterization result. Incubation of the specimen container occurs prior to and during the sequential sampling of the specimen container.

Q. Coupling to automated detection instrument.

[0470] In some embodiments, the automated identification instrument 104 of the first and second embodiments is tightly coupled to an automated detection instrument configured to determine whether a specimen container 500 is positive for presence of a microbial agent. This tight coupling preferably provides for automated hand-off of positive specimen containers 500 from a detection instrument to the automated identification instrument 104 as soon as the specimen container is tested “positive.”

[0471] A variety of instrument configurations for achieving such coupling are described in our prior U.S. provisional application serial. 61/216,339 filed May 15, 2009 and above. A few options are shown in Figures 77 and 78. In Figure 77, an automated detection instrument 102 is linked via conveyer 4702 to the automated identification and/or characterization instrument 104. Bottles arriving at the automated identification and/or characterization instrument 104 are picked up by the robotic transfer mechanism 1910 and loaded into the racks. In Figure 78, the bottles are provided to a combined detection and automated identification and characterization instrument (e.g., as set forth above for the second embodiment, see Figure 58 and the above discussion). In this configuration, the racks holding the incoming specimen containers 500 include detection instrumentation for interrogating colorimetric sensors incorporated in the bottom of the bottles. Further, the combined instrument 102 + 104 is provided with incubation features, such as providing the incubation enclosure 1812 of Figures 57 and 67.

[0472] Still other configurations are possible, as described above in Part 1. Figure 79 shows an embodiment in which the combined instrument 102 + 104 includes a door 4902 for manual loading of bottles into the racks of the combined detection and identification/characterization instrument.

5 [0473] In the embodiments of Figure 77-79, a drawer 4702 is provided to provide access to remove waste from the instrument, e.g., specimen containers, sampling devices and separation devices.

[0474] The physical configuration of the external panels for the instruments of Figures 77-79 are not particularly important and can vary widely. The instruments
10 include a graphical user interface and display 4704 which can also vary widely.

R. Computer system schematic

[0475] Figure 80 is schematic block diagram showing the identification and/or characterization instrument 104 and its associated computer control system.
15 The details shown in Figure 80 can vary widely and are not particularly important, and therefore are provided here by way of example and not limitation.

[0476] A computer 4902 running LabVIEW (National Instruments) is connected to two computers: (1) a computer 4904 via a serial connection, and (2) a robot control computer 4906 via an Ethernet connection. The computer 4904 controls
20 the racks 1906 and associated detection subsystem for detecting whether bottles are positive, controls the stepper motors which agitates (oscillates) the rack 1906 to provide agitation during incubation via a motion controller 4908. A stepper motor (not shown) allows for the rack to be precisely put in position for venting and sampling by the robot transfer mechanism 1910.

25 [0477] The LabVIEW 4902 computer queries the computer 4904 for positive bottles. The computer 4904 computer replies through the serial connection and the bottle ID, time of positive and bottle position are parsed by the LabVIEW computer 4902. The bottle position is sent to the robot controller 4906 which opens the door to the racks (Figure 27, 1810) through a digital signal to a relay controlling pneumatic
30 cylinders connected to the door. The robot 1910 acquires a sampling device 1902 and vents the bottle and samples as described above.

[0478] A digital signal from the robot controller 4906 is sent to relays to open and close the lid of the centrifuge 1916, start the centrifuge 1916 and control the

vortexer 1816. Motion control of the linear actuator on the rolling diaphragm pump is controlled by the LabVIEW computer 4902 via a motion controller 4908.

[0479] Interrogation measurements (e.g., intrinsic fluorescence and/or diffuse reflectance measurements) captured by the identification module 1918 are sent to the LabVIEW computer 4902. The computer 4902 compares the measured spectra with stored reference spectra from known samples to identify and/or characterize the microbial agent in the sample as described above. To do this comparison, the computer 4902 includes a memory (e.g., hard disk) containing the reference spectra data and machine-readable code storing software instructions to perform the comparison, e.g., the algorithms described previously. The computer 4902 includes a conventional central processing unit which operates on the acquired data and stored reference data using the algorithm(s) to generate a result for the sample under test and provides a report e.g., via a user interface on the instrument or attached peripherals 4910. The computer 4902 can communicate over an Internet Protocol network 4914 with other remotely located computers 4912, e.g., to share identification and/or characterization results, store the results in a remote database, or interface to other laboratory information systems.

S. Combination of separation and sampling devices into a single disposable device.

[0480] As described previously, the identification and/or characterization instrument 104 includes a sample removal apparatus 1912 which holds or grasps a disposable sampling device 1902. Together, they operate to remove a portion of the biological sample in the positive detection container 500 and add the portion to a separation device 1904. The functions of separation and sampling could be performed in a single disposable device.

[0481] Referring to Figures 90-94, a separation device 6000 includes a body 6002, generally in the shape of a block, a top plate 6004 and a base plate 6006. The body contains an optical window used for intrinsic fluorescence measurements; the material forming the window optically clear and non-fluorescing. In general, the body 6002 can be molded or otherwise formed from any known plastic material known in the art. As shown in Figures 62-64, the body 6002 of the separation device 6000 encloses a lytic chamber 6020, a venting channel 6030, a fluid transfer channel 6034 and a separation chamber 6040. The lytic chamber 6020 and separation

chamber 6040 are orientated along two parallel and adjacent vertical axes 6022, 6042, defined in the body 6002, each chamber having top and bottom terminal ends. The venting channel 6030 provides a first fluid communication channel connecting the bottom end of the lytic chamber to a vent or pump port 6018 in the top plate 6004. As shown in Figures 93-94, the first fluid communication channel further comprises a venting fluid flow groove 6032 contained in the upper surface 6034 of the body 6002 and providing fluid communication between the lytic chamber 6020 and the venting channel 6030. The fluid transfer channel 6036 provides a second fluid communication channel connecting the bottom end of the lytic chamber 6020 to the top end of the separation chamber 6040 for transferring a lytic buffer and sample from the lytic chamber 6020 to the separation chamber 6040. As shown in Figures 93 and 95, the second fluid communication channel further comprises a venting fluid flow groove 6038 contained in the upper surface 6034 of the body 6002 and providing fluid communication between the lytic chamber 6020 and the separation chamber 6040. The lytic chamber 6020, venting channel 6030 and fluid transfer channel 6036 are open to a bottom surface 6010 of the body 6002 of the device 6000, as shown in Figure 91. The bottom surface 6010 of the body 6002 may further comprise a lower fluid flow groove 6024 providing fluid communication between the bottom of the lytic chamber 6020 and the venting channel 6030 and fluid transfer channel 6036 through a valve well 6026 (described further below). The top plate 6004 and base plate 6006 can be attached to the body 6002 by any known means in the art to close or otherwise seal the chambers 6020, 6040 and channels 6030, 6034. For example, the top plate 6004 and/or base plate 6006 can be affixed to the body by welding or by the use of an adhesive.

[0482] As shown in Figure 91, the separation device 6000 includes a valve 6012 and a valve actuator port 6008 that runs through the top plate 6004. The valve 6012 is contained within a valve well 6026 in the bottom surface 6010 of the body 6002, and is operable between a first position and a second position via an external actuator (not shown). When the valve 6012 is in a first position, a first fluid communication channel is “open” from the bottom of the lytic chamber 6020 through the venting channel 6030 to the vent or pump port 6018. This open first fluid communication channel is operable to vent excess pressure from the device 6000 or to provide a vacuum to the lytic chamber 6020 through the use of a pump (not shown). When the valve 6012 is in a second position, a second fluid communication channel is

“open” from the bottom of the lytic chamber 6020 through the fluid transfer channel 6036 to the separation chamber 6040. This open second fluid communication channel is operable for transferring the lytic buffer and sample from the lytic chamber 6020 to the separation chamber 6040. As shown in Figure 92, the vent or pump port 6018 and sample entry port 6016 comprise open channels through the top plate 6004. In one possible embodiment, the sample entry port 6016 further comprises a pierceable septum (not shown). In another embodiment, a syringe needle (not shown) can be attached or affixed to the sample entry port 6016, thereby allowing the lytic and separation device to operate as the sampling device for directly obtaining a sample from a specimen container 500.

[0483] As shown in Figure 93, the separation chamber 6040 may further comprise an upper reservoir, a middle tapered section and a lower capillary tube 6048 all arranged around a central vertical axis. As shown, the middle tapered section connects the wider diameter upper reservoir and the smaller diameter capillary tube 6048. In one embodiment, the bottom wall of the capillary tube 6048 is made of an optically transparent material for facilitating optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6048. In another embodiment, the separation device 6000 is made of an optically transparent material to facilitate optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6048. As shown, the bottom wall opposite the capillary tube 6048 may be of a reduced thickness to facilitate optical interrogation as indicated in Figure 92. In yet another embodiment, optical interrogation can occur from the side of the device 6000. In accordance with this embodiment, the block will comprise a notch section 6010 and a reduced thickness side wall juxtaposed the capillary tube 6048. In accordance with this embodiment, the separation device 6000 is made of an optically transparent material to facilitate optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6048.

[0484] In operation, the lytic chamber 6020 can be loaded with a lysis buffer and a sample taken from a positive culture container. For example, a sampling device 1902, as described elsewhere herein, can be used to deposit separately or in combination a lysis buffer and a sample from a positive culture container into the lytic chamber 6020. In another embodiment, the lysis buffer can be added to the lytic

chamber 6020 of the separation device 6000 within the characterization/identification subsystem. For example, the sampling device 1902 can be used to obtain an aliquot of lysis buffer (e.g., from a lysis buffer reservoir) that can be subsequently deposited into the lytic chamber 6020 through the sample entry port 6016 (e.g., a pierceable septum) in the body 6002. Next, the sampling device 1902 can be used to obtain a sample from a positive specimen container 500 and deposit that sample into the lytic chamber 6020 through the lytic chamber port 6016. The lysis buffer and sample are then mixed within the lytic chamber 6020, e.g., by agitation and/or vortexing of the sampling device 6000. The selective lysis step is allowed to proceed for a sufficient time to allow the lysis reaction to be substantially completed (e.g., from 1 to 5 minutes). This selective lysis step selectively lyses undesired cells (i.e., non-microorganism cells) that may be present in the sample, e.g., blood cells and/or tissue cells. In another embodiment, the lytic chamber 6020 can be pre-loaded with a lysis buffer and the sample loaded to the lytic chamber prior to agitation and/or vortexing. In one embodiment, the sampling device 6000 can optionally be incubated to allow the selective lysis step to proceed more quickly.

[0485] After the lysis step, the lysed sample and lysis buffer can be transferred to the separation chamber 6040 through the a fluid flow channel 6030 for the separation of any microorganisms over a pre-loaded a density cushion, as described herein. The valve 6012 is pressed down externally by a mechanical actuator (not shown), thereby opening the fluid flow channel 6030 between the lytic chamber 6020 and the separation chamber 6040. A pump above the separation chamber 6040 draws the mixture through the fluid flow channel 6030 to the top of the separation chamber 6040. In one embodiment, by holding the separation device 6000 at an angle, the fluid can flow gently down the interior wall of the separation chamber 6040 and onto the density gradient.

[0486] The identification/characterization instrument 104 further includes a separation and/or concentration station, optionally in the form of a centrifuge, which operates on the separation device 6000 so as to separate the microbial agent from other products in the portion of the biological sample and concentrate the microbial agent within the separation device 6000. In one example, the microbial agent is concentrated in the form of a pellet or pellet-like mass in the bottom of the capillary tube 6060 of the separation device 6000.

[0487] The identification/characterization instrument further includes a identification and/or characterization module or read station (see, e.g., Figure 31, 1918) which interrogates the concentrated microbial agent to identify and/or characterize the microbial agent.

5 [0488] Another embodiment having a stacked chamber design is shown in Figures 98-108B.

[0489] Figures 95-99 illustrate a combined sampling and separation device 6100. As shown in Figures 95-96 and 98, the combined sampling and separation device 6100 includes an upper housing 6102, a lower housing 6104, and a flexible
10 pinch valve 6108 connecting the upper housing 6102 and lower housing 6104. As shown in Figures 96 and 97, the upper housing encloses an upper lytic chamber 6120, the lower housing encloses a lower separation chamber 6140, and the flexible pinch valve 6108 defines a fluid transfer channel 6130 therethrough. The upper lytic chamber 6120, fluid transfer channel 6130 and lower separation chamber 6140 can be
15 orientated around a central axis 6122.

[0490] The combined sampling and separation device 6100 further includes a pair of opposable compression tabs 6110, a valve actuator block 6106 and opposable actuator arms 6118 operable to “open” and “close” the flexible pinch valve 6110. In operation, the valve actuator block 6106 can be moved in a first direction (e.g.,
20 towards the compression tabs 6110, as represented by arrow 6107) to “open” the valve 6100. By moving the actuator block 6106 towards the compression tabs 6110 the actuator arms 6118 push up the compression tabs 6110 moving the compression tabs 6110 away from the flexible pinch valve thereby open the valve 6108. In the open position, the fluid flow channel 6130 is opened allowing fluid communication
25 between the upper lytic chamber 6120 and the lower separation chamber 6140 (as shown in Figure 97). The valve actuator block 6106 can also be moved in a second direction (e.g., away from the compression tabs 6110, as represented by arrow 6109) to “close” the valve 6108. When the actuator block 6106 is moved away from the compression tabs 6110 the actuator arms 6118 move the pair of opposable
30 compression tabs 6110 to a “closed” position, thereby pinching closed the flexible pinch valve 6108 (as shown in Figure 99).

[0491] As shown in Figures 95-96 and 98, the combined sampling and separation device 6100 also includes a syringe needle 6112 for obtaining a sample from a specimen container, and a vacuum port 6114 for pulling a vacuum within the

lytic chamber 6120, thereby assisting with loading of the device 6100. Optionally the syringe may further comprise a sheath (not shown) to protect the syringe needle from damage and/or contamination. Also, as shown in Figures 95-96 and 98, the combined sampling and separation device 6100 includes a vacuum port 6114. The vacuum port will include a gas permeable filter or hydrophobic membrane 6116 that allows gases to pass but prevents contamination. In operation, the vacuum port can be connected to a pump (not shown) that can apply a vacuum to the sampling and separation device 6100 for the uptake of a sample from a positive specimen container.

[0492] As shown in Figures 97 and 99, the separation chamber 6140 includes an upper reservoir 6142, a middle tapered section 6144 and a lower capillary tube 6146 all arranged around axis 6122 below the lytic chamber 6120. As shown, the middle tapered section 6144 connects the wider diameter upper reservoir 6142 and the smaller diameter capillary tube 6146. In one embodiment, the bottom wall 6150 of the capillary tube 6146 is made of an optically transparent material for facilitating optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6146. In another embodiment, the separation device 6100 is made of an optically transparent material to facilitate optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6146. As shown, the bottom wall 6150 opposite the capillary tube 6146 may be of a reduced thickness to facilitate optical interrogation as indicated in Figure 41.

[0493] In operation, with the flexible pinch valve 6108 in the closed position, the lytic chamber 6120 can be loaded with a lysis buffer and a sample taken from a positive culture container. In one embodiment, the lysis buffer can be added to the lytic chamber 6120 of the separation device 6100 using the syringe needle 6112. For example, the syringe needle 6112 can be used to obtain an aliquot of lysis buffer (e.g., from a lysis buffer reservoir), depositing the lysis buffer into the lytic chamber 6120. Next, the syringe needle 6112 can be used to obtain a sample from a positive specimen container 500, depositing that sample into the lytic chamber 6120. The lysis buffer and sample are then mixed within the lytic chamber 6120, e.g., by agitation and/or vortexing of the sampling device 6100. The selective lysis step is allowed to proceed for a sufficient time to allow the lysis reaction to be substantially completed (e.g., from 1 to 5 minutes). This selective lysis step selectively lyses undesired cells (i.e., non-microorganism cells) that may be present in the sample, e.g., blood cells

and/or tissue cells. In another embodiment, the lytic chamber 6120 can be pre-loaded with a lysis buffer and the sample loaded to the lytic chamber prior to agitation and/or vortexing. In still another embodiment, the sampling device 6100 can optionally be incubated to allow the selective lysis step to proceed more quickly.

5 **[0494]** After the lysis step, the lysed sample and lysis buffer can be transferred to the separation chamber 6140 through the a fluid flow channel 6130 for the separation of any microorganisms over a pre-loaded a density cushion, as described herein. To transfer the lysed sample and lysis buffer to the separation chamber 6140, the pair of opposable compression tabs 6110 are moved to the open
10 position, thereby opening the flexible pinch valve 6108 and allowing fluid communication between the lytic chamber 6120 and the separation chamber 6140 through the fluid flow channel 6130. With the flexible valve 6108 in the open position, the lysed sample and lysis buffer will flow via gravity through the fluid flow channel 6130 and onto the density cushion (not shown) contained in the separation
15 chamber 6140. In one embodiment, by holding the separation device 6100 at an angle, the fluid can flow gently down the interior wall of the separation chamber 6140 and onto the density gradient.

[0495] The identification/characterization instrument 104 further includes a separation and/or concentration station, optionally in the form of a centrifuge, which
20 operates on the separation device 6100 so as to separate the microbial agent from other products in the portion of the biological sample and concentrate the microbial agent within the separation device 6100. In one example, the microbial agent is concentrated in the form of a pellet or pellet-like mass in the bottom of the capillary tube 6160 of the separation device 6100.

25 **[0496]** The identification/characterization instrument further includes a identification and/or characterization module or read station (see, e.g., Figure 31, 1918) which interrogates the concentrated microbial agent to identify and/or characterize the microbial agent as described previously.

[0497] Another embodiment of the combined sampling and separation device
30 6300 is shown in Figures 100-102. Like the combined sampling and separation device shown in Figures 95-99, the combined sampling and separation device 6300 includes an upper housing 6302 enclosing a lytic chamber 6320, a lower housing 6104 enclosing a separation chamber 6340, and a flexible pinch valve 6308 defining therethrough a fluid transfer channel 6130.

[0498] The combined sampling and separation device 6300 further comprises a pair of opposable compression tabs 6310, a valve actuator block 6306 and opposable actuator arms 6318 operable to “open” and “close” the flexible pinch valve 6308. In operation, the valve actuator block 6306 can be moved in a first direction (e.g.,
5 towards the compression tabs 6310, as represented by arrow 6307) to “open” the valve 6308. By moving the actuator block 6306 towards the compression tabs 6310 the actuator arms 6318 push up the compression tabs 6310 moving the compression tabs 6310 away from the flexible pinch valve thereby open the valve 6308. In the open position, the fluid flow channel 6330 is opened allowing fluid communication
10 between the upper lytic chamber 6320 and the lower separation chamber 6140 (as shown in Figure 101). The valve actuator block 6306 can also be moved in a second direction (e.g., away from the compression tabs 6306) to “close” the valve 6308. When the actuator block 6306 is moved away from the compression tabs 6310 the actuator arms 6318 move the pair of opposable compression tabs 6310 to a “closed”
15 position, thereby pinching closed the flexible pinch valve 6308 (not shown).

[0499] As shown in Figures 100-102, the combined sampling and separation device 6300 also comprises a syringe needle 6312 for obtaining a sample from a positive specimen container, a valve port 6314 for pulling a vacuum within the lytic chamber 6320, thereby assisting with loading of the device 6300. Optionally the
20 syringe may further comprise a sheath (not shown) to protect the syringe needle from damage and/or contamination. The vacuum port will include a gas permeable filter or hydrophobic membrane 6116 that allows gases to pass but prevents contamination of the environment. The combined sampling and separation device further comprises a vacuum chamber, which is optionally pre-charged with a vacuum and operable
25 connected to the sampling and separation device 6300 via a valve 6360 to apply a vacuum to the sampling and separation device 6300 for the uptake of a sample from a positive specimen container.

[0500] Referring to Figure 102, the valve mechanism 6360 of this embodiment comprises a pump port 6370 that allows a pump (not shown) to operate
30 the plunger 6380 between a vacuum position and a venting position. The valve 6360 further comprises an interior chamber 6374, a vacuum port 6376, and a venting port 6378. In operation, the pump (not shown) can move the plunger to a first position or a vacuum position (as shown in Figure 104) thereby opening a fluid communication channel from said valve port 6372, through the interior chamber 6374 and through the

vacuum port 6376 to the vacuum chamber 6362. In the first position or vacuum position, the valve 6360 allows a vacuum to be applied to the sampling and separation device 6300, thereby controlling the uptake of a sample from positive specimen container. The plunger 6380 can also be moved to a second position or venting position thereby opening a fluid communication channel from said valve port 6372, through the interior chamber 6374 and through the vacuum port 6378, thereby allowing the sampling and separation device to vent a specimen container prior to the uptake of a sample via the vacuum.

[0501] As shown in Figure 101, the separation chamber 6340 may further comprise an upper reservoir 6342, a middle tapered section 6344 and a lower capillary tube 6346 all arranged around axis 6322 below the lytic chamber 6320. As shown, the middle tapered section 6344 connects the wider diameter upper reservoir 6342 and the smaller diameter capillary tube 6346. In one embodiment, the bottom wall 6350 of the capillary tube 6346 is made of an optically transport material for facilitating optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6346. In another embodiment, the separation device 6300 is made of an optically transparent material to facilitate optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6346. As shown, the bottom wall 6350 opposite the capillary tube 6346 may be of a reduced thickness to facilitate optical interrogation as indicated in Figure 101.

[0502] As one of skill in the art would appreciate, the sampling and separation device 6300 of this embodiment operates in a similar manner as the sampling and separation device 6100 of the first embodiment. Accordingly, a detailed description of the operation of this specific embodiment is excluded. After the lysis step has been carried out, the sampling and separation device 6300 of this embodiment can be centrifuged for separation and/or pelleting of any microorganisms contained therein. The sampling and separation device 6300 of this embodiment may be pre-loaded with a lysis buffer and/or a density cushion.

[0503] Referring now to Figures 103-104, a third embodiment of a combined sampling and separation device 6200 is shown. The combined sampling and separation device 6200 includes an upper housing 6202, a lower housing 6204, and a rotary connection 6206 connecting the upper housing 6202 and lower housing 6204. As shown in Figure 104, the upper housing encloses an upper lytic chamber 6220, the

lower housing encloses a lower separation chamber 6240, and the rotary connection 6206 defines a fluid transfer channel 6130 therethrough. The upper lytic chamber 6220, fluid transfer channel 6230 and lower separation chamber 6240 can be orientated around a central axis 6222, as shown in Figure 74.

5 **[0504]** In operation, the rotary connection 6206 can be rotated to an “open” position. In the open position, the fluid flow channel 6230 is opened allowing fluid communication between the upper lytic chamber 6220 and the lower separation chamber 6240 (as shown in Figure 104). The rotary connection 6206 can also be rotated to a “closed” position to close the fluid flow channel 6230. As shown in
10 Figure 104, the fluid flow channel comprises an upper opening or channel 6232 through the upper portion of the rotary connection 6208 and a lower opening or channel 6234 through the lower portion of the rotary connection 6210. The rotary connection 6206 of this embodiment may further comprise a sealing gasket 6218 between the upper portion of the rotary connection 6208 and the lower portion of the
15 rotary connection 6210, as shown in Figure 105, to prevent leaks.

[0505] As shown in Figures 103-105, the combined sampling and separation device 6200 also comprises a syringe needle 6212 for obtaining a sample from a positive specimen container, and a vacuum port 6214 for pulling a vacuum within the lytic chamber 6220, thereby allowing a sample to be loading into the lytic chamber
20 6260 of the device 6200. Optionally the syringe may further comprise a sheath (not shown) to protect the syringe needle from damage and/or contamination. The vacuum port 6214 will include a gas permeable filter or hydrophobic membrane 6216 that allows gases to pass but prevents contamination. In operation, the vacuum port 6214 can be connected to a pump (not shown) that can apply a vacuum to the sampling and
25 separation device 6200 for the uptake of a sample from a positive specimen container.

[0506] As shown in Figure 104, the separation chamber 6240 may further comprise an upper reservoir 6242, a middle tapered section 6244 and a lower capillary tube 6246 all arranged around axis 6222 below the lytic chamber 6220. As shown, the middle tapered section 6244 connects the wider diameter upper reservoir 6242 and the
30 smaller diameter capillary tube 6246. In one embodiment, the bottom wall 6250 of the capillary tube 6246 is made of an optically transparent material for facilitating optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6246. In another embodiment, the separation device 6200 is made of an optically transparent material to facilitate optical interrogation of a

concentrated microbial agent (not shown) located at the bottom of the capillary tube 6246. As shown, the bottom wall 6250 opposite the capillary tube 6246 may be of a reduced thickness to facilitate optical interrogation as indicated in Figure 104.

5 [0507] As one of skill in the art would appreciate, the sampling and separation device 6200 of this embodiment operates in a similar manner as the sampling and separation device 6100 of the first embodiment. Accordingly, a detailed description of the operation of this specific embodiment is excluded. After the lysis step has been carried out, the sampling and separation device 6200 of this embodiment can be centrifuged for separation and/or pelleting of any microorganisms
10 contained therein. The sampling and separation device 6200 of this embodiment may be pre-loaded with a lysis buffer and/or a density cushion.

[0508] Referring now to Figures 106-108B, another embodiment of a combined sampling and separation device 6400 is shown. The combined sampling and separation device 6400 includes an upper housing 6402, a lower housing 6404,
15 and a rotary valve 6406 connecting the upper housing 6402 and lower housing 6404. As shown in Figures 107B and 108B, the upper housing encloses an upper lytic chamber 6420, the lower housing encloses a lower separation chamber 6440, and the rotary valve 6406 defines a fluid transfer channel 6430 therethrough. The upper lytic chamber 6420, fluid transfer channel 6430 and lower separation chamber 6440 can be
20 orientated around a central axis 6422, as shown in Figures 77B and 78B.

[0509] In operation, the rotary valve 6406 can be rotated via a valve handle 6408 to an “open” position 6434 (see Figure 108B). In the open position, the fluid flow channel 6430 is opened allowing fluid communication between the upper lytic chamber 6420 and the lower separation chamber 6440 (as shown in Figure 108B).
25 The rotary valve 6406 can also be rotated to a “closed” position 6434 (see Figure 107B) to close the fluid flow channel 6430.

[0510] As shown in Figures 106-108B, the combined sampling and separation device 6400 also comprises a syringe needle 6412 for obtaining a sample from a positive specimen container, and a vacuum port 6414 for pulling a vacuum within the
30 lytic chamber 6420, thereby allowing a sample to be loading into the lytic chamber 6460 of the device 6400. Optionally the syringe may further comprise a sheath (not shown) to protect the syringe needle from damage and/or contamination. The vacuum port 6414 will include a gas permeable filter or hydrophobic membrane 6416 that

allows gases to pass but prevents contamination. In operation, the vacuum port 6414 can be connected to a pump (not shown) that can apply a vacuum to the sampling and separation device 6400 for the uptake of a sample from a positive specimen container.

[0511] As shown in Figures 107B and 108B, the separation chamber 6440 may further comprise an upper reservoir 6442, a middle tapered section 6444 and a lower capillary tube 6446 all arranged around axis 6422 below the lytic chamber 6420. As shown, the middle tapered section 6444 connects the wider diameter upper reservoir 6442 and the smaller diameter capillary tube 6446. In one embodiment, the bottom wall 6450 of the capillary tube 6446 is made of an optically transparent material for facilitating optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6446. In another embodiment, the separation device 6400 is made of an optically transparent material to facilitate optical interrogation of a concentrated microbial agent (not shown) located at the bottom of the capillary tube 6446. As shown, the bottom wall 6450 opposite the capillary tube 6446 may be of a reduced thickness to facilitate optical interrogation as indicated in Figures 107B and 108B.

[0512] As one of skill in the art would appreciate, the sampling and separation device 6400 of this embodiment operates in a similar manner as the sampling and separation device 6100 of the first embodiment. Accordingly, a detailed description of the operation of this specific embodiment is excluded. After the lysis step has been carried out, the sampling and separation device 6400 of this embodiment can be centrifuged for separation and/or pelleting of any microorganisms contained therein. The sampling and separation device 6400 of this embodiment may be pre-loaded with a lysis buffer and/or a density cushion.

T. Further Advantages and Features

[0513] A number of further advantages and features are obtained by the systems and methods described herein:

[0514] 1. The system detects the growth of microorganisms and facilitates sampling of a container once adequate microbial growth occurs so the microorganisms can be isolated, purified and characterized from blood (or other sample) and prepared for use in and tested in an ID, AST, molecular or other system.

[0515] 2. The system can provide for:

- Automated loading and unloading
- Automated incubation
- Automated agitation of culture specimen containers to accelerate antibiotic neutralization
- 5 - Automated detection system for improved time to detection,
- Sampling of the positive detection container at the time of detection and automatically prepare a purified sample and present the sample to an optical interrogation unit.
- 10 - Optional second detection technology for characterization of the purified sample,
- Automated calibration of optical detection system
- Automated waste disposal system
- [0516] 3. Automated clinical Gram, species-level identification antibiotic resistance marker and/or characterization within 15 min of positive bottle detection, with attendant significant clinical benefits
- 15 [0517] 4. Characterization and/or identification testing only performed on positive specimen containers.
- [0518] 5. More reliable characterization result (immediate sample during growth acceleration phase)
- 20 [0519] 6. Characterization during exponential phase cultures is possible, as is characterization during a stationary or stable phase.
- [0520] 7. Rapid Blood Culture Possibilities:
 - Opportunity/benefits for multiple samples of same bottle.
 - Incubate 4-8 hrs and then sample (stat mode)
 - 25 - Septicemia and/or screening negative specimen containers
- [0521] 8. Major workflow improvement
 - automate Gram result for blood cultures.
 - automated identification and/or characterization

- possible to provide purified sample for AST or molecular testing

[0522] 9. Supply of disposables to the identification and/or characterization instrument in a cartridge for easy loading

[0523] 10. Added disposable cost is only incurred for positive specimen containers (where there is clinical value)

[0524] 11. Potential to save cost for negative samples via sensorless bottle.

[0525] 12. Only one system for characterization and identification.

[0526] 13. Low complexity blood culture detection system.

[0527] 14. The identification and/or characterization system could be configured as an external, separate system but the advantage of being able to immediately sample positive specimen containers would be lost. Hence, the preferred embodiments couple the identification and/or characterization instrument to the detection instrument to enable automated transfer of positive specimen containers. The system can operate 24/7 with little or no human involvement.

[0528] 15. Potential for complete characterization at time of detection (intrinsic fluorescence spectroscopy, Raman spectroscopy, mass spectroscopy or other technology)

[0529] 16. Simplified manufacturing process for culture bottle.

[0530] 17. A combined CO₂ or other sensor could be included for:

- Compatibility with previous systems,
- Contamination detection during manufacturing, transport or storage,
- Accommodate delayed entry of specimen containers into a incubation/reading system.

[0531] 18. A memory device (such as RFID) could be included with the system to store:

- Data from an initial read of the bottle at time of sample collection (including time),
- Information from a test (could be used for post characterization),

- Manufacturing information (lot, date, expiration, initial readings, etc),
- Patient and sample information at time acquired at the time of collecting the sample.

5 [0532] 19. Conveyer input/output facilitating automation and high capacity installations.

[0533] 20. Automated loading/unloading (via robot transfer mechanism or conveyor).

[0534] 21. Design of the detection instrument without drawers improves internal system thermal stability by not exposing the incubator area to ambient air.

10 [0535] 22. Automatic moving of specimen containers from one position to another or from one rack to another if a rack fails (fault tolerance).

[0536] 23. Video camera with image analysis on the robot transfer mechanism in either the detection instrument and/or the identification/characterization instrument to aid in:

- 15
- location of specimen containers/disposables,
 - recovering from error conditions,
 - detecting spills,
 - for troubleshooting (field service can connect to the camera for remote diagnosis & repair).

20 [0537] 24. Expandability:
a) Internal capacity/functionality by adding racks/modules.
b) External by adding other instruments

[0538] 25. Measuring volume of blood present in the detection container
a) by weight or optically
25 b) or acoustically
c) or ultrasound scanning
d) or other method.

[0539] 26. Automation promotes "Load and Go" operation of the system. Once the specimen containers are supplied to an input conveyer or robotic transfer
30 mechanism, the rest of the operation is automated and the operator can attend to other tasks.

[0540] 27. Presenting the bottle at input or output to a fixed point in space for interface to another system.

[0541] 28. Automated preplanning before loading and rejecting error specimen containers to a return station.

[0542] 29. Verifying authentication of a product to ensure counterfeit specimen containers are not being used.

5 -- using a specific authentication method

-- using the internal camera to look for manufacturer logo, label features, etc.

[0543] 30. Password protection for access to positive specimen containers.

[0544] 31. Automated dispensing of negatives into bottle waste.

10 [0545] 32. Safety:

a) Elimination of sharps exposure for venting and sampling specimen containers

b) Reduction in laboratory personnel to biohazardous materials

c) Manual/Automated decontamination of disposables and/or system

15 d) Automated decontamination of stoppers prior to sampling

e) Automated venting of specimen containers to eliminate exposure to risk to laboratory personnel handling specimen containers that have high internal pressure due to gas producing organisms.

20

[0546] From the foregoing description, it will be appreciated that we have disclosed an automated system for rapid detection of a microbial agent in a sample and identifying and/or characterizing the microbial agent, comprising, in combination:

[0547] a detection instrument (100/102, Figures 1-30) receiving a specimen
25 container 500 containing a sample, the detection instrument including a heated enclosure for incubating the specimen container and a detection unit interrogating the specimen container to detect whether the specimen container is positive for the presence of a microbial agent in the sample; a supply of disposable separation devices (1904, could also be one of the combined sampling and separation devices of Figures
30 90-108); and an identification/characterization instrument (104, Figures 31-77) receiving a specimen container detected positive by the detection instrument, the characterization/identification instrument comprising: (a) a sample removal apparatus (1912, Figure 31) operative to remove a portion of the sample from the specimen container and add the portion to a separation device (1904) obtained from the supply

of separation devices; (b) a separation and concentration station (1916, Figure 31) operative on the separation device after receiving the portion of the sample so as to separate the microbial agent from other products in the sample and concentrate the microbial agent within the separation device; and (c) an identification and/or characterization module (1918, Figure 31) interrogating the concentrated microbial agent to identify and/or characterize the microbial agent.

[0548] In another aspect, we have disclosed a method for rapid identification and/or characterization of a microbial agent present in a sample, the sample loaded into a specimen container, comprising performing the following steps in an automated manner:

(a) incubating the specimen container 500; (b) periodically interrogating the specimen container to determine whether it is positive for presence of a microbial agent within the specimen container; (c) when the specimen container is determined positive, withdrawing a portion of the sample from the specimen container; (d) introducing the portion of the biological sample into a disposable separation device (1904); (e) lysing components in the portion of the biological sample, either before or after performing step (d); (f) separating and concentrating the microbial agent within the separation device; and (g) analyzing the concentrated microbial agent to identify and/or characterize the microbial agent. Steps (a)-(g) can be performed automatically within an integrated detection and identification/characterization instrument 104 as described above.

[0549] In one embodiment the analyzing step (g) comprises the step of performing a spectroscopic analysis of the concentrated microbial agent. In another embodiment the spectroscopic analysis comprise one of (i) intrinsic fluorescence spectroscopy or (ii) Raman spectroscopy.

[0550] The method may further include step (f)(1) withdrawing the concentrated microbial agent from the separation device and wherein step (g) is performed on the concentrated microbial agent after withdrawal from the separation device.

[0551] The analyzing step (g) may include at least one of (i) performing a spectroscopic analysis on the concentrated microbial agent, (ii) performing mass spectrometry on the concentrated microbial agent, (iii) performing molecular testing of the concentrated microbial agent and (iv) introducing the concentrated microbial

agent into a microbial characterization test device and subjecting the test device to analysis.

[0552] In the disclosed embodiments, steps (a) and (b) are performed in an automated detection instrument (100/102, Figures 1-30) and wherein steps (c), (d), (e) and (f) are performed in an automated identification and/or characterization instrument 104 (Figures 31-77).

[0553] In yet another aspect, an automated method for rapid characterization and/or identification of a microbial agent has been disclosed, comprising the steps of: (a) providing a detection subsystem (100/102) having an entrance location for receiving specimen containers 500 containing samples to be tested, and detection apparatus for detecting when a specimen container has become positive for presence of a microbial agent in a sample within the specimen container; (b) when a specimen container has become positive, automatically moving the specimen container to an identification and/or characterization subsystem remote from the detection subsystem (Figure 77); (c) within the characterization and/or identification subsystem, automatically performing the steps of: (1) withdrawing a portion of the sample from the specimen container; (2) dispensing the withdrawn portion of the sample into a disposable separation device; (3) concentrating the microbial agent within the withdrawn portion of the sample within the separation device; and (4) analyzing the concentrated microbial agent to characterize and/or identify the microbial agent.

[0554] In yet another aspect, an automated method for rapid characterization and/or identification of a microbial agent has been disclosed, comprising the steps of: (a) receiving a specimen container containing a sample to be tested in a detection instrument (100/102, Figures 1-30); (b) detecting when the specimen container has become positive for presence of a microbial agent in the sample; (c) when such detection has occurred, automatically removing a portion of the sample from the specimen container and placing the portion of the sample into a disposable separation device (1904); (d) separating and concentrating the microbial agent in a disposable separation device, and (e) analyzing the concentrated microbial agent to identify and/or characterize the microbial agent.

[0555] In still another aspect, a sample testing system has been disclosed, comprising, in combination: a detection subsystem (100/102, Figures 1-30) having an entrance location for receiving specimen containers containing samples to be tested, and detection apparatus for detecting when a specimen container has become positive

for presence of a microbial agent in the sample; an identification and/or characterization subsystem receiving a positive specimen container and having modules for automatically characterizing and/or identifying the microbial agent in the positive specimen container; and apparatus for automatically transferring a positive
5 detection container from the detection subsystem to the identification and/or characterization subsystem.

[0556] The apparatus for automatically transferring can take the form of a conveyor system as shown in Figure 77. The automatically transferring can also take the form of a a robotic arm assembly as shown in Figures 32, 35 and 57. In still
10 another embodiment, the conveyor system includes a first conveyor and a second conveyor, and wherein the system comprises two detection subsystems daisy chained and coupled to each other by the first conveyor, and wherein the one of the detection subsystems is coupled to the identification and/or characterization subsystem by the second conveyor, and wherein a positive specimen detected in either the two detection
15 subsystems is moved to the identification and/or characterization subsystem by means of the second conveyor.

[0557] Presently preferred and alternative embodiments of the inventive automated identification and/or characterization instrument have been described with particularity. However, persons skilled in the art will understand that variation from
20 the details of the disclosed embodiments may be made. All questions concerning the scope of the invention are to be answered by reference to the appended claims.

CLAIMS

We claim:

1. An automated system for rapid detection of a microbial agent in a
5 sample and identifying and/or characterizing the microbial agent, comprising, in combination:

a detection instrument receiving a specimen container containing a sample, the detection instrument including a heated enclosure for incubating the specimen container and a detection unit interrogating the specimen container to detect whether
10 the specimen container is positive for the presence of a microbial agent in the sample;

a supply of disposable separation devices; and

an identification/characterization instrument receiving a specimen container detected positive by the detection instrument, the characterization/identification instrument comprising:

15 (a) a sample removal apparatus operative to remove a portion of the sample from the specimen container and add the portion to a separation device obtained from the supply of separation devices;

(b) a separation and concentration station operative on the separation device after receiving the portion of the sample so as to separate the microbial agent from
20 other products in the sample and concentrate the microbial agent within the separation device; and

(c) an identification and/or characterization module interrogating the concentrated microbial agent to identify and/or characterize the microbial agent.

25 2. The system of claim 1, further comprising a robotic transfer mechanism for transferring the positive specimen container from the detection instrument to the identification/characterization instrument.

30 3. The system of claim 2, wherein the detection instrument and the identification/characterization instrument are integrated into a single system and operate in a substantially fully automated manner to detect a specimen container for being positive for microbial growth and the identify and/or characterize the microbial agent.

4. The system of claim 2, wherein the specimen container comprises a blood culture bottle and wherein the biological sample comprises a sample of blood.

5. The system of claim 1, wherein the specimen container comprises a pierceable element, and wherein the identification/characterization instrument includes a supply of disposable sampling devices and operates to remove the portion of the sample from the specimen container using one of the sampling devices.

6. The system of claim 5, wherein the sampling device contains a selective lysis buffer.

7. The system of claim 1, wherein the separation and concentration station comprises a centrifuge, and wherein the centrifuge concentrates the microbial agent in a portion of the separation device.

8. The system of claim 7, wherein the separation device contains at least one of a density cushion and a selective lysis buffer.

9. The system of claim 7, wherein the separation device comprises an internal capillary tube and wherein a peripheral portion of the capillary tube contains the concentrated microbial agent, and wherein the identification and/or characterization module operates to interrogate the concentrated microbial agent within the separation device.

10. The system of claim 9, wherein the separation device is made at least in part from a transparent material.

11. The system of claim 10, wherein the identification and/or characterization module includes instrumentation to detect intrinsic fluorescence of the concentrated microbial agent.

12. The system of claim 1, wherein the identification and/or characterization module comprises a system for performing Raman Spectroscopy on the concentrated microbial agent.

13. The system of claim 1, wherein the identification and/or characterization module includes a sub-system for obtaining a sample of the concentrated microbial agent from the separation device and interrogates the concentrated microbial agent after removal from the separation device.

14. The system of claim 1, wherein the identification and/or characterization instrument comprises a source of disposable sampling devices pre-loaded with a selective lysis buffer.

15. The system of claim 1, wherein the identification and/or characterization instrument includes a plurality of containers each containing a different selective lysis buffer.

16. A method for rapid identification and/or characterization of a microbial agent present in a sample, the sample loaded into a specimen container, comprising performing the following steps in an automated manner:

- (a) incubating the specimen container;
- (b) periodically interrogating the specimen container to determine whether it is positive for presence of a microbial agent within the specimen container;
- (c) when the specimen container is determined positive, withdrawing a portion of the sample from the specimen container;
- (d) introducing the portion of the biological sample into a disposable separation device;
- (e) lysing components in the portion of the biological sample, either before or after performing step (d);
- (f) separating and concentrating the microbial agent within the separation device; and
- (g) analyzing the concentrated microbial agent to identify and/or characterize the microbial agent.

17. The method of claim 16, wherein steps (a)-(g) are performed automatically within an integrated detection and identification/characterization instrument.

18. The method of claim 16, wherein the analyzing step (g) comprises the step of performing a spectroscopic analysis of the concentrated microbial agent.

5 19. The method of claim 18, wherein the spectroscopic analysis comprise one of (i) intrinsic fluorescence spectroscopy or (ii) Raman spectroscopy.

20. The method of claim 16, further comprising step (f)(1) withdrawing the concentrated microbial agent from the separation device and wherein step (g) is
10 performed on the concentrated microbial agent after withdrawal from the separation device.

21. The method of claim 18, wherein the analyzing step (g) comprises at least one of (i) performing a spectroscopic analysis on the concentrated microbial
15 agent, (ii) performing mass spectrometry on the concentrated microbial agent, (iii) performing molecular testing of the concentrated microbial agent and (iv) introducing the concentrated microbial agent into a microbial characterization test device and subjecting the test device to analysis.

20 22. The method of claim 16, wherein step (f) comprises the step of centrifuging the separator device.

23. The method of claim 16, wherein step (c) comprises the step of withdrawing a portion of the sample into a disposable sampling device and wherein
25 step (d) comprises dispensing the portion of the sample into the separation device.

24. The method of claim 23, wherein one of the disposable sampling device or the separation device is pre-loaded with a selective lysis buffer.

30 25. The method of claim 23, further comprising the step of mixing the portion of the sample with a selective lysis buffer within a disposable sampling device used to withdraw the portion of the sample from the specimen container, wherein the mixing step is performed prior to performing step (d).

26. The method of claim 16, wherein steps (a) and (b) are performed in an automated detection instrument and wherein steps (c), (d), (e) and (f) are performed in an automated identification and/or characterization instrument.

5 27. The method of claim 26, further comprising the step of automatically transferring a specimen container from the automated detection instrument to the automated identification and/or characterization instrument.

10 28. The method of claim 27, wherein the automated detection instrument is incorporated within the automated identification and/or characterization instrument.

29. An automated method for rapid characterization and/or identification of a microbial agent, comprising the steps of:

15 (a) providing a detection subsystem having an entrance location for receiving specimen containers containing samples to be tested, and detection apparatus for detecting when a specimen container has become positive for presence of a microbial agent in a sample within the specimen container;

20 (b) when a specimen container has become positive, automatically moving the specimen container to an identification and/or characterization subsystem remote from the detection subsystem;

 (c) within the characterization and/or identification subsystem, automatically performing the steps of:

 (1) withdrawing a portion of the sample from the specimen container;

25 (2) dispensing the withdrawn portion of the sample into a disposable separation device;

 (3) concentrating the microbial agent within the withdrawn portion of the sample within the separation device; and

 (4) analyzing the concentrated microbial agent to characterize and/or identify the microbial agent.

30

30. The method of claim 29, wherein the sample comprises a biological sample and wherein the method further comprises the step of lysing components present in the withdrawn portion of the sample.

31. The method of claim 29, wherein the method further comprises the step of removing the concentrated microbial agent from the separation device and performing the analyzing step (e)(4) after removal of the concentrated microbial agent from the separation device.

5

32. The method of claim 29, wherein step (e)(4) is performed while the microbial agent is concentrated within the separation device.

33. The method of claim 31, wherein the analyzing step (e)(4) comprises performing a spectroscopic analysis on the concentrated microbial agent.

10

34. The method of claim 32, wherein the spectroscopic analysis comprises intrinsic fluorescence spectroscopy.

35. The method of claim 29, wherein the concentrating step (e)(3) comprises centrifuging the separator device.

15

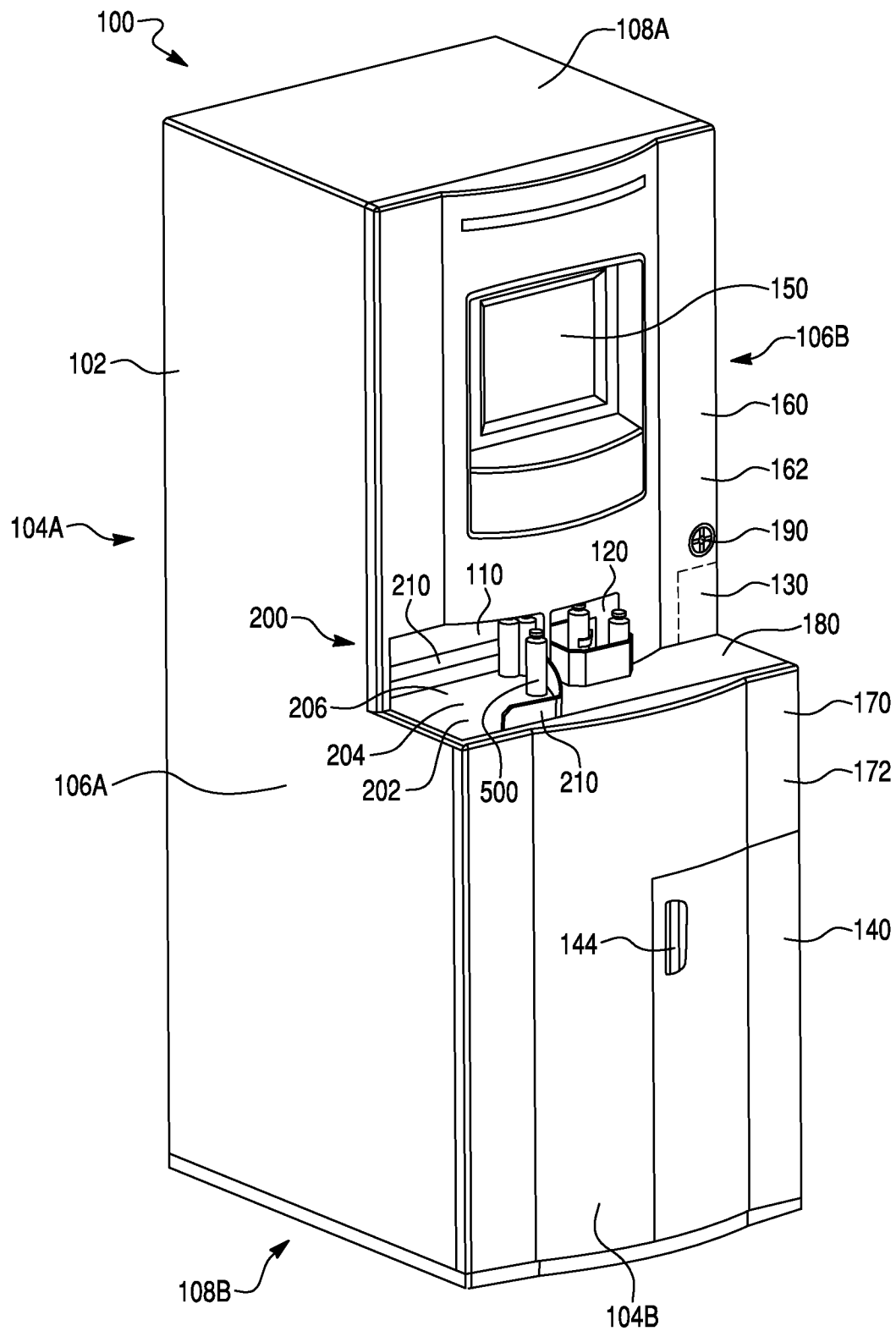
36. The method of claim 29, wherein the sample comprises a blood and wherein the specimen container comprises a blood culture bottle.

20

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Fig. 1



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Fig. 2

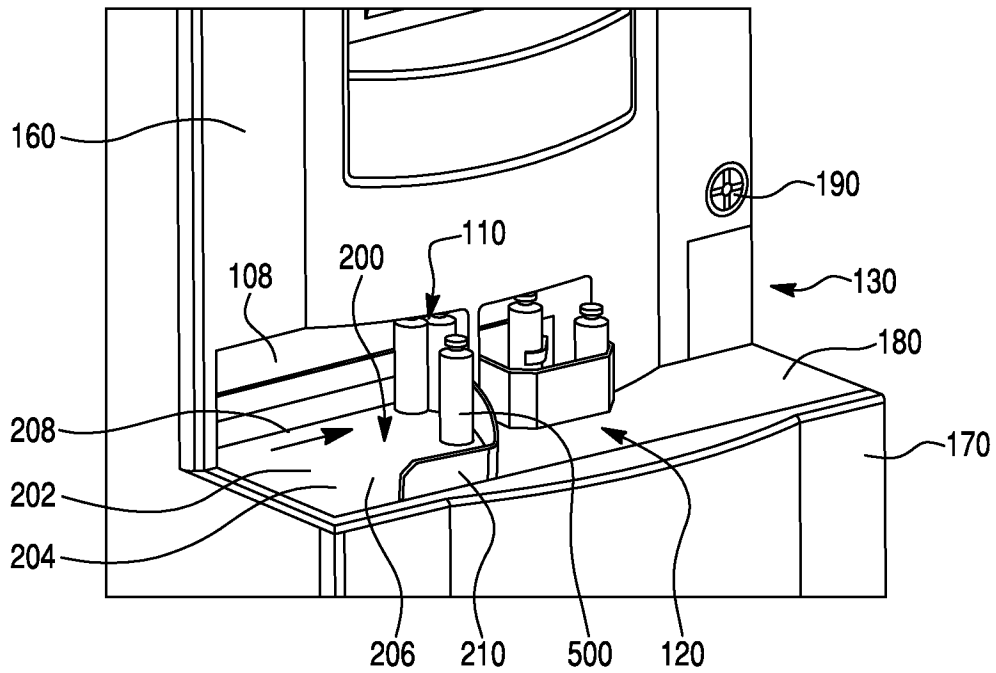


Fig. 3

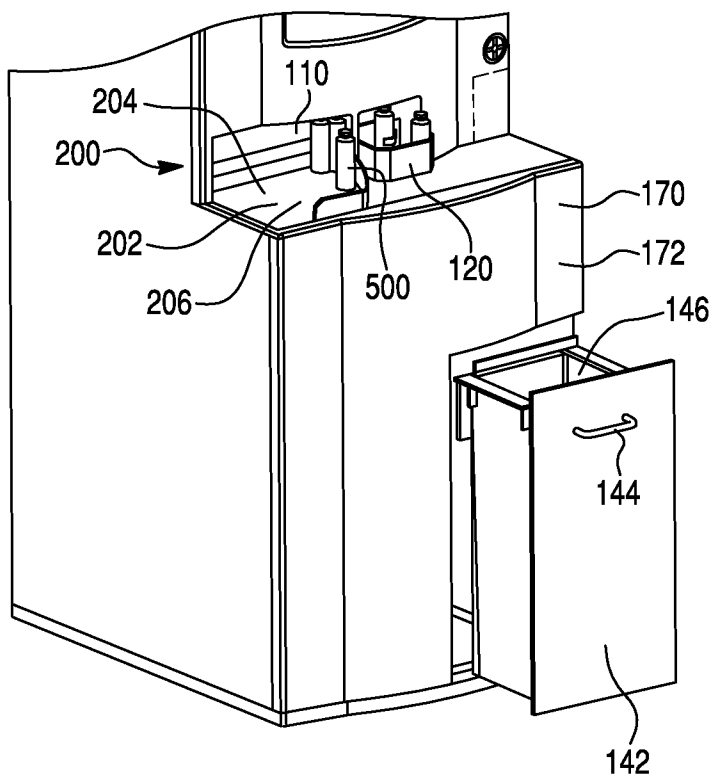


Fig. 4

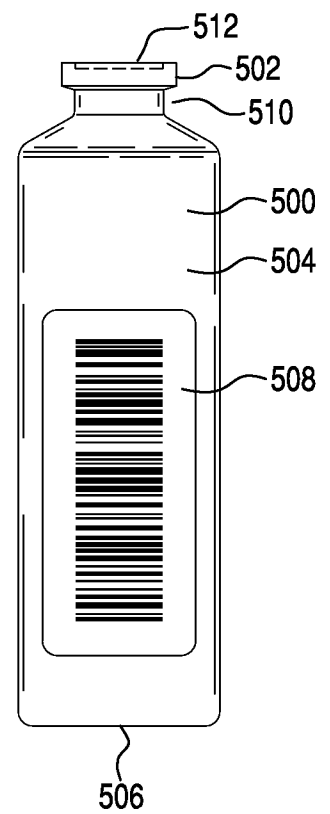


Fig. 5B

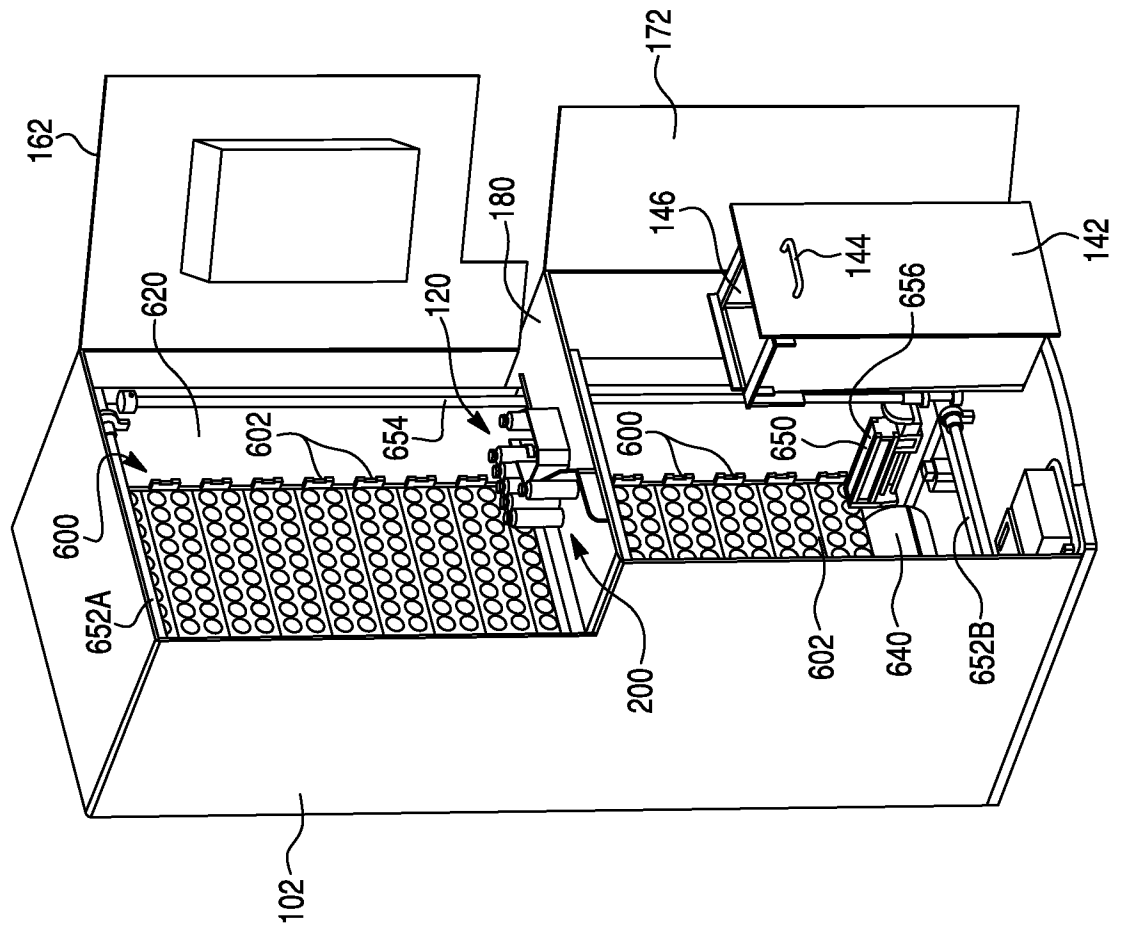
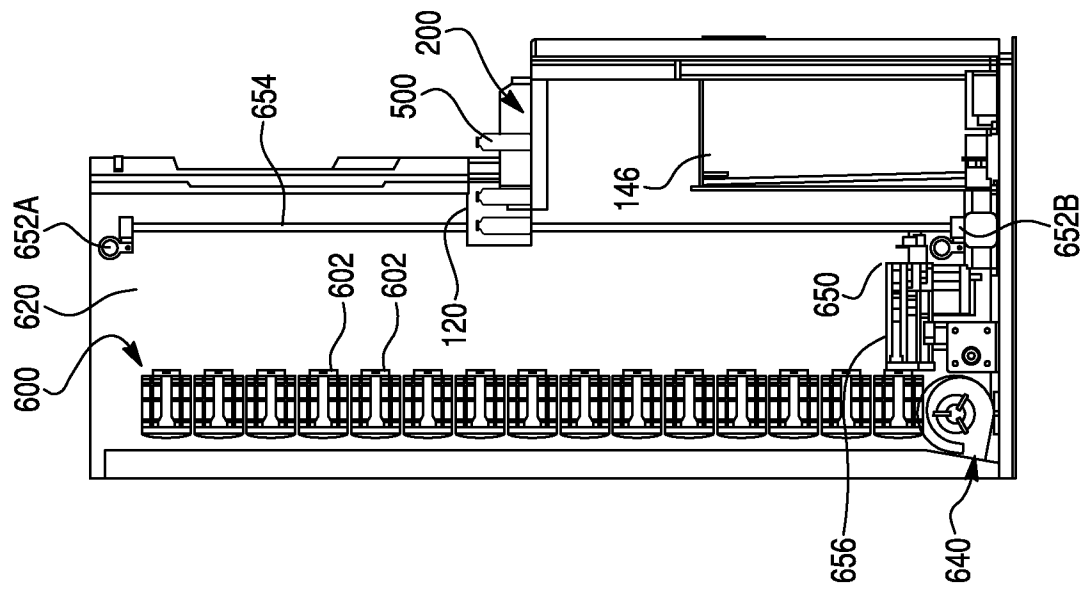
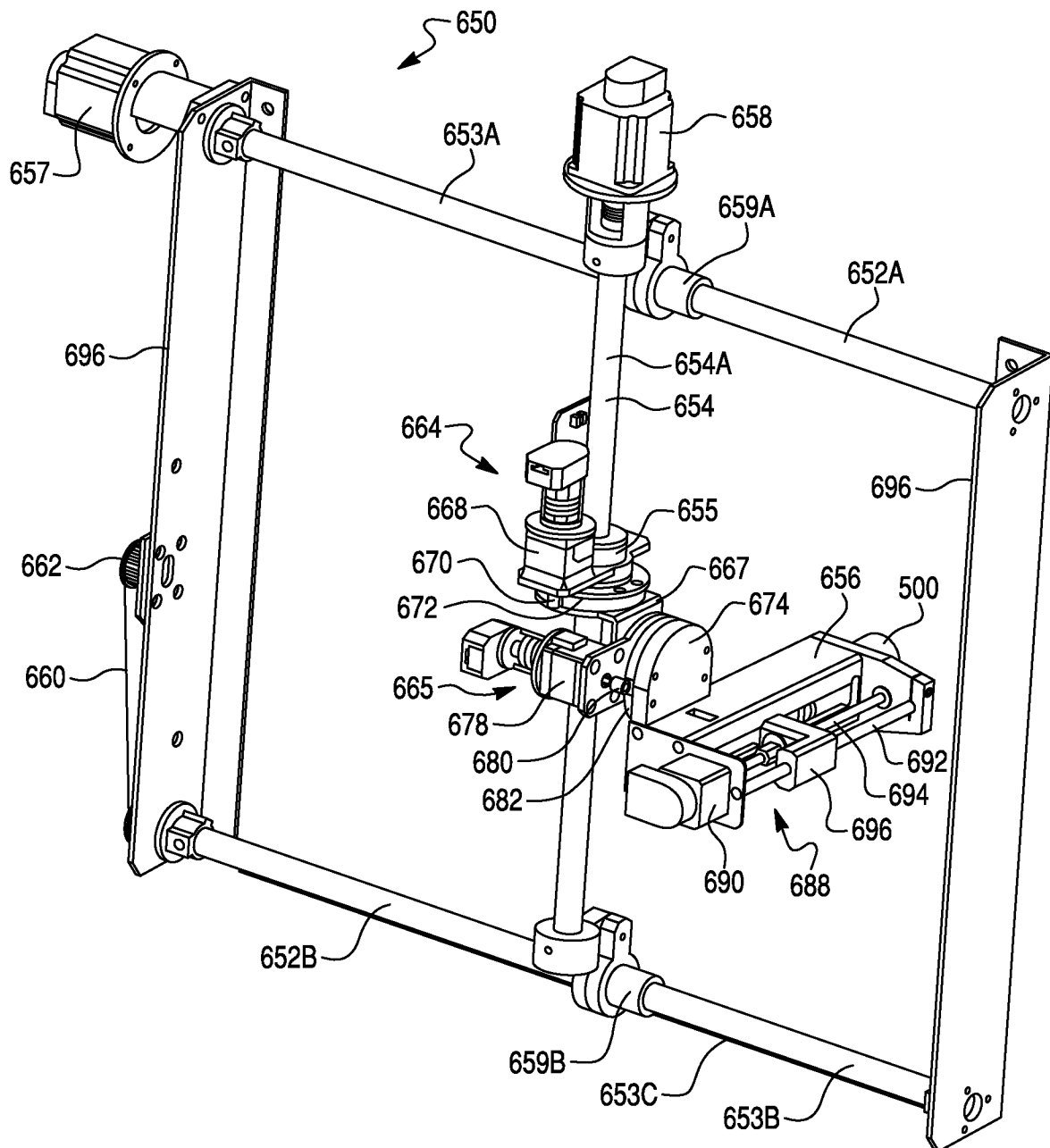


Fig. 5A



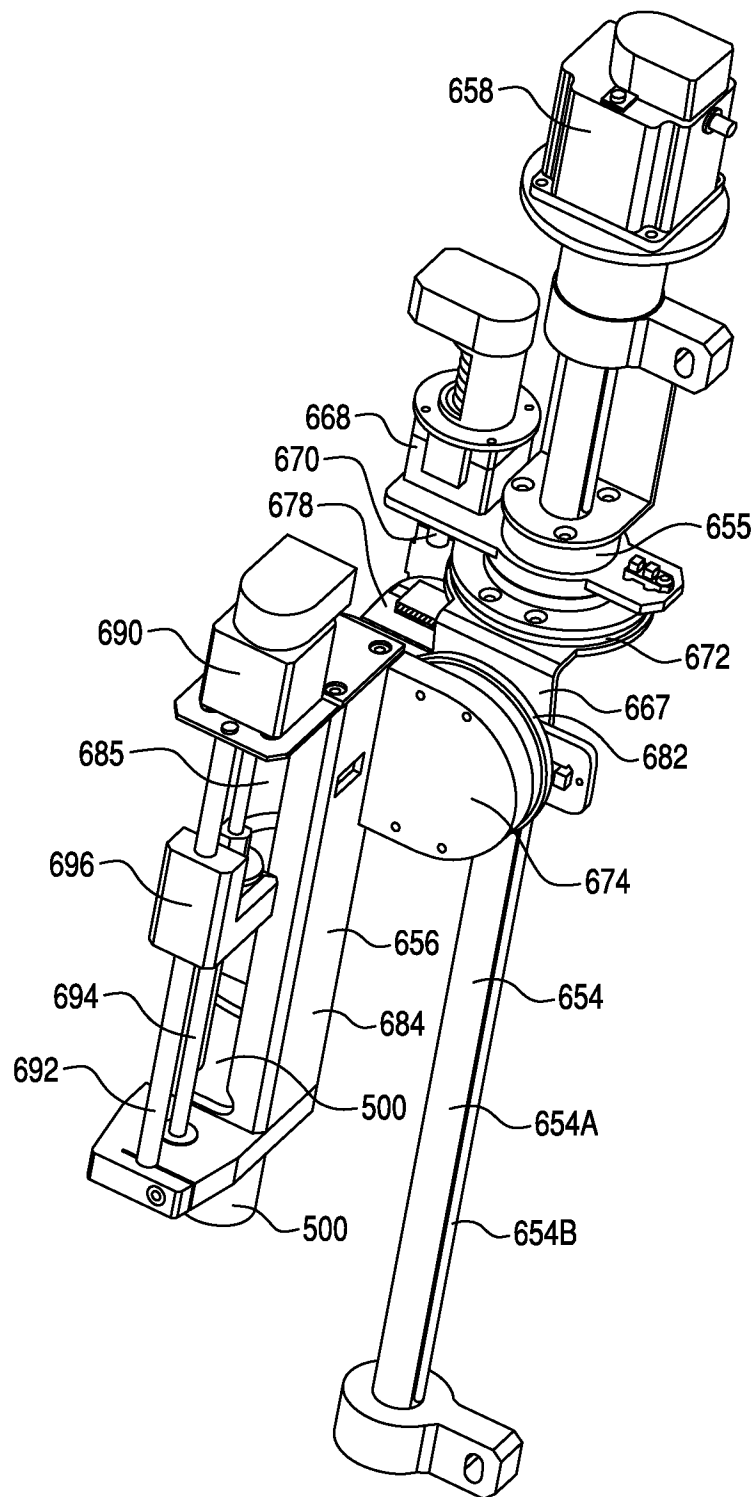
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Fig. 6



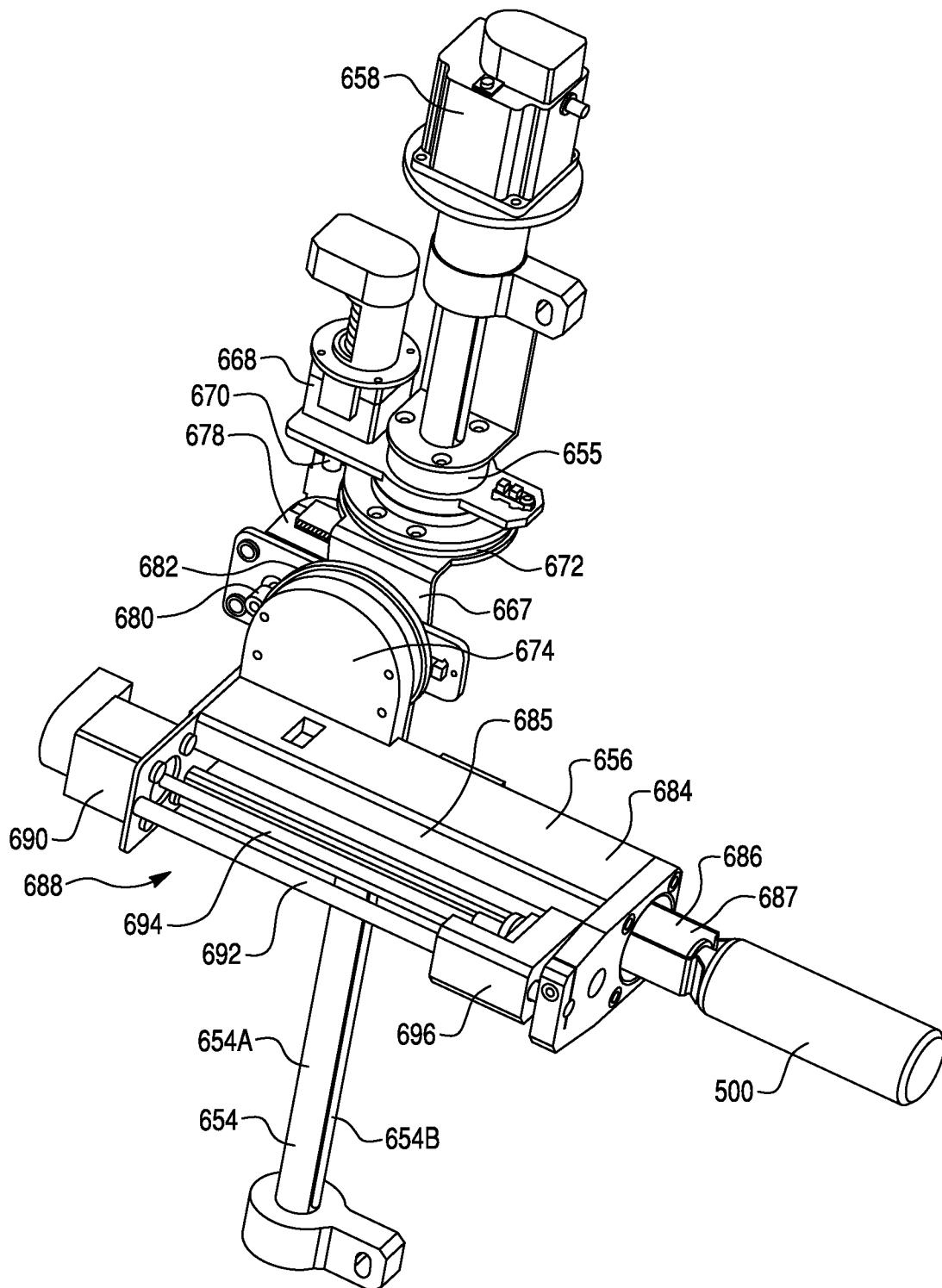
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Fig. 7A



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Fig. 7B



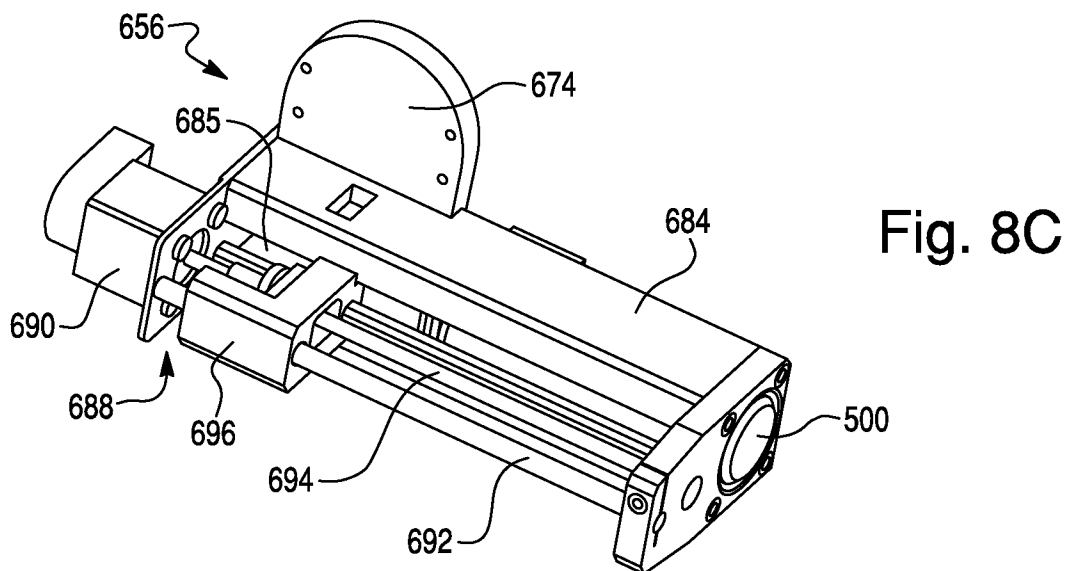
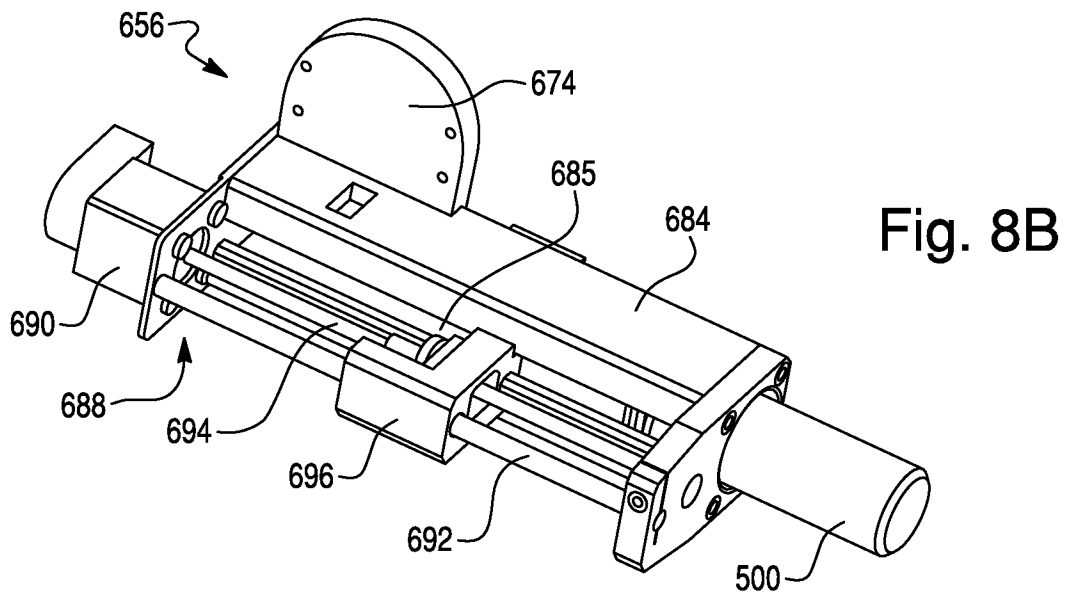
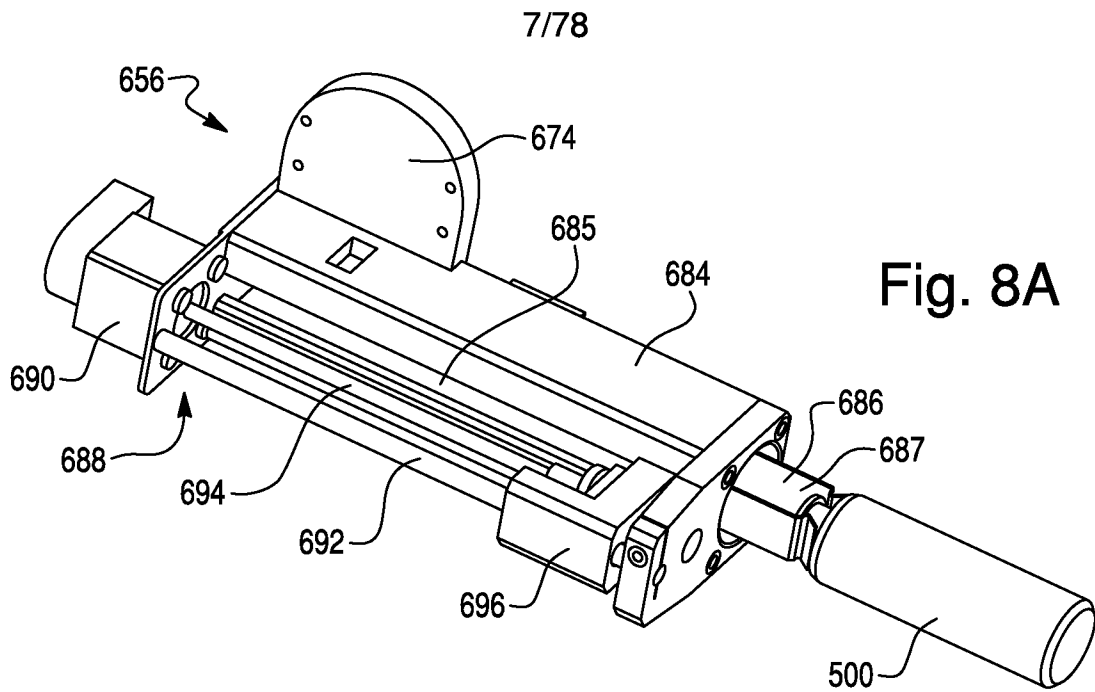


Fig. 9B

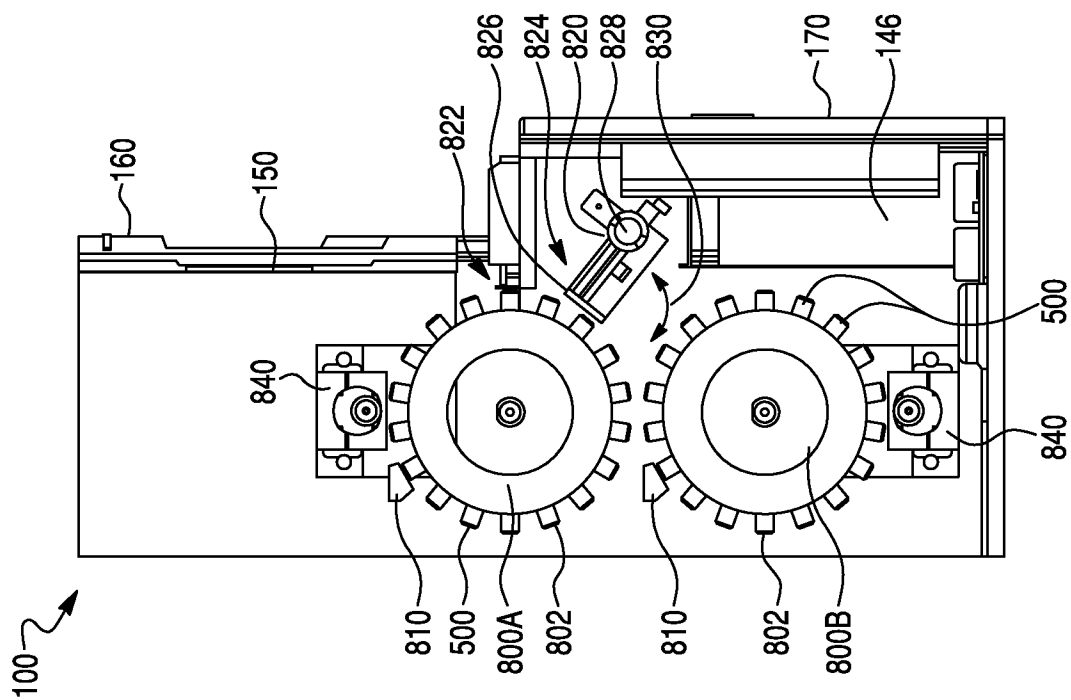
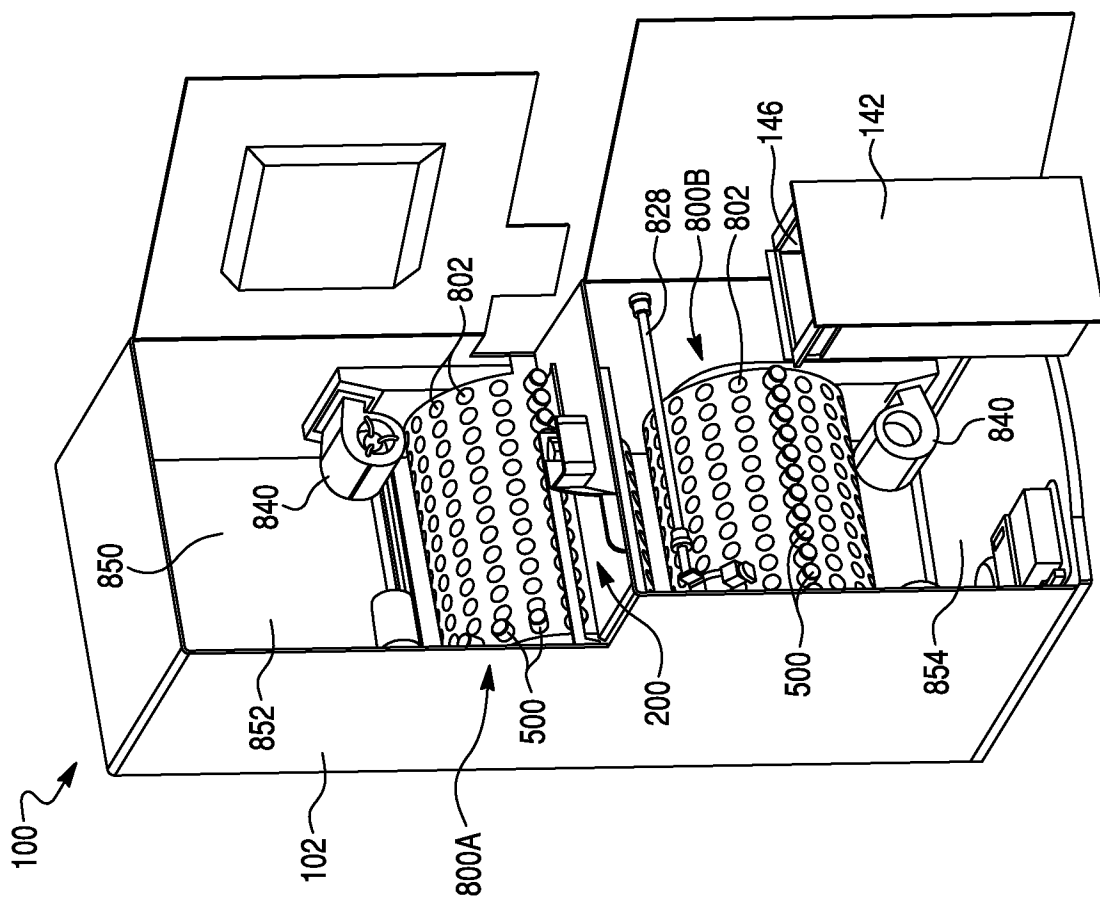


Fig. 9A



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Fig. 10

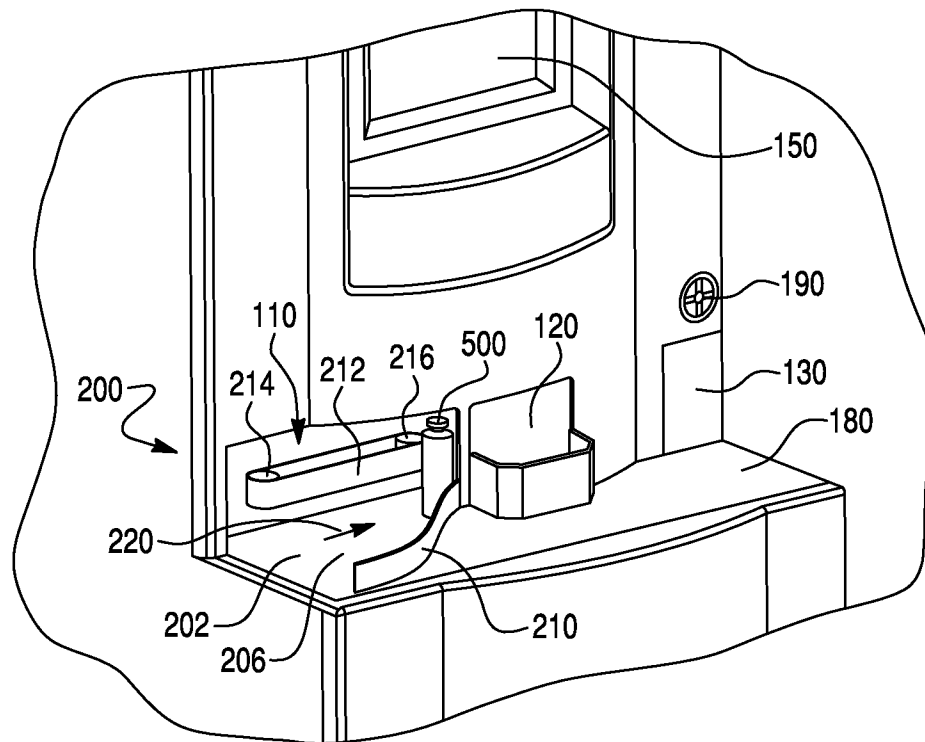
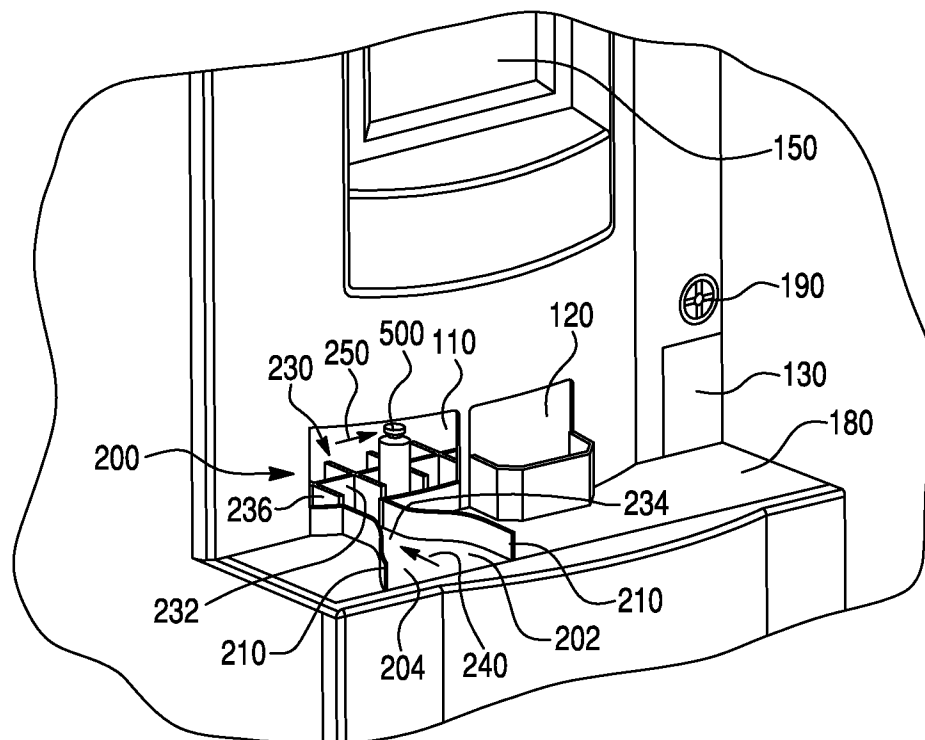
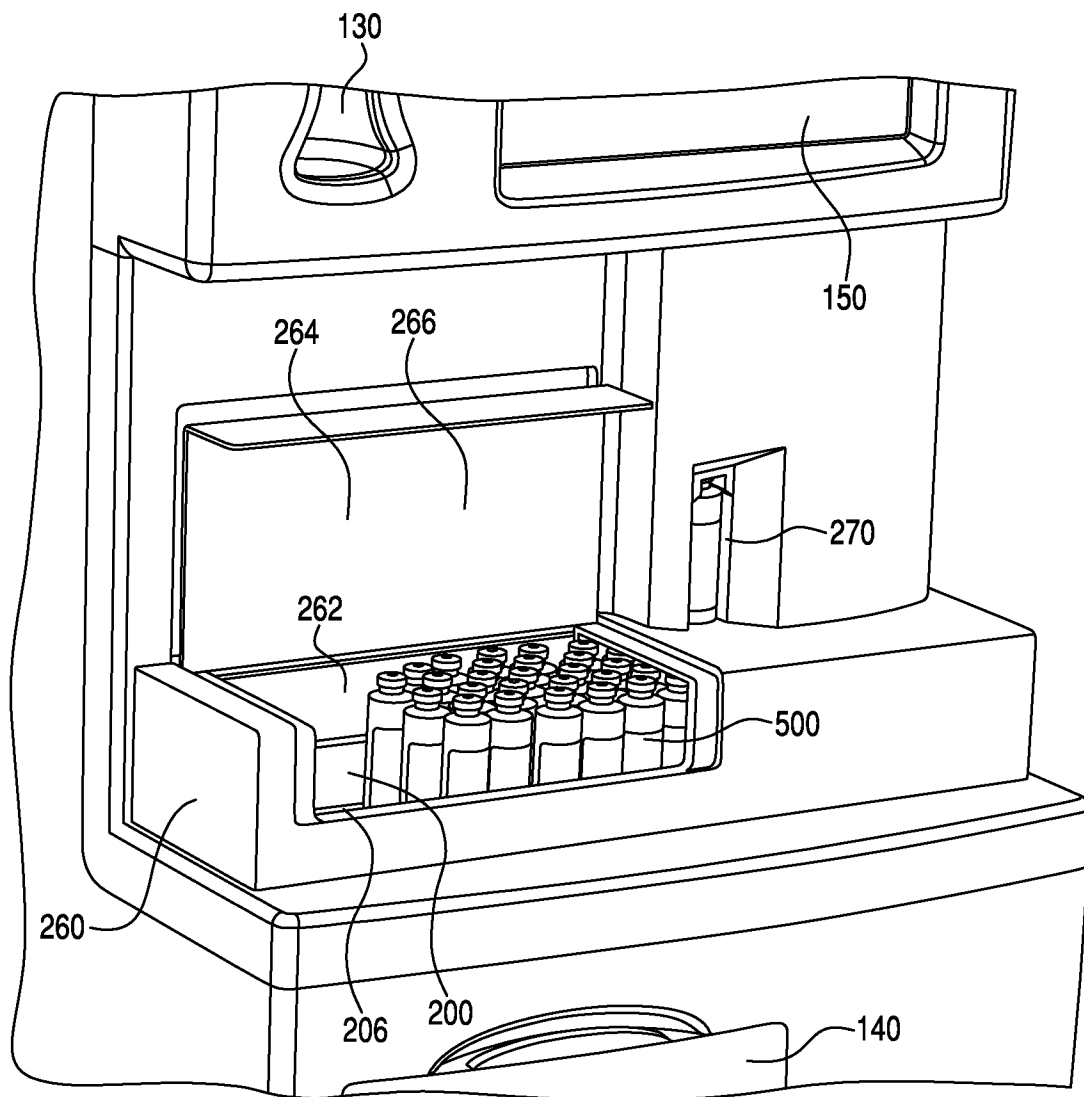


Fig. 11



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Fig. 12



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Fig. 13

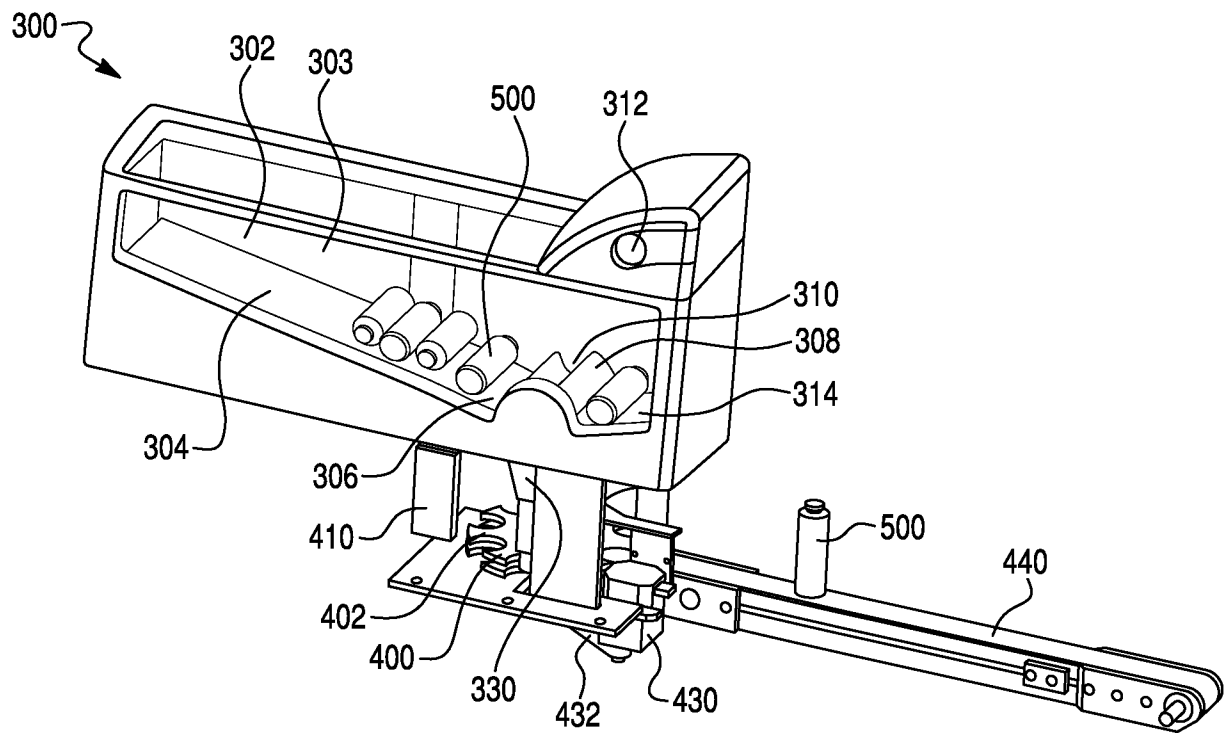


Fig. 14

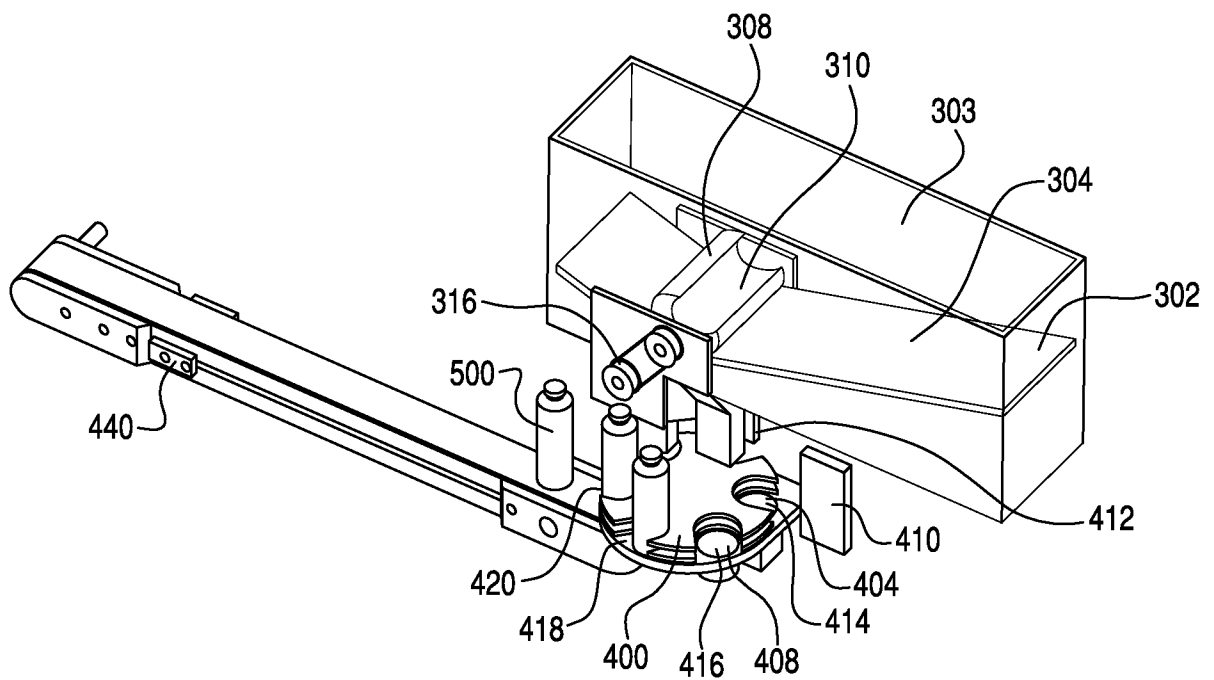


Fig. 15

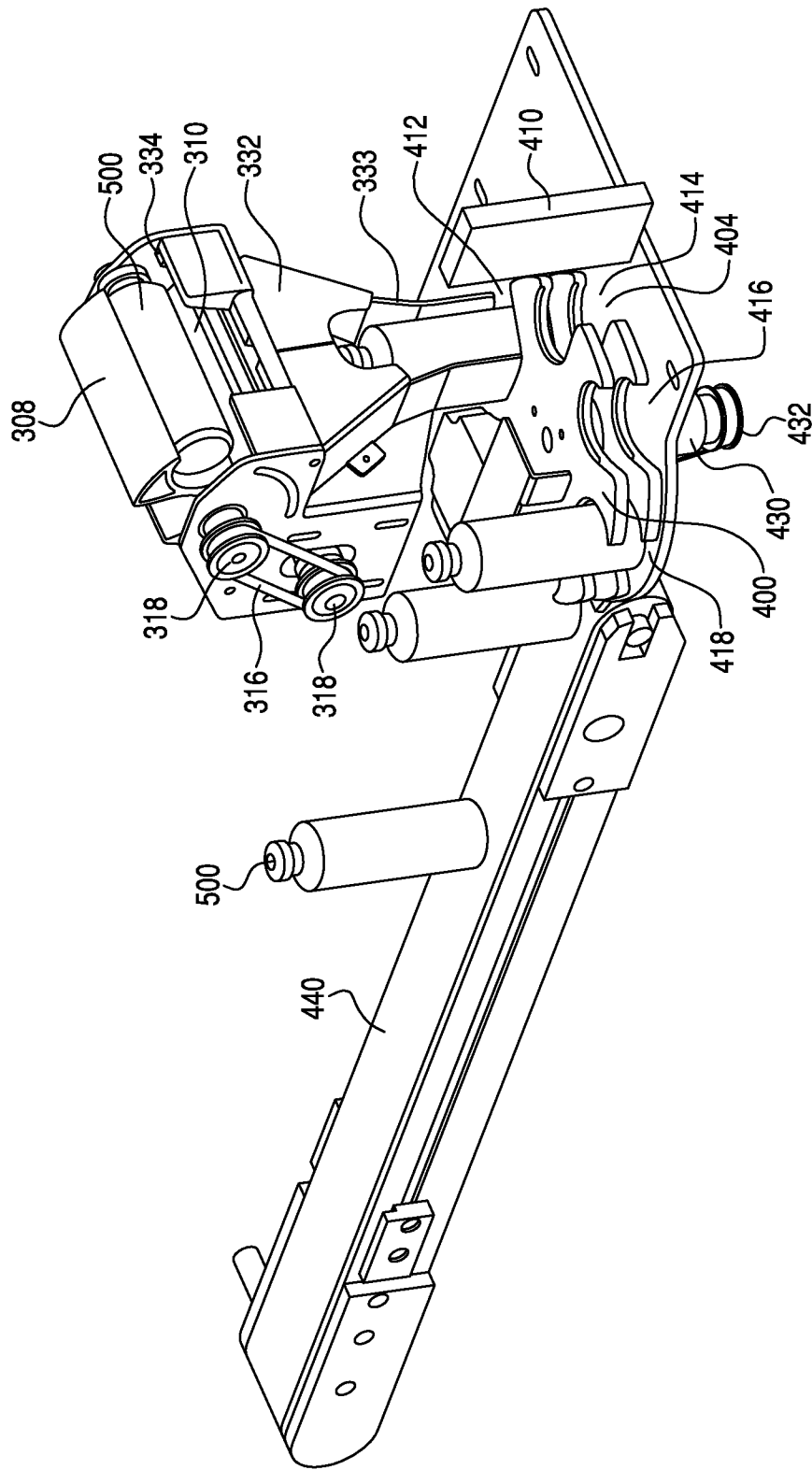


Fig. 16

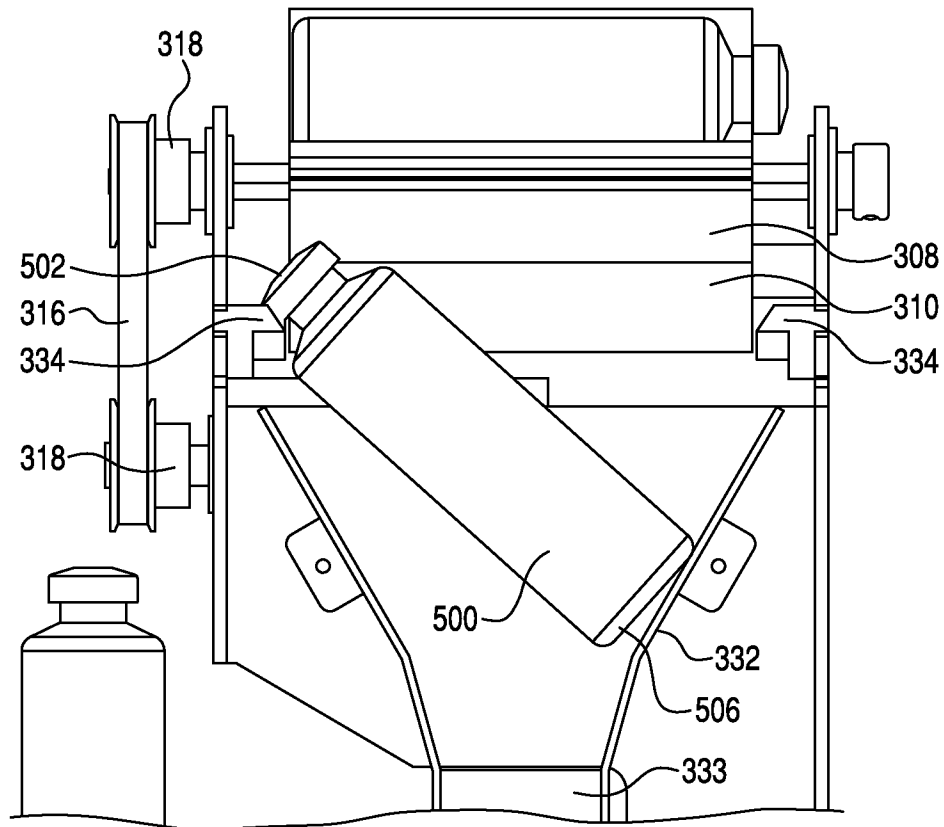


Fig. 17

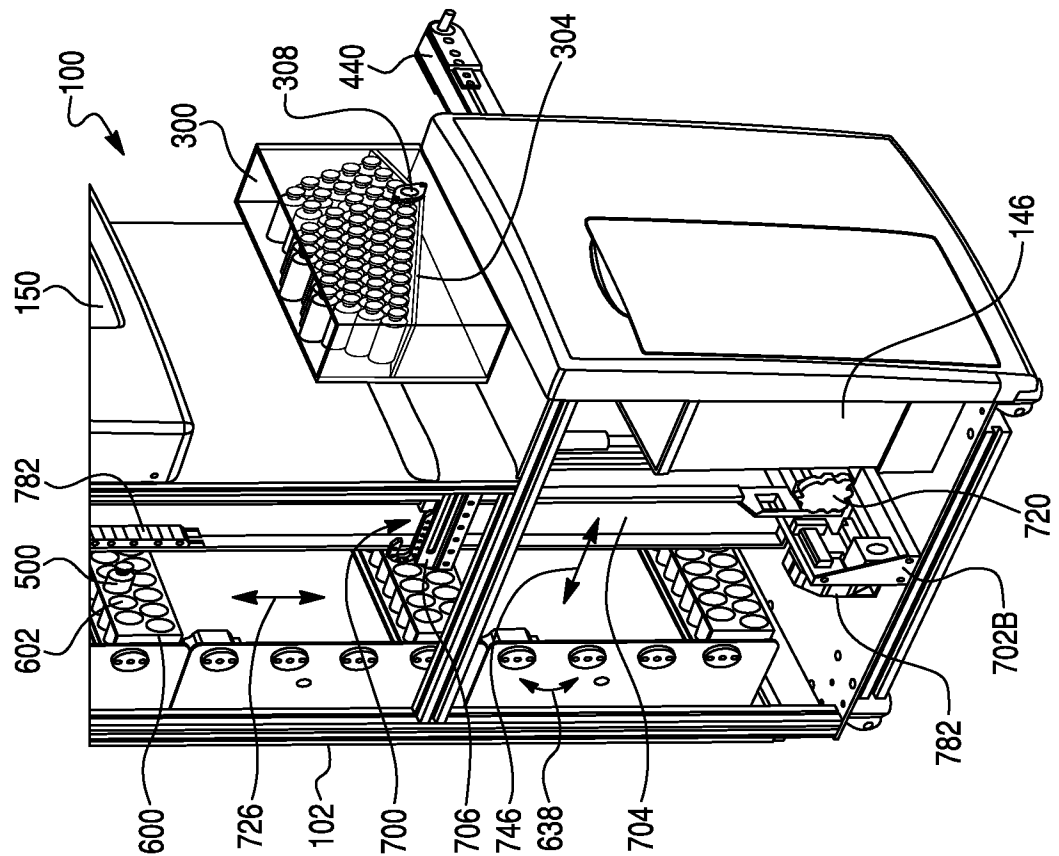


Fig. 18

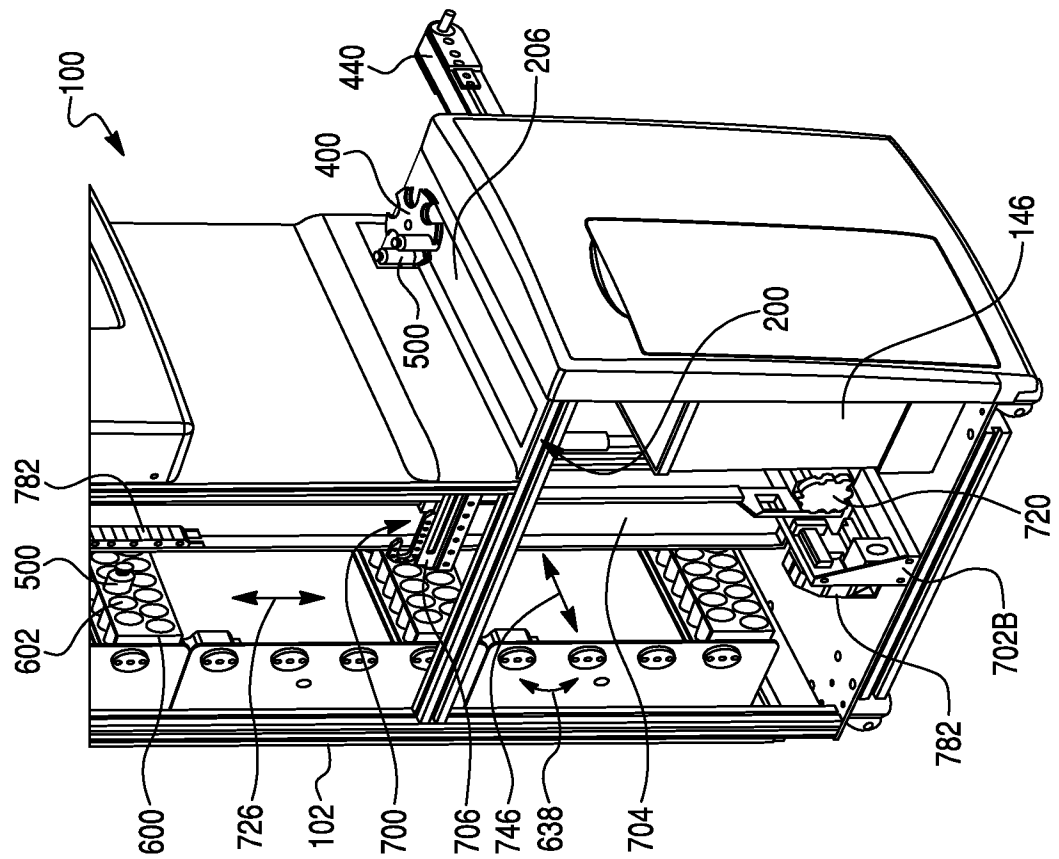
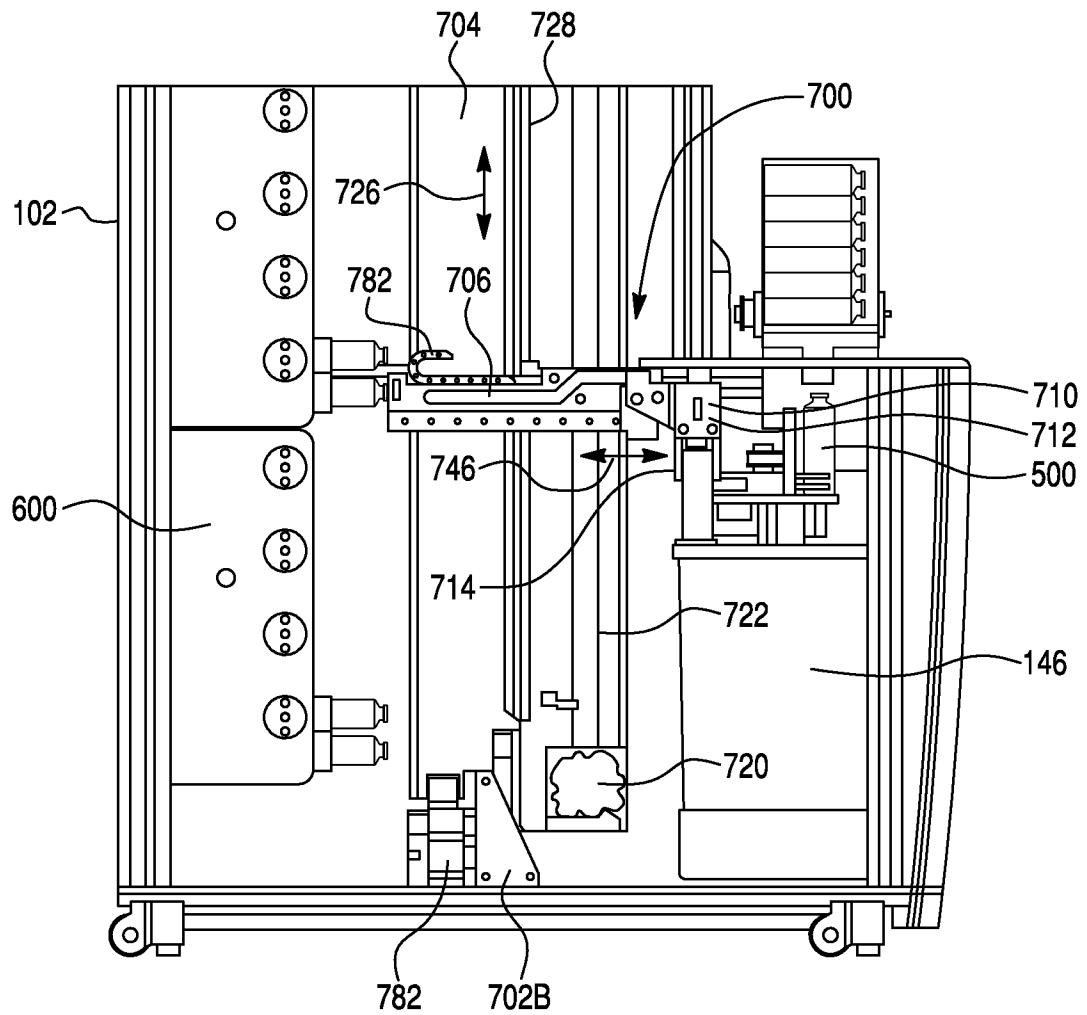
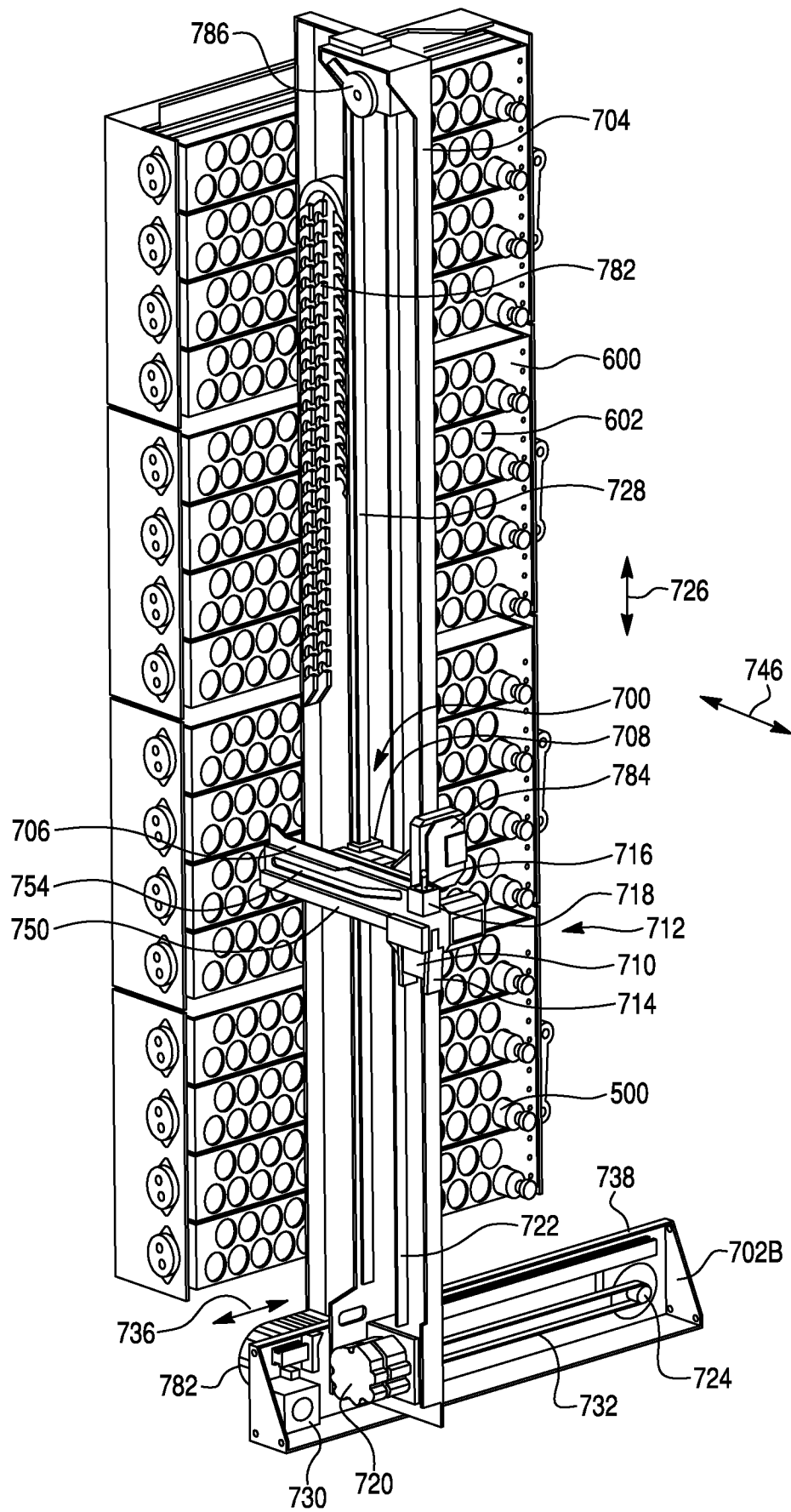


Fig. 19



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Fig. 20



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Fig. 21A

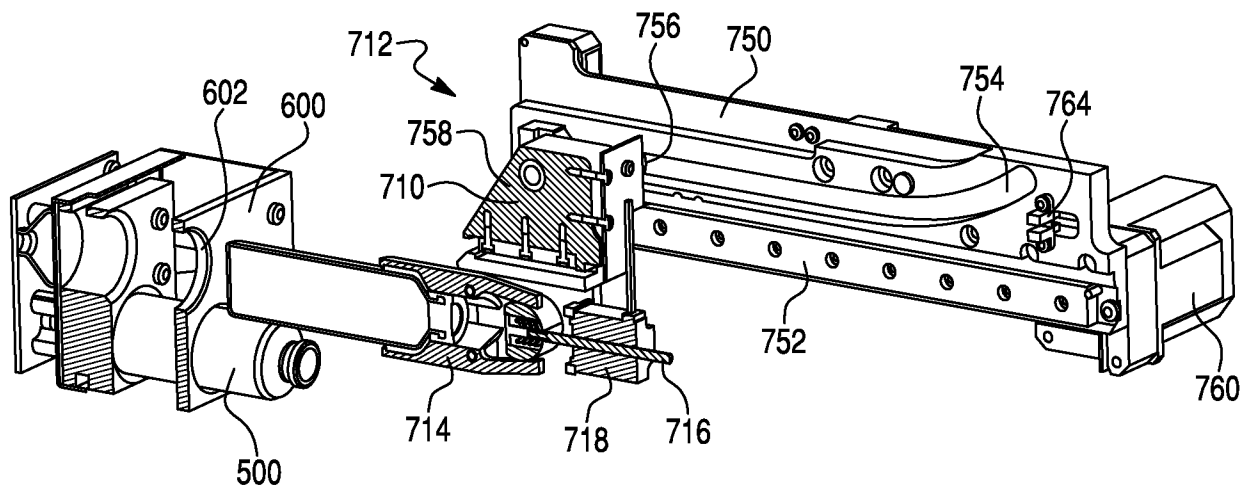
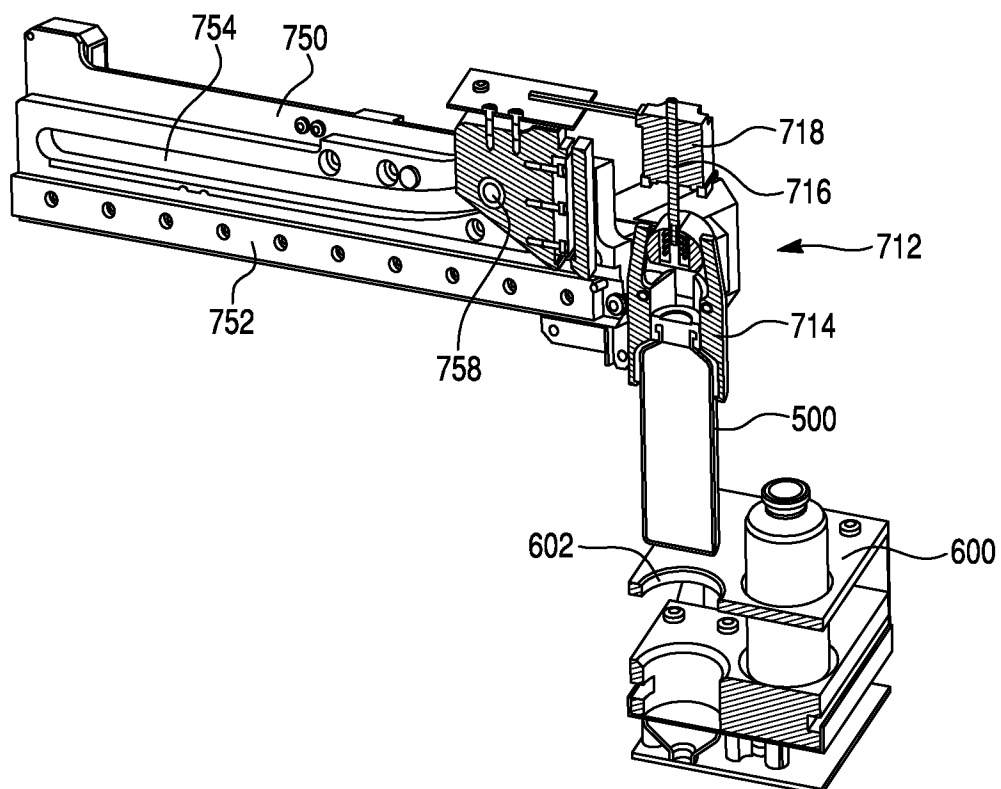


Fig. 21B



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Fig. 22

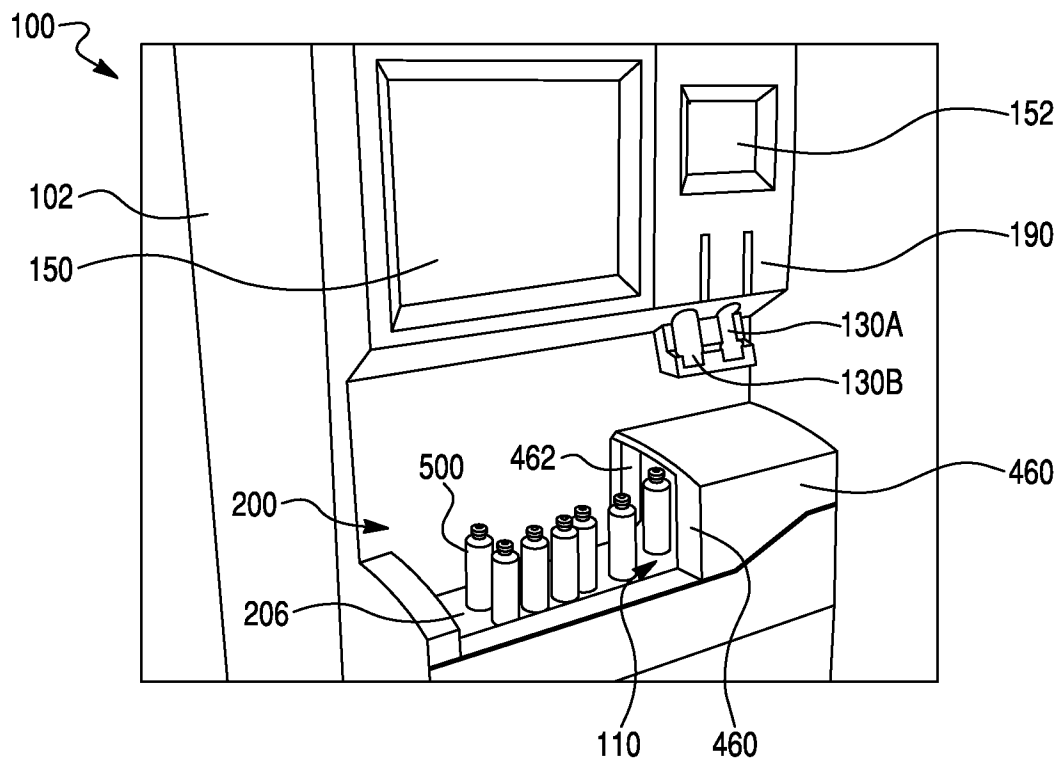


Fig. 23

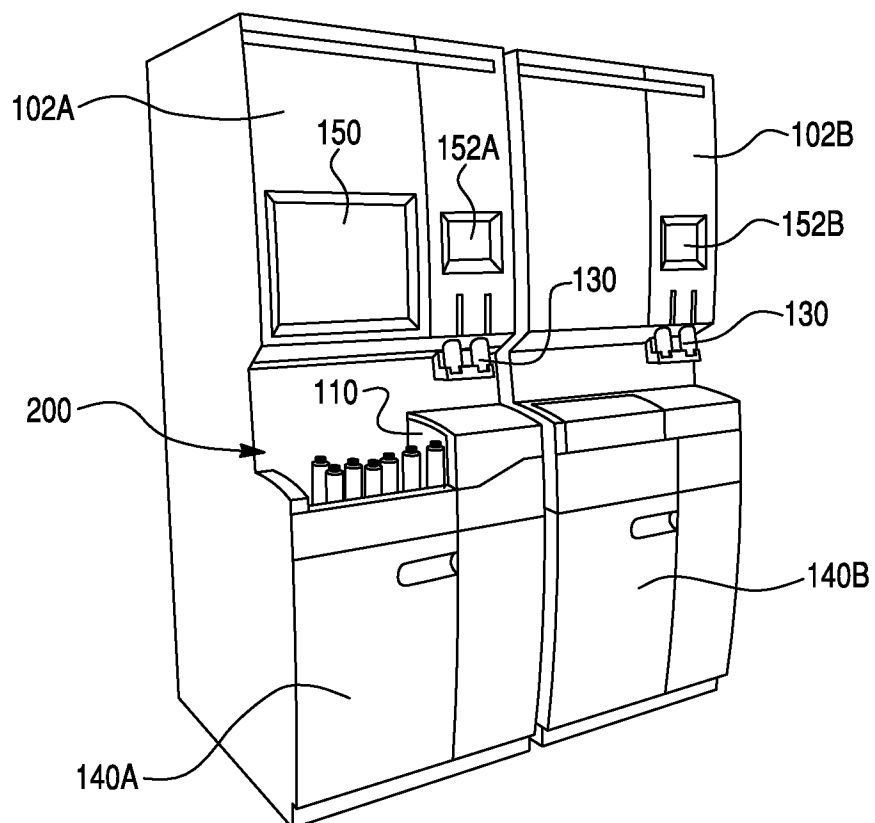
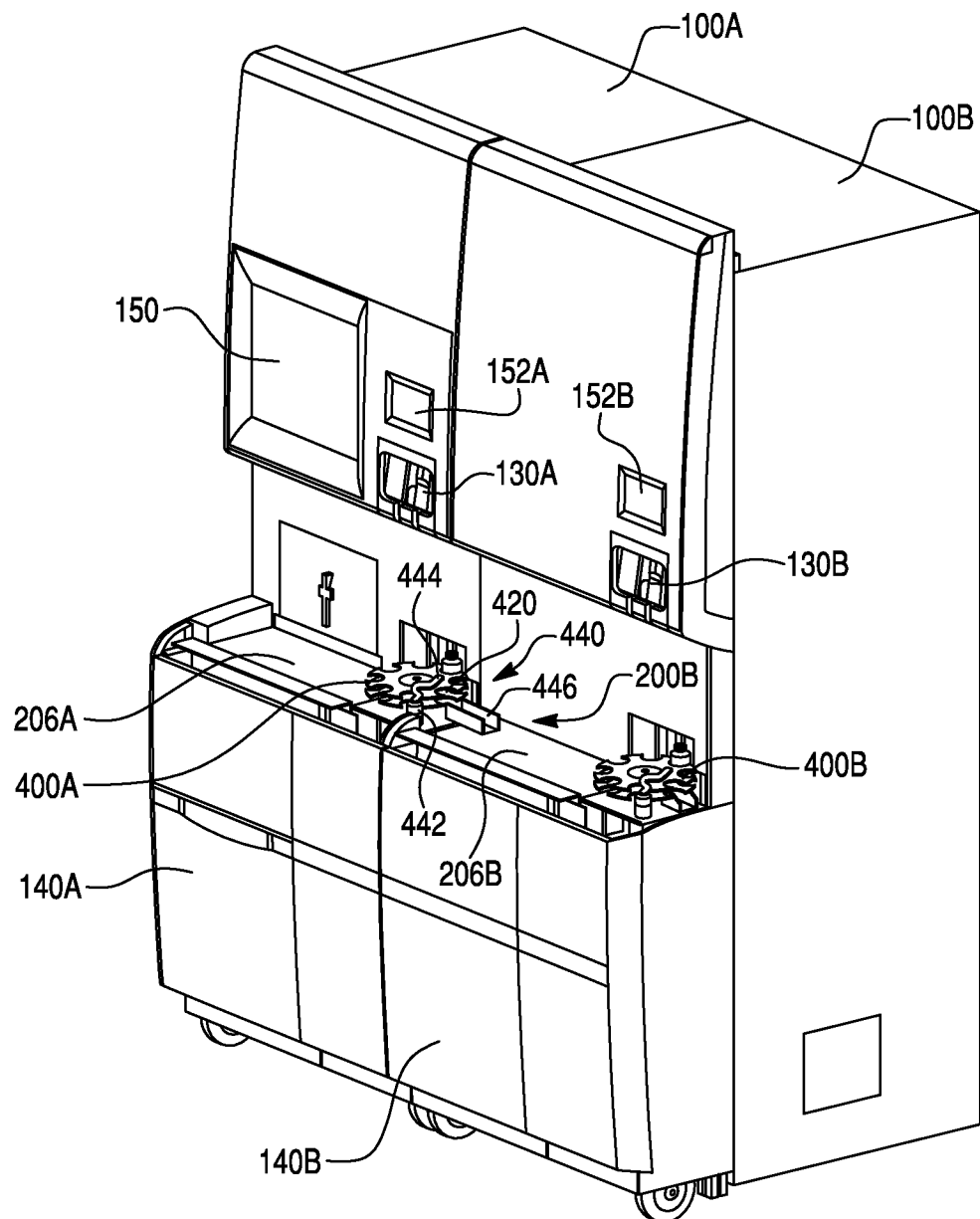
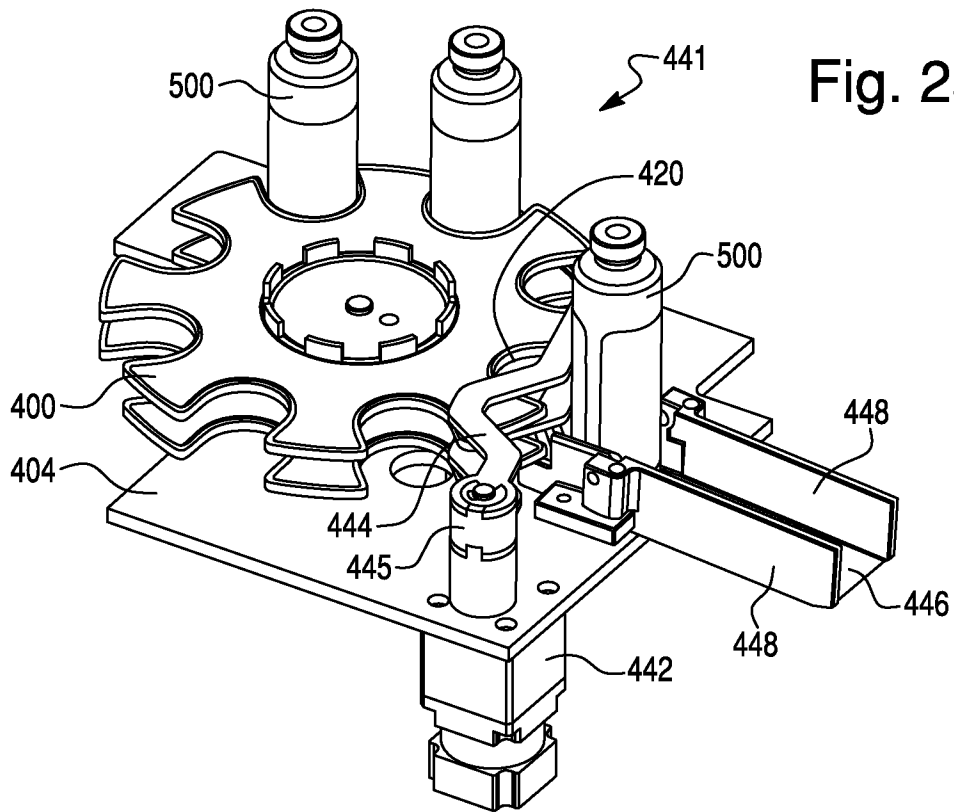


Fig. 24





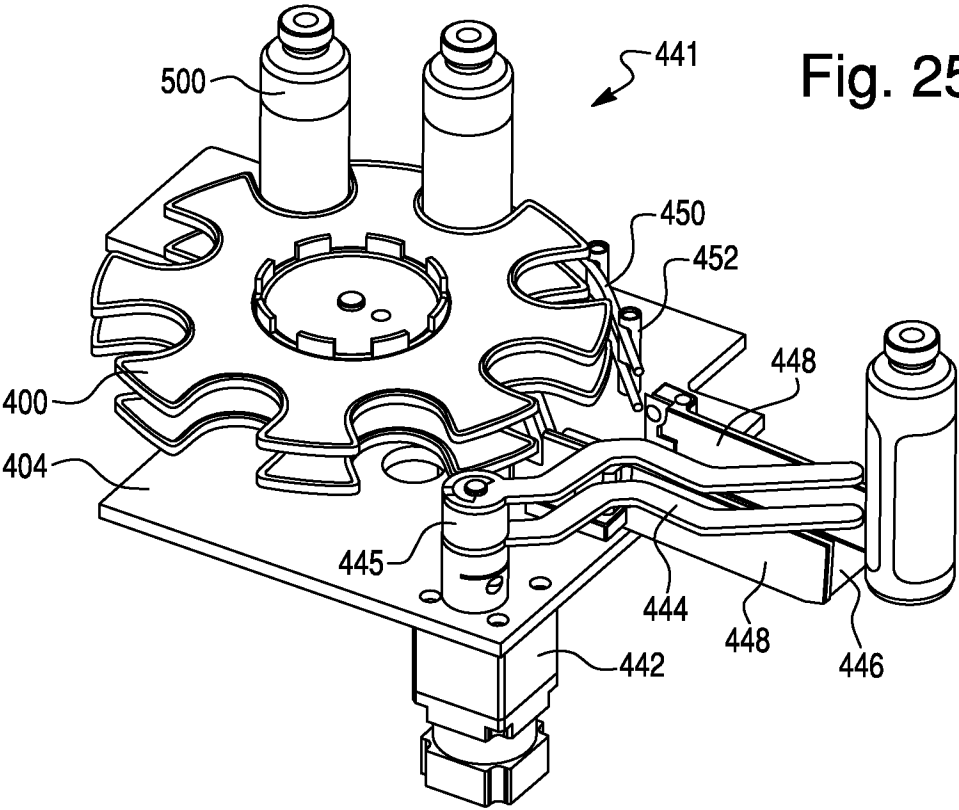


Fig. 26

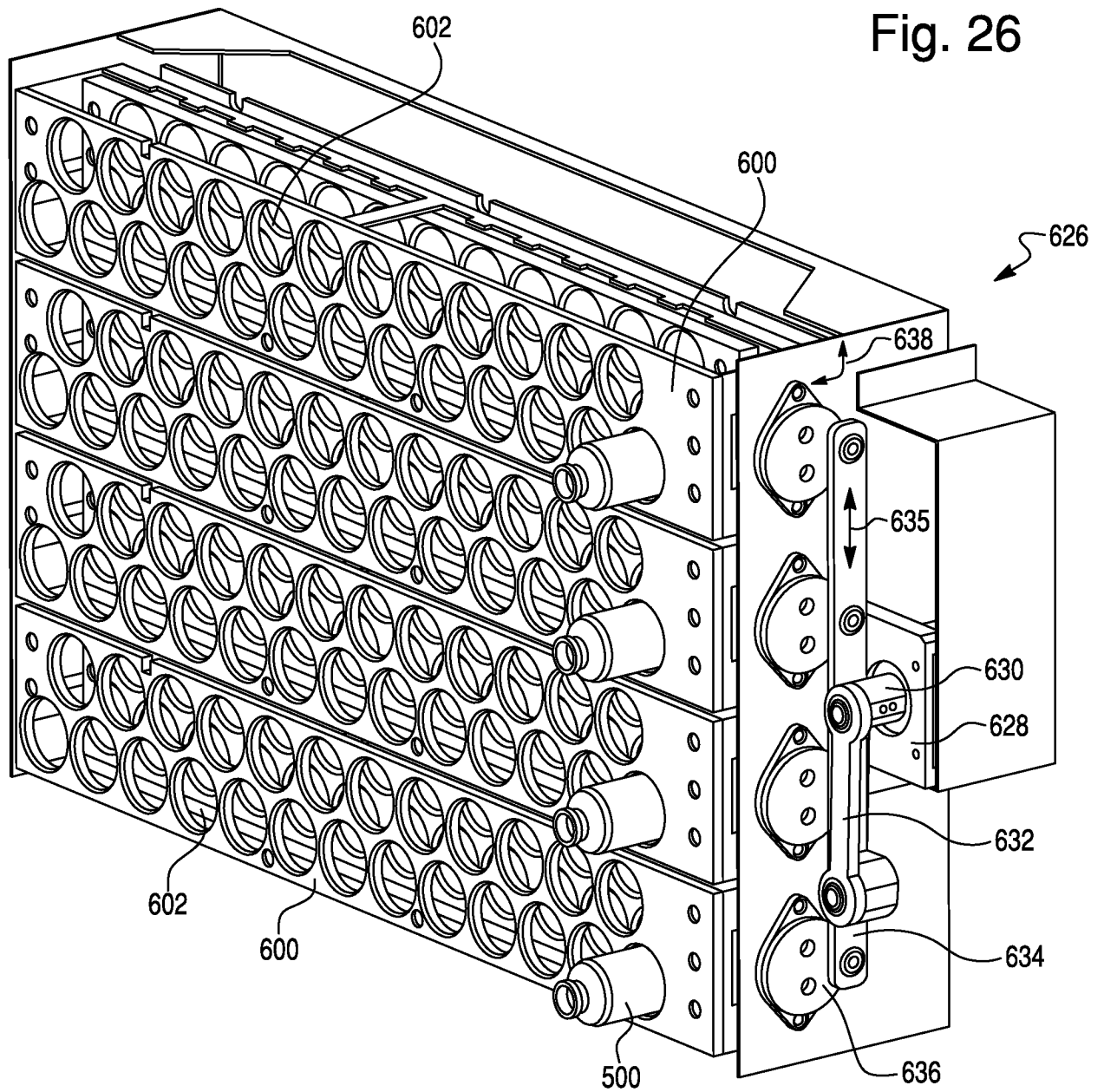


Fig. 27B

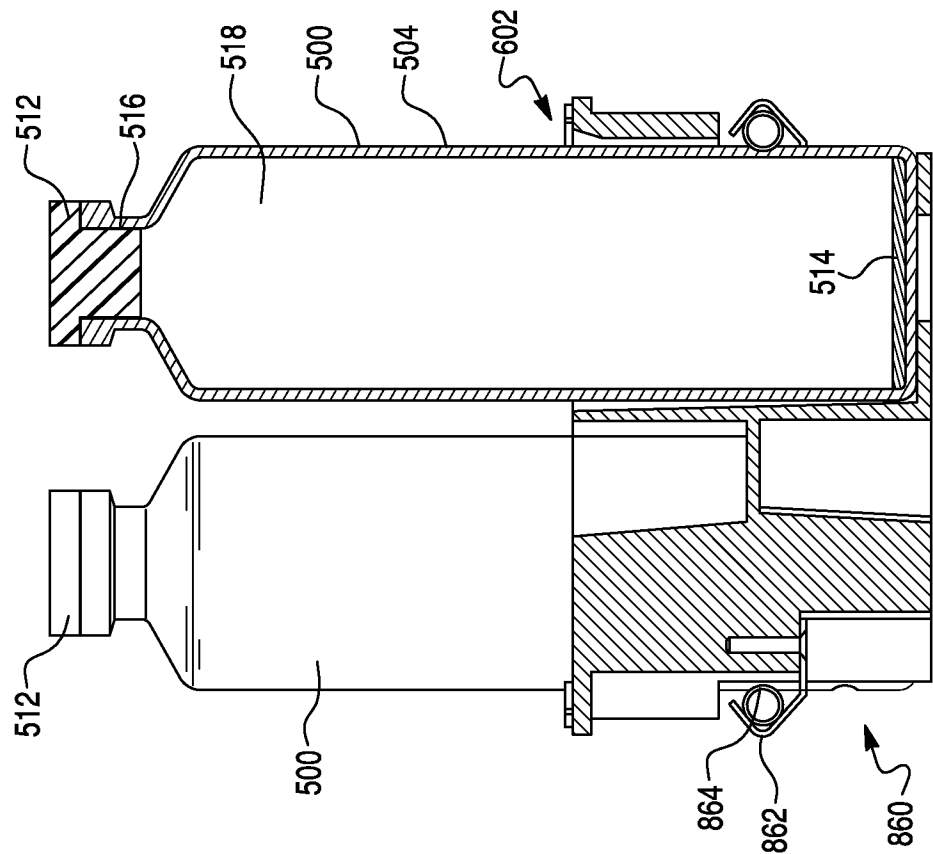


Fig. 27A

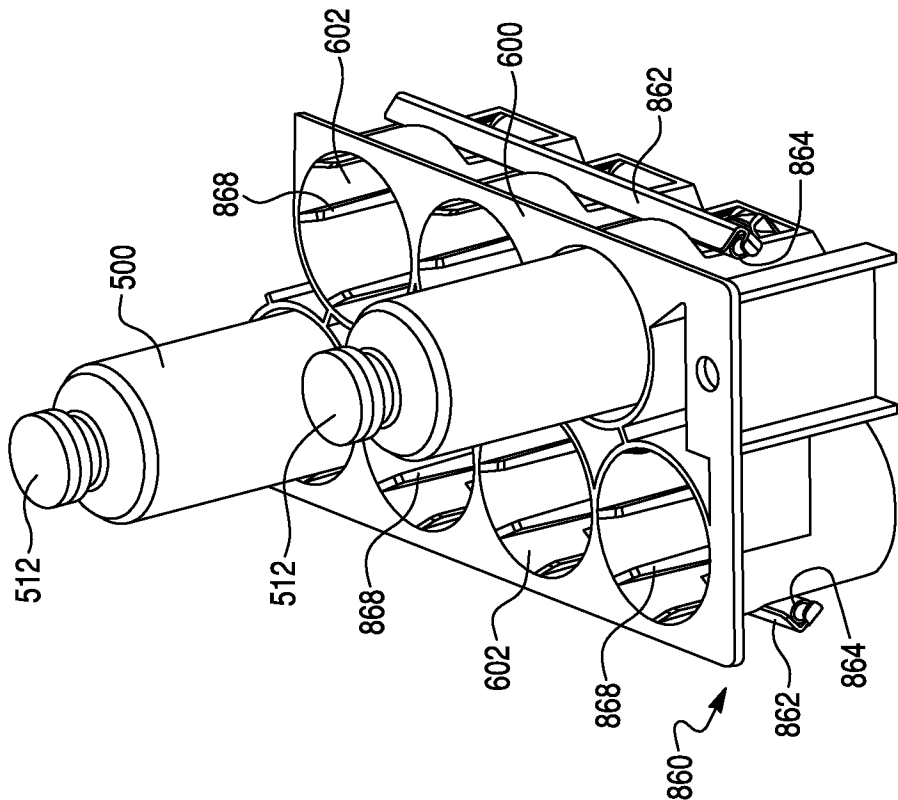
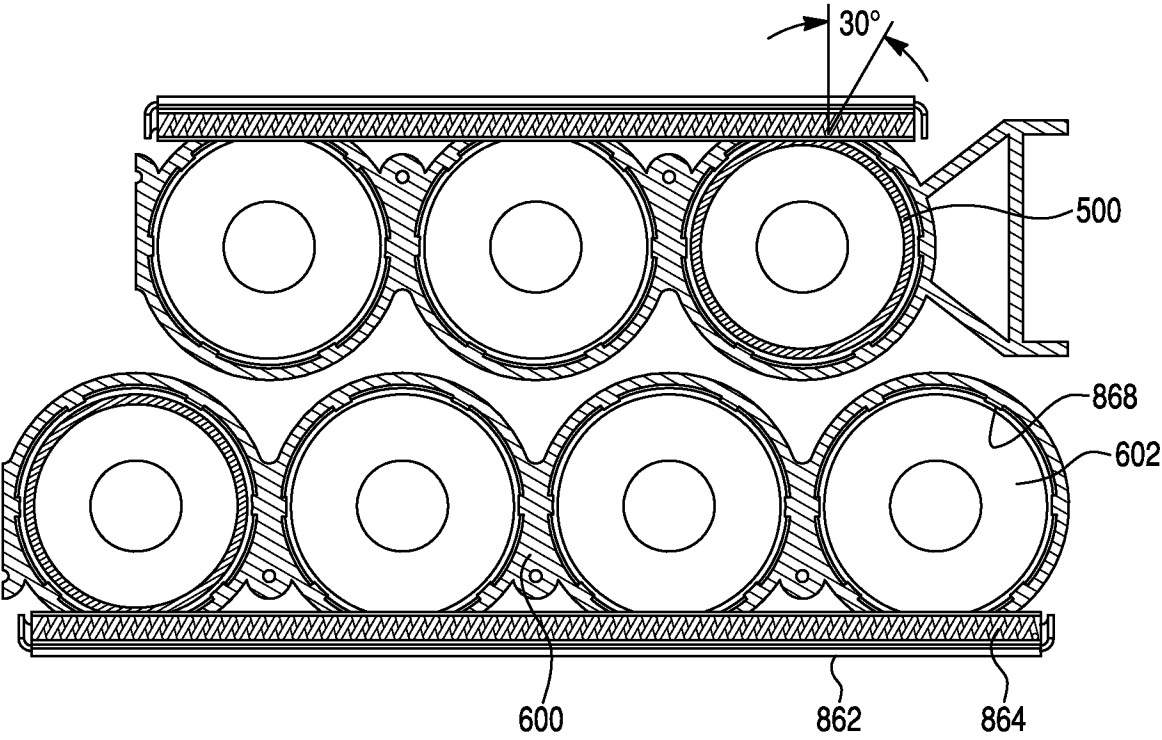


Fig. 27C



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Fig. 28A

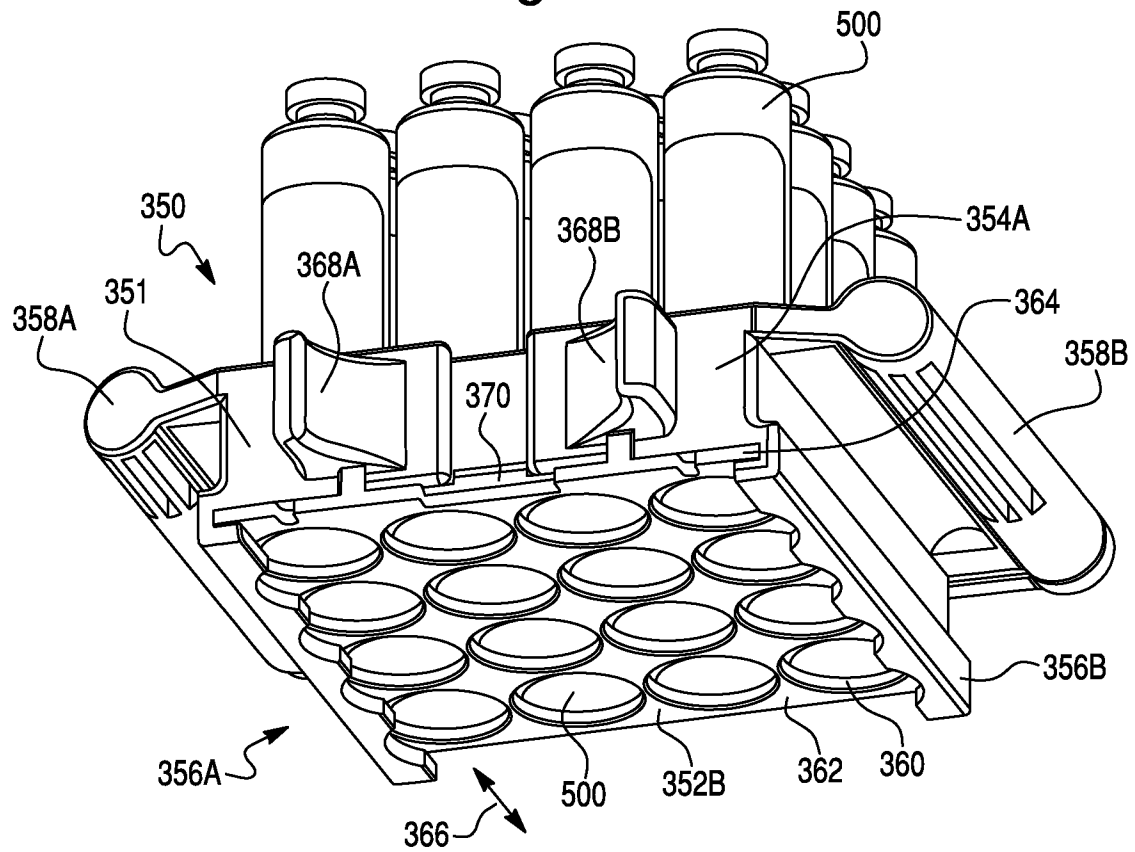


Fig. 28B

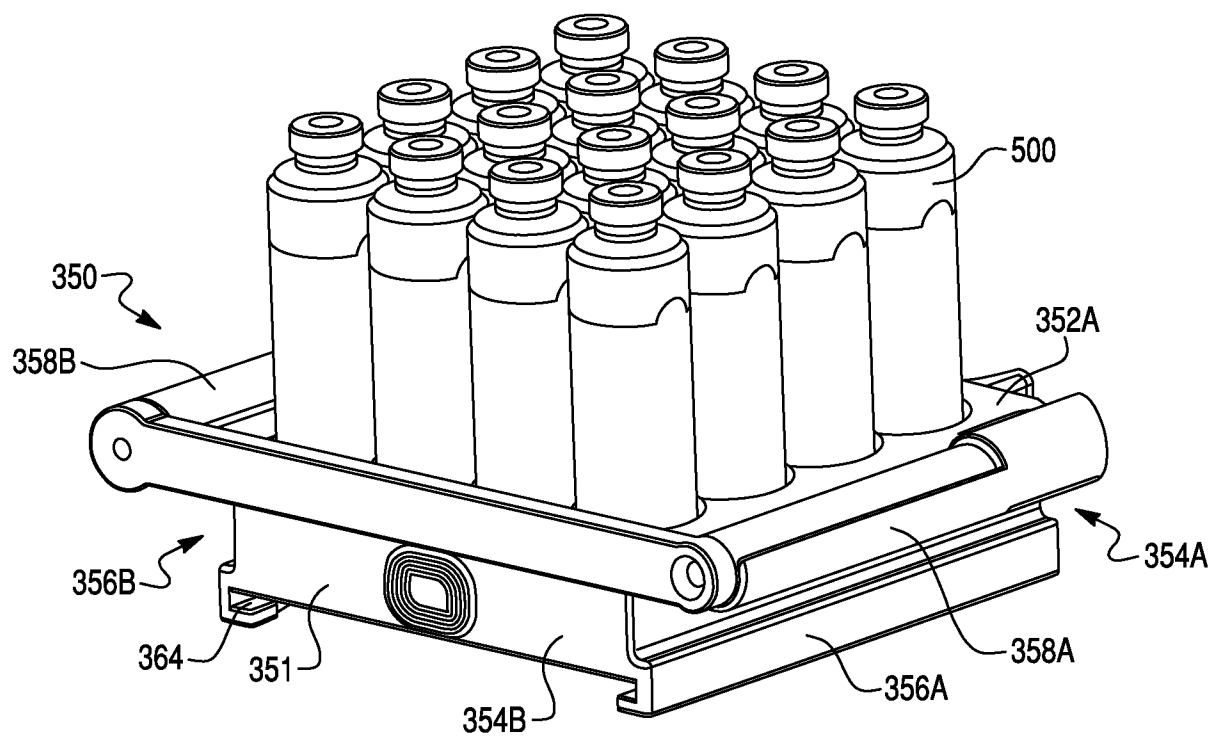
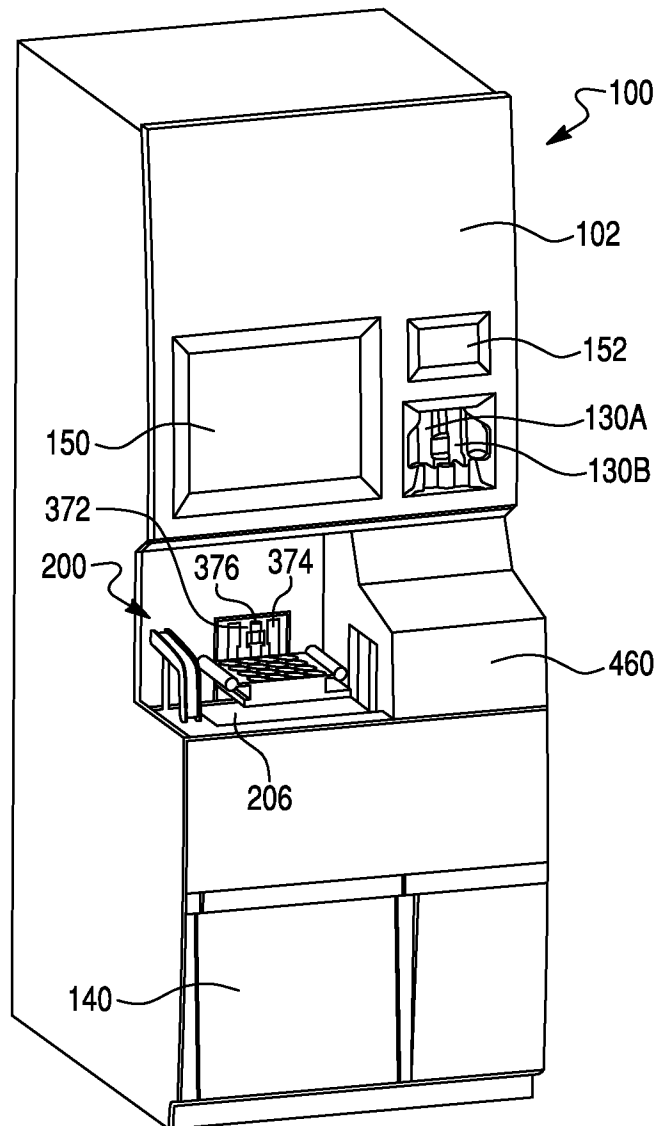


Fig. 29



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Fig. 30

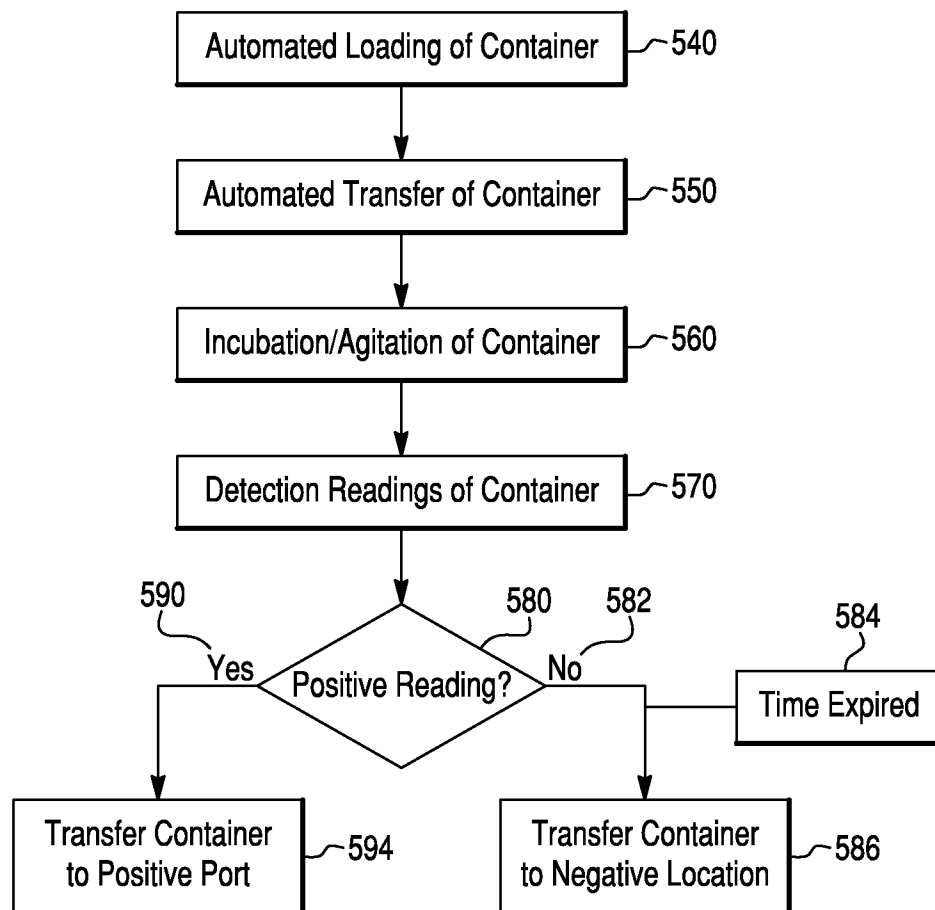
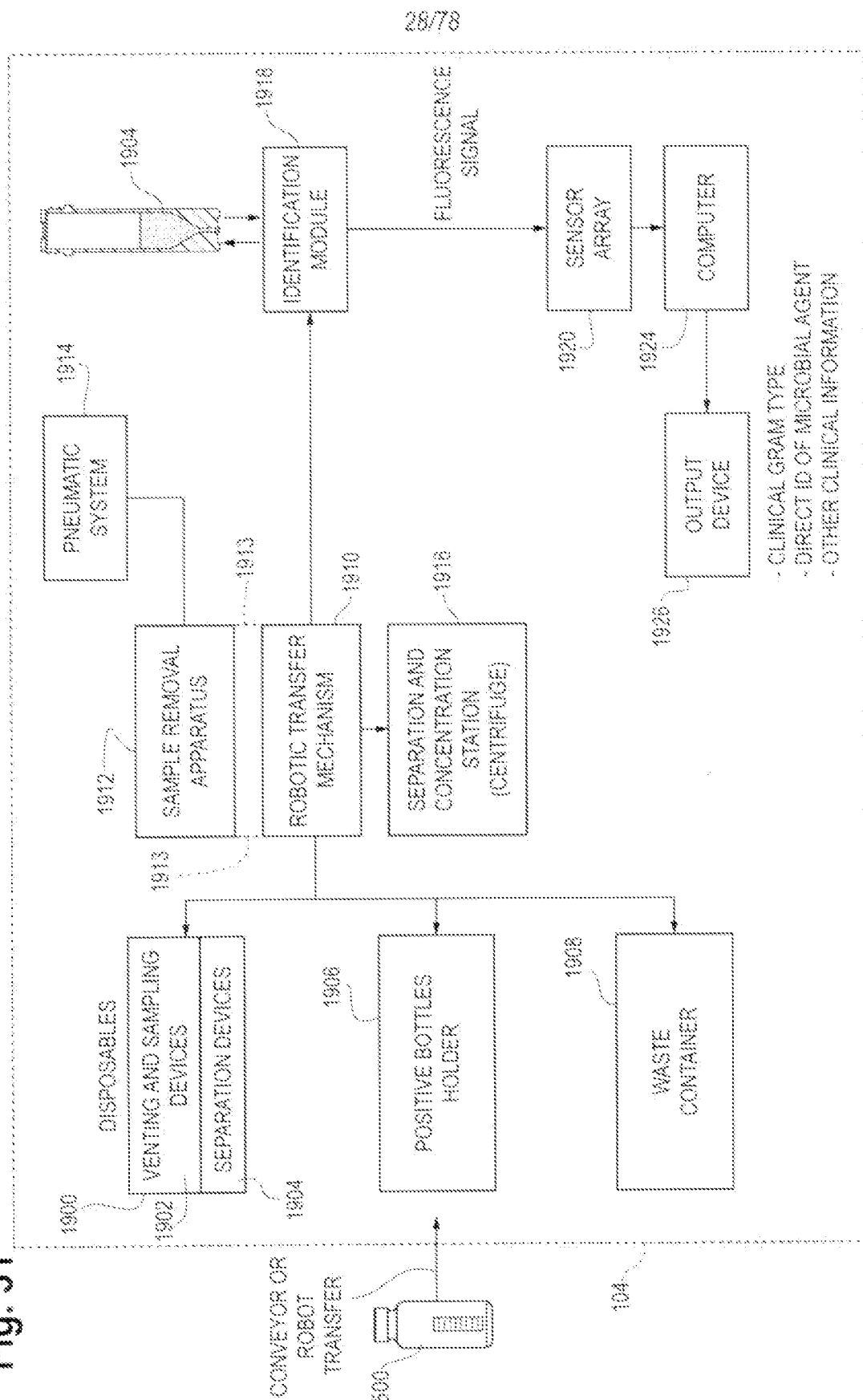


Fig. 31



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Fig. 32

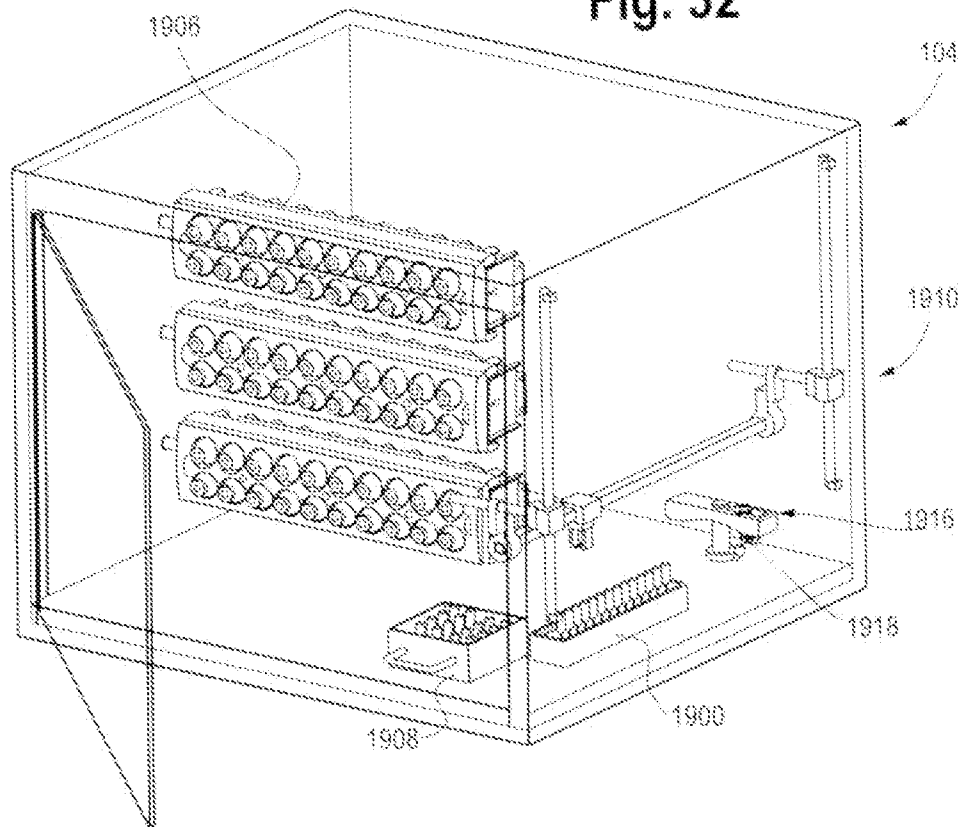


Fig. 33

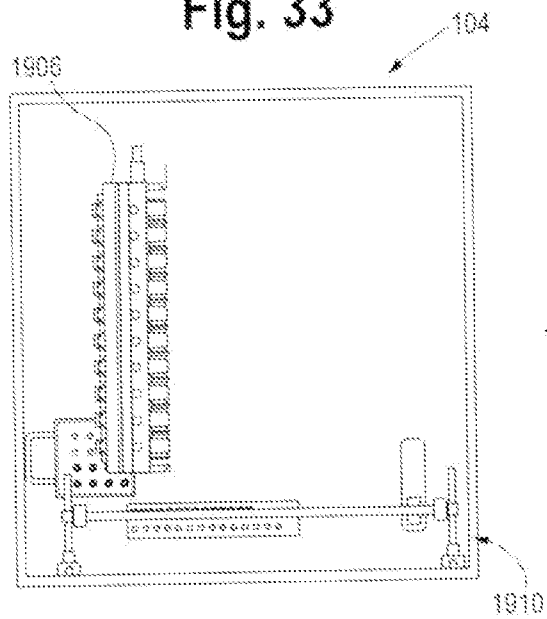
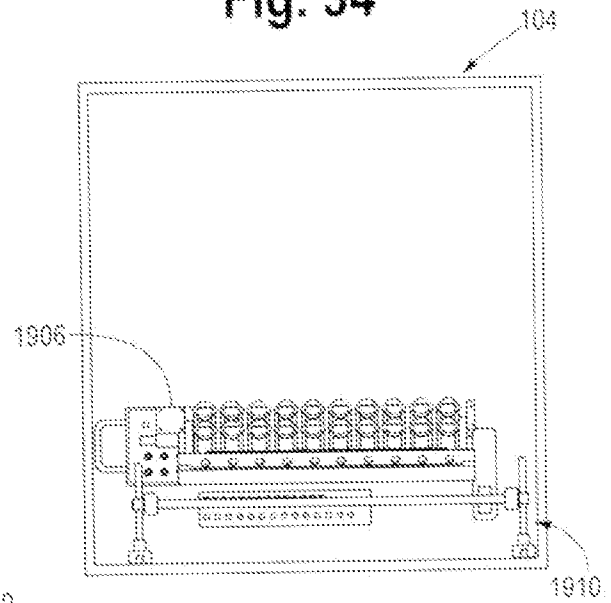


Fig. 34



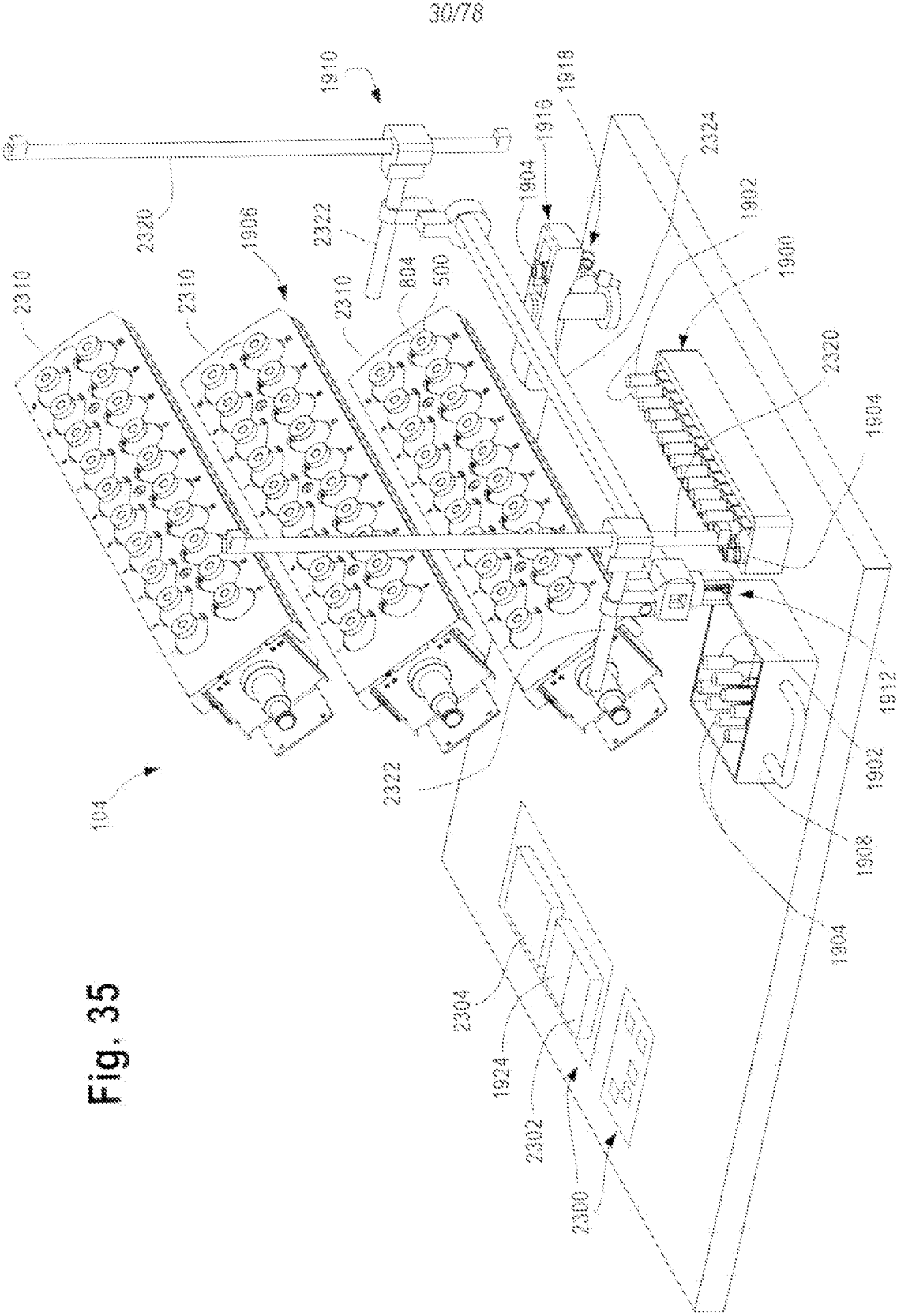
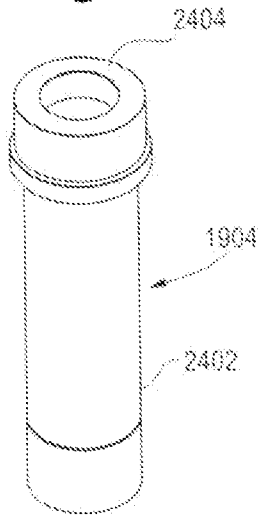


Fig. 35

Fig. 36



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Fig. 37

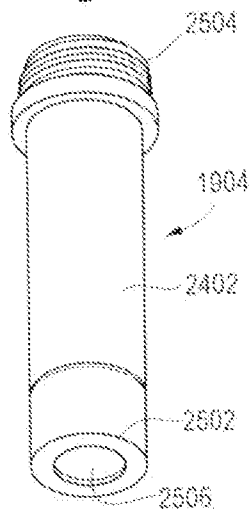


Fig. 38

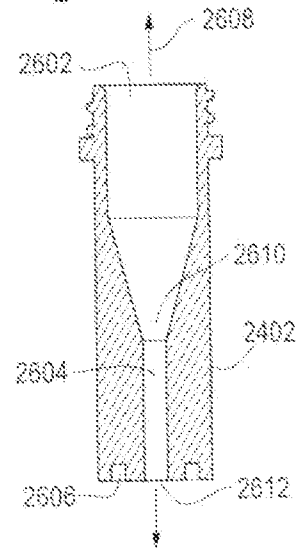


Fig. 39

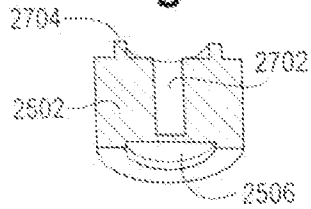


Fig. 40

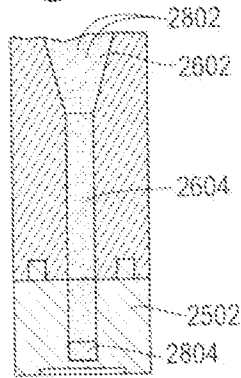


Fig. 41

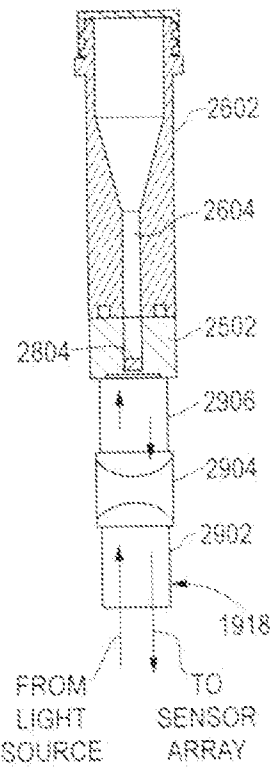


Fig. 42

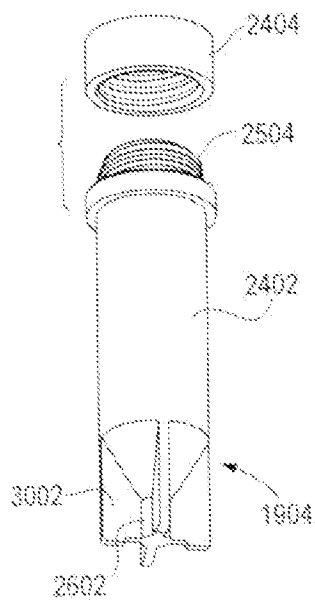


Fig. 43

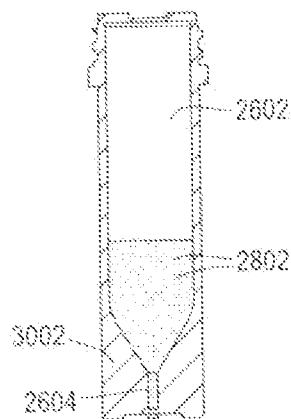


Fig. 44

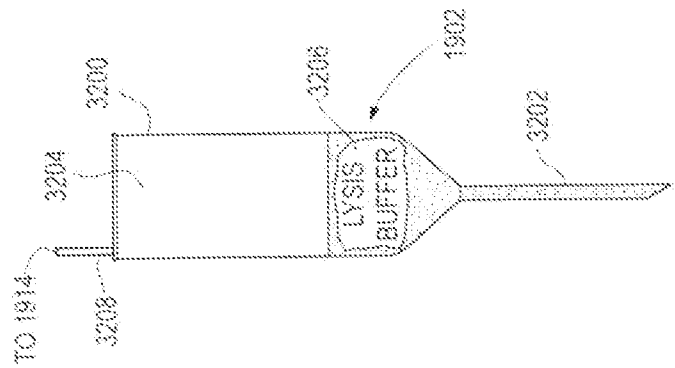


Fig. 45

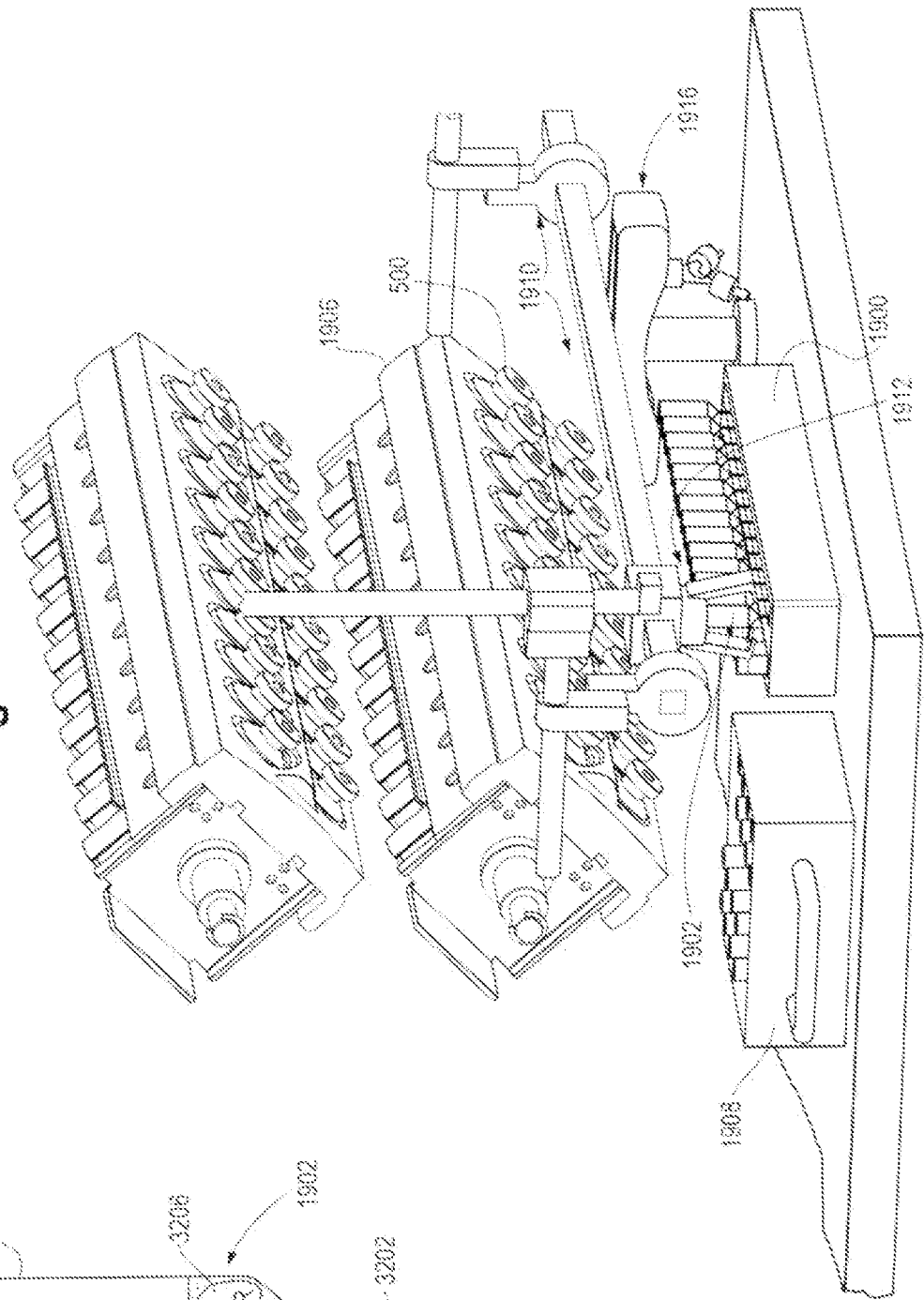


Fig. 46

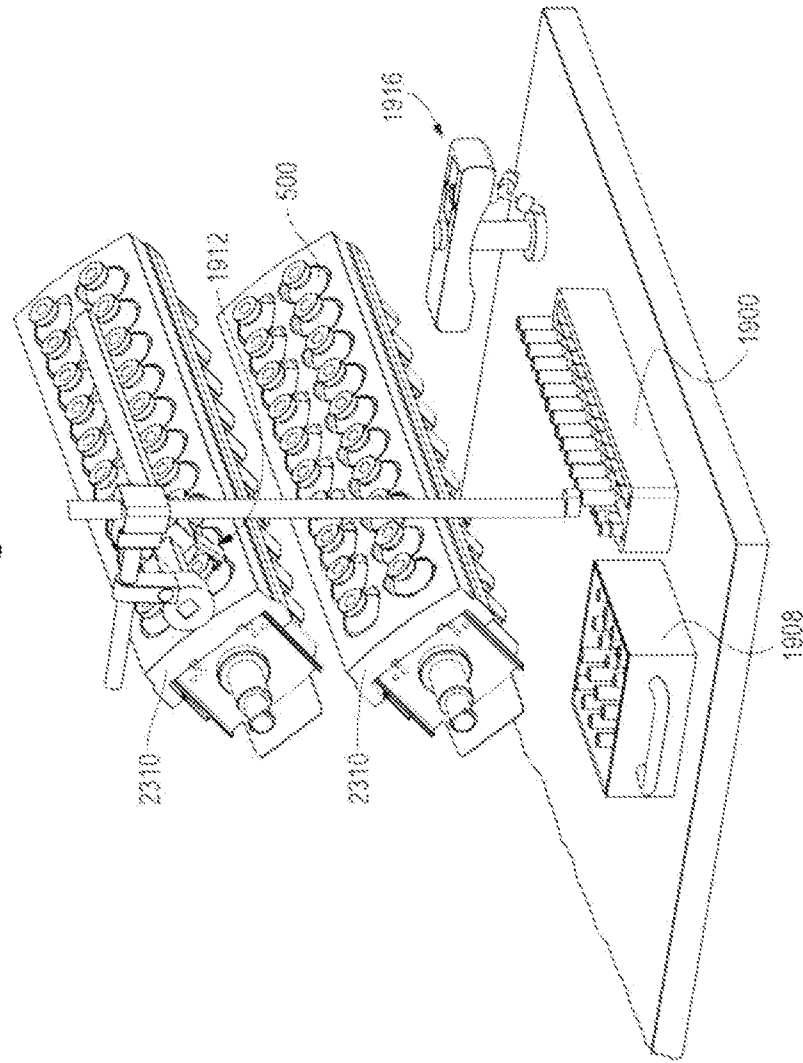


Fig. 47

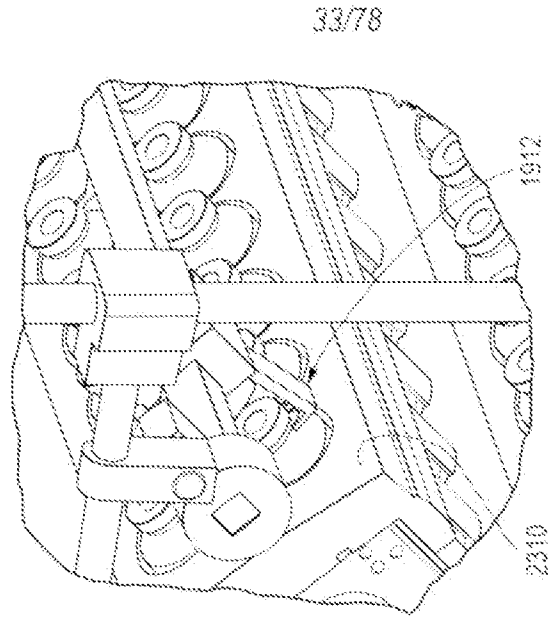


Fig. 48

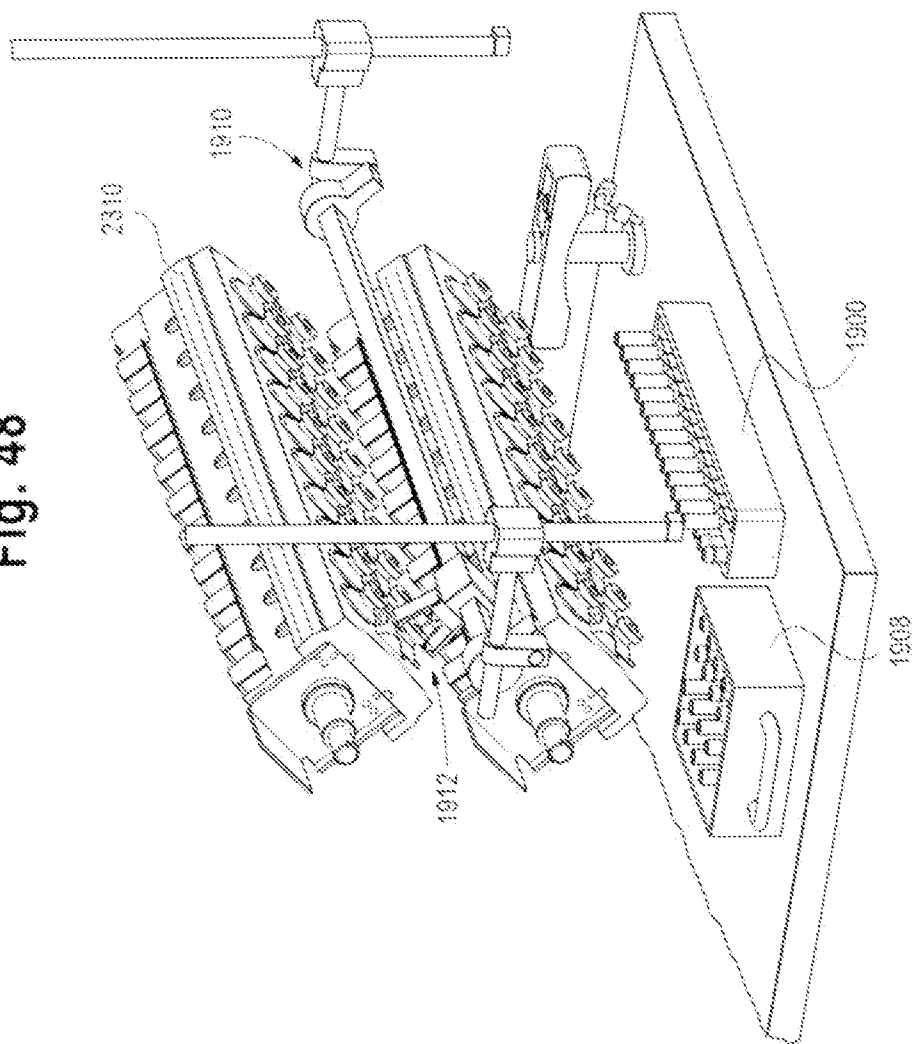
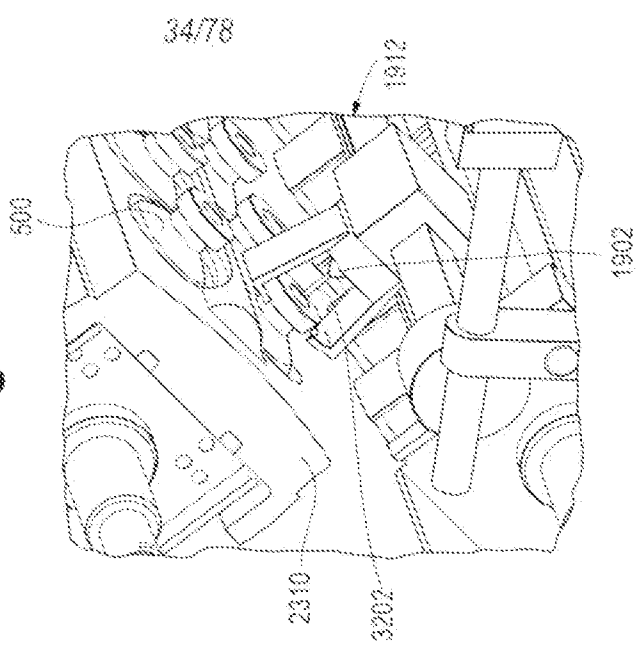


Fig. 49



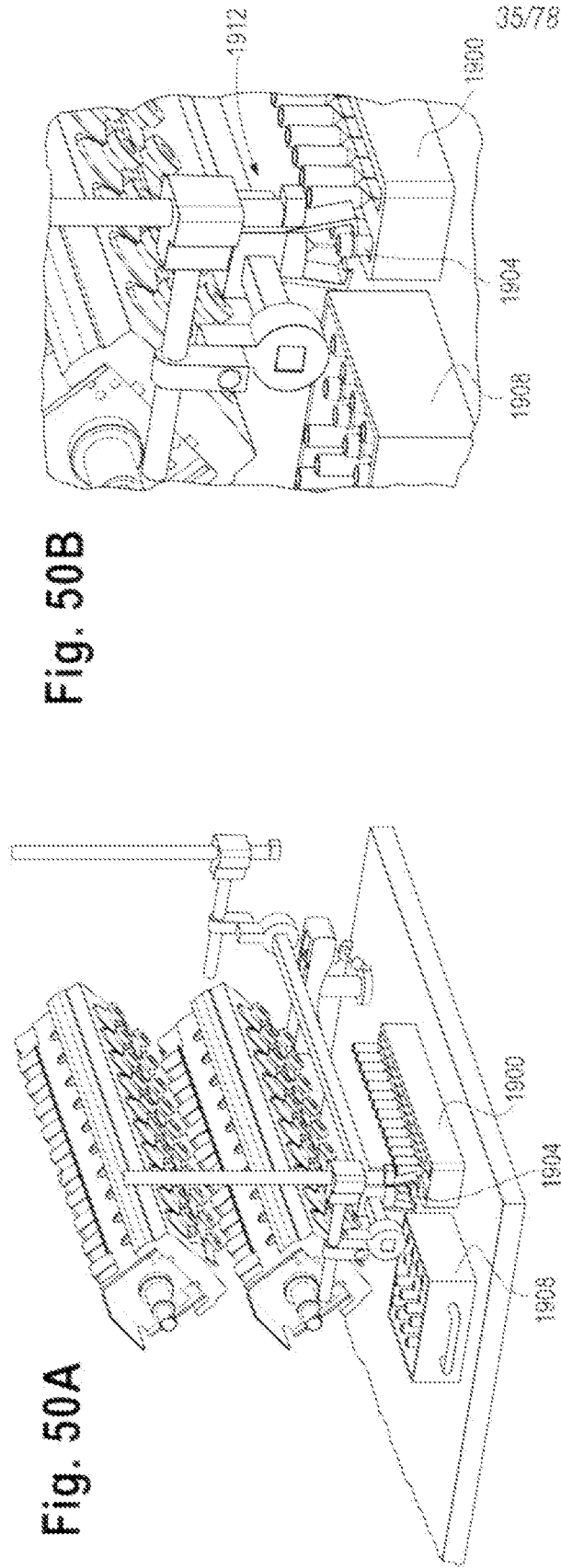


Fig. 50B

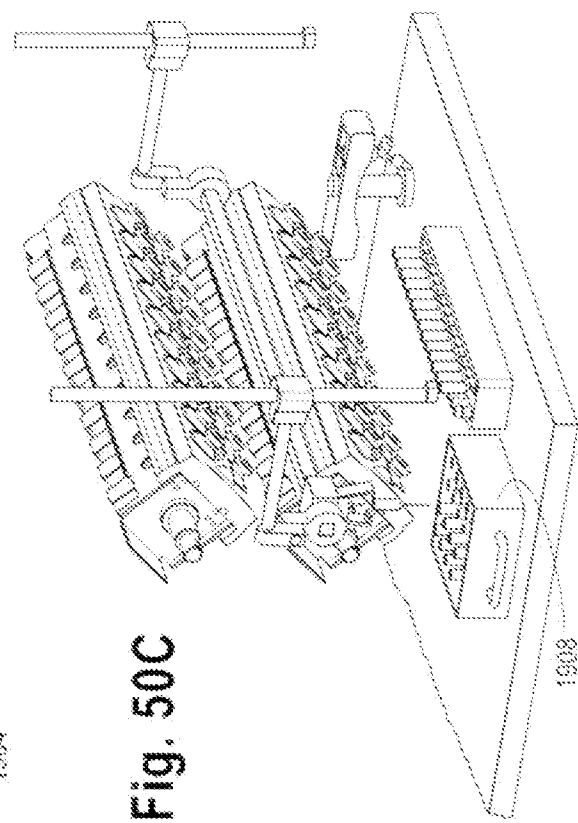


Fig. 51

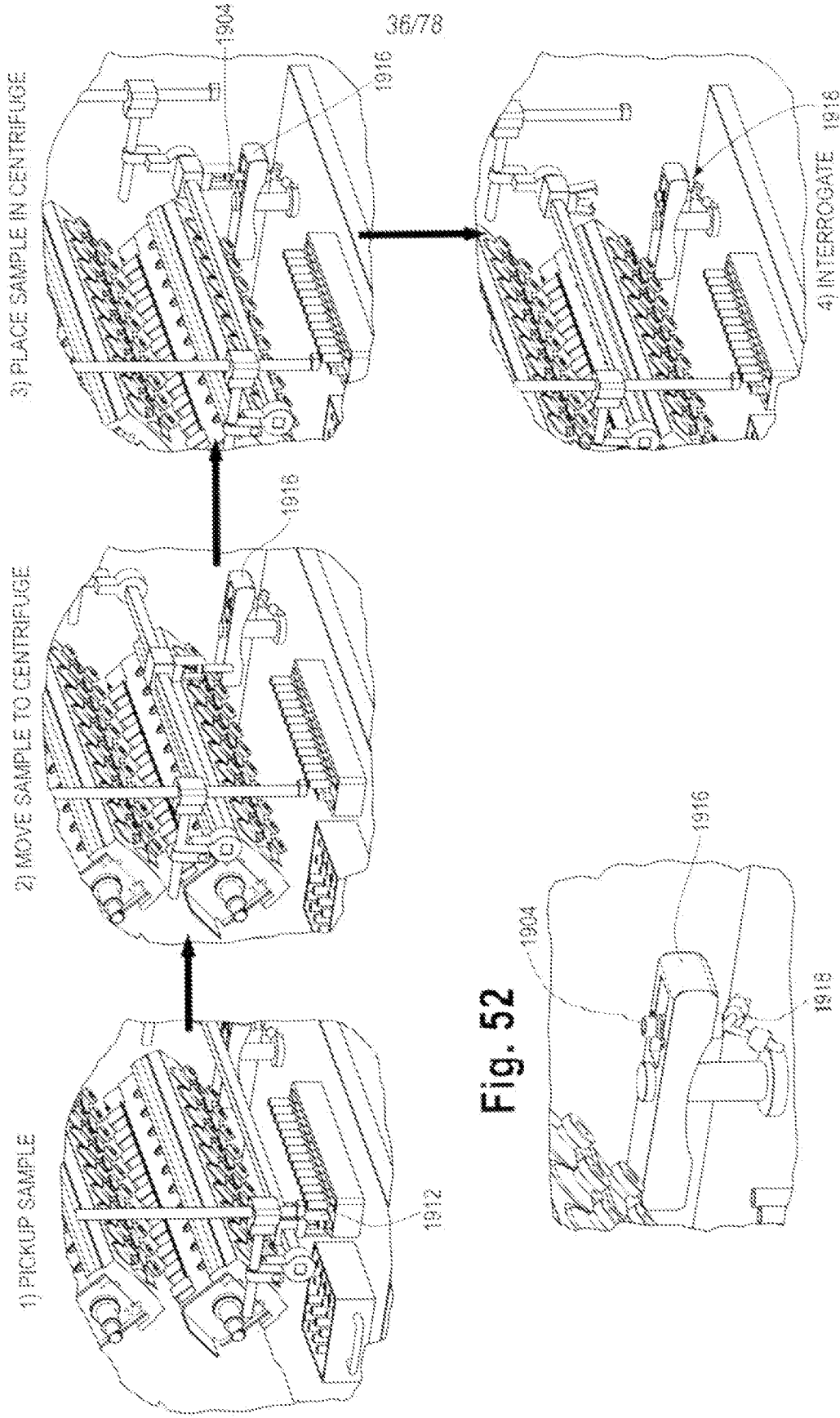
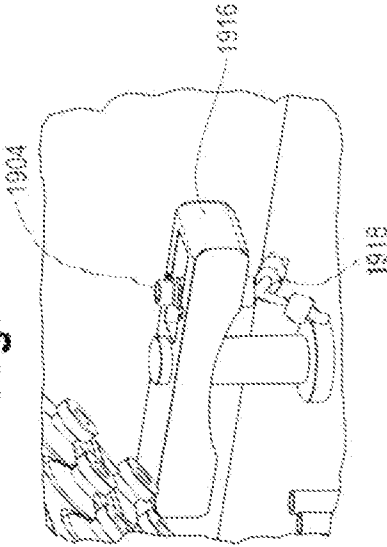


Fig. 52



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Fig. 53

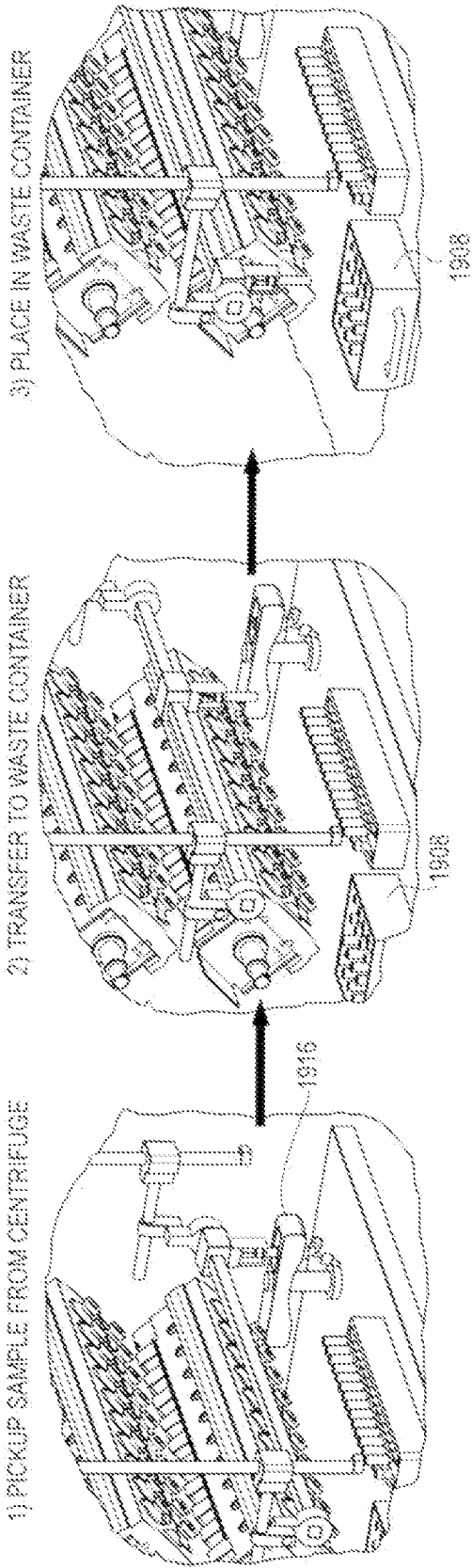
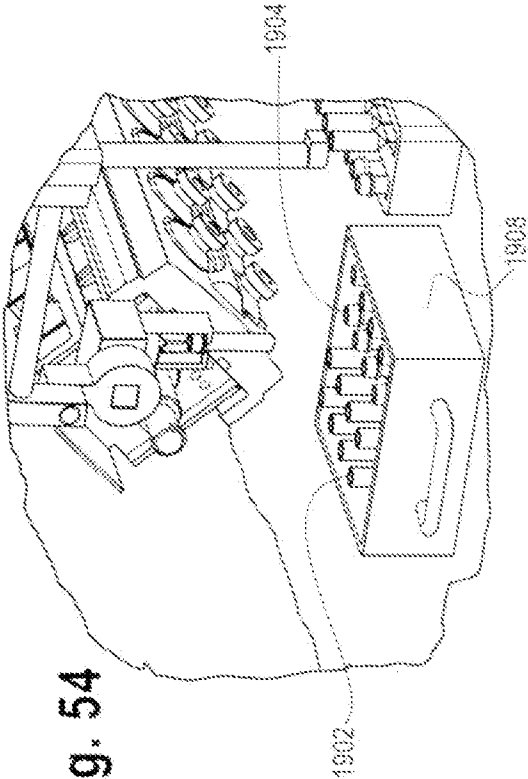
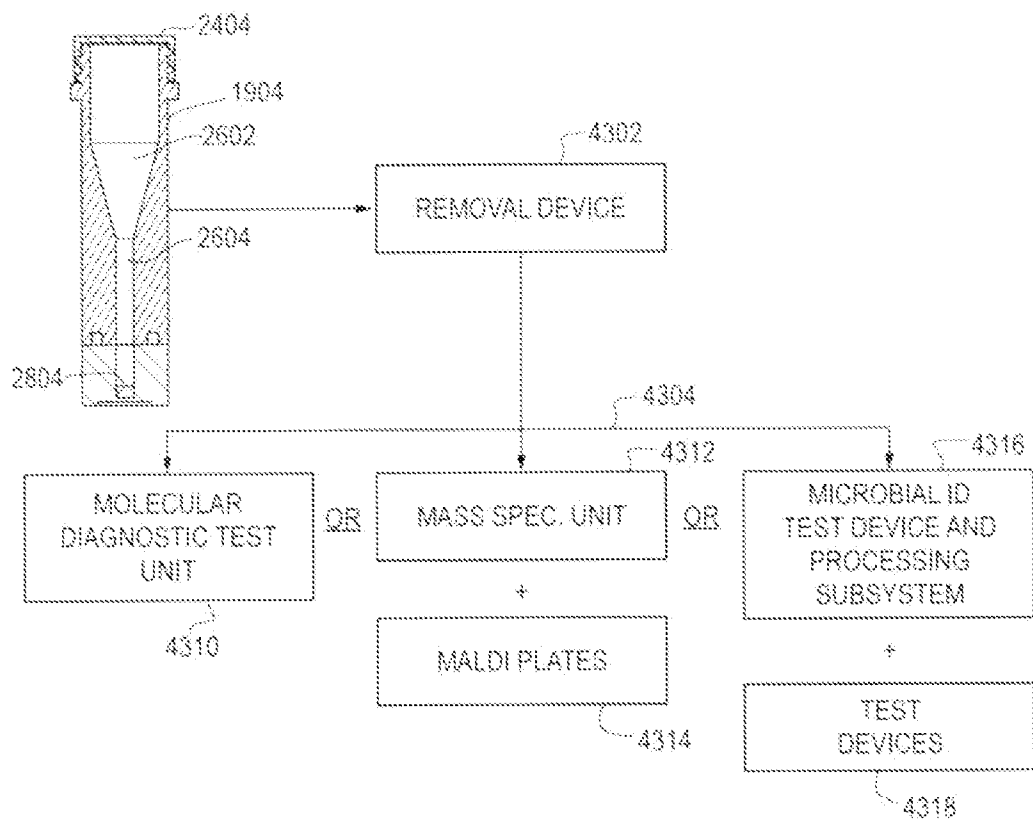


Fig. 54



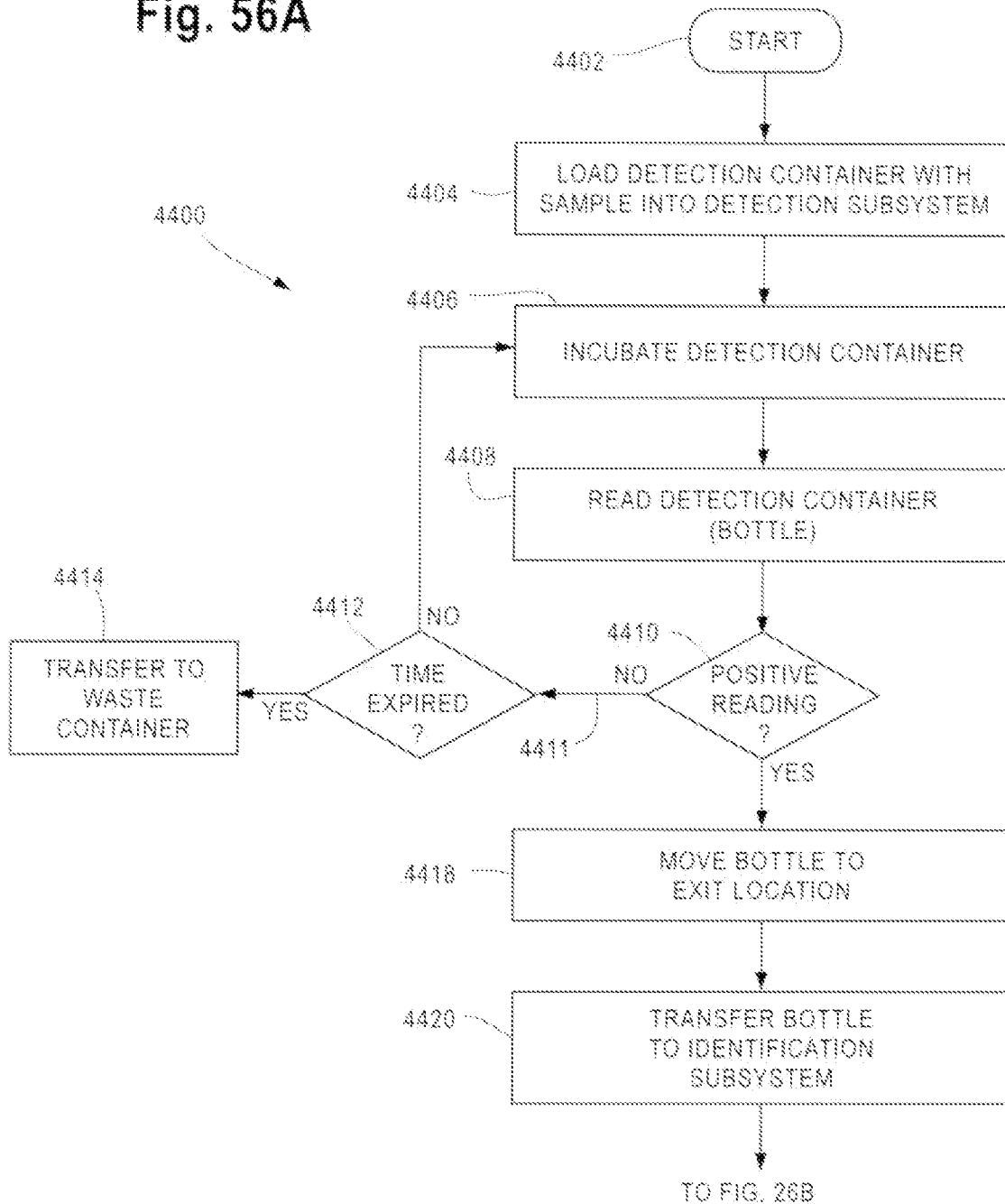
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Fig. 55



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Fig. 56A



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Fig. 56B

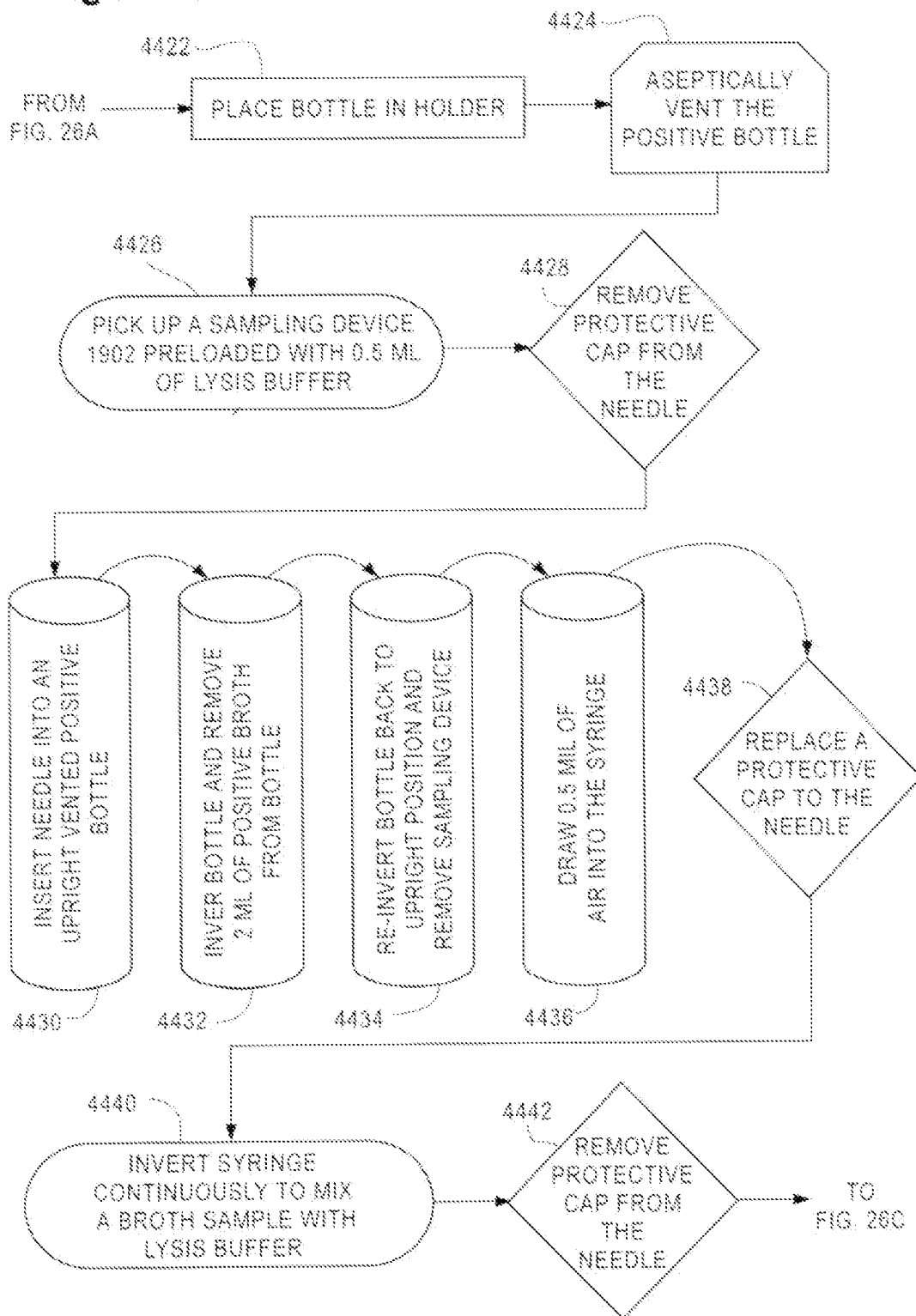
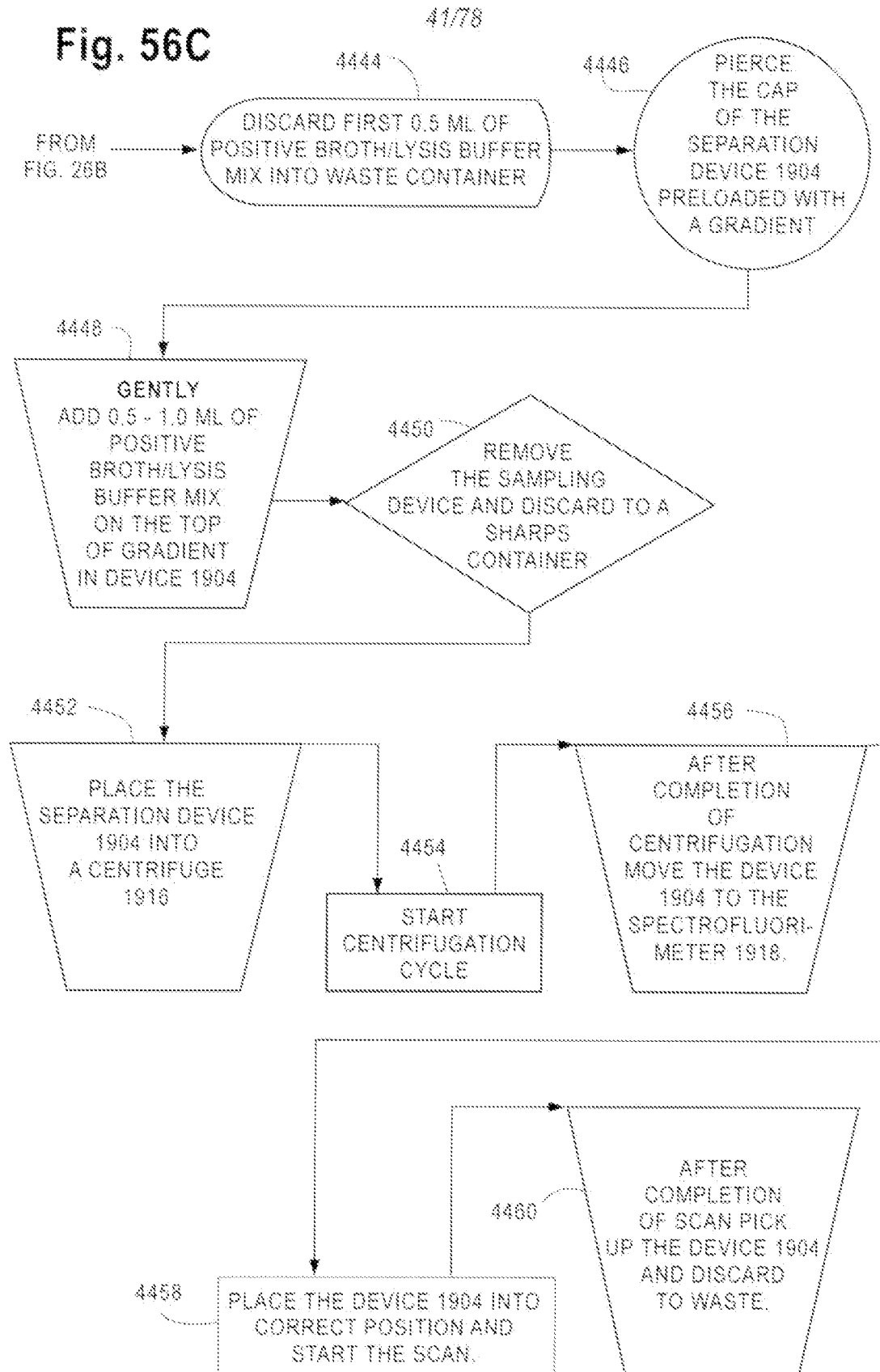
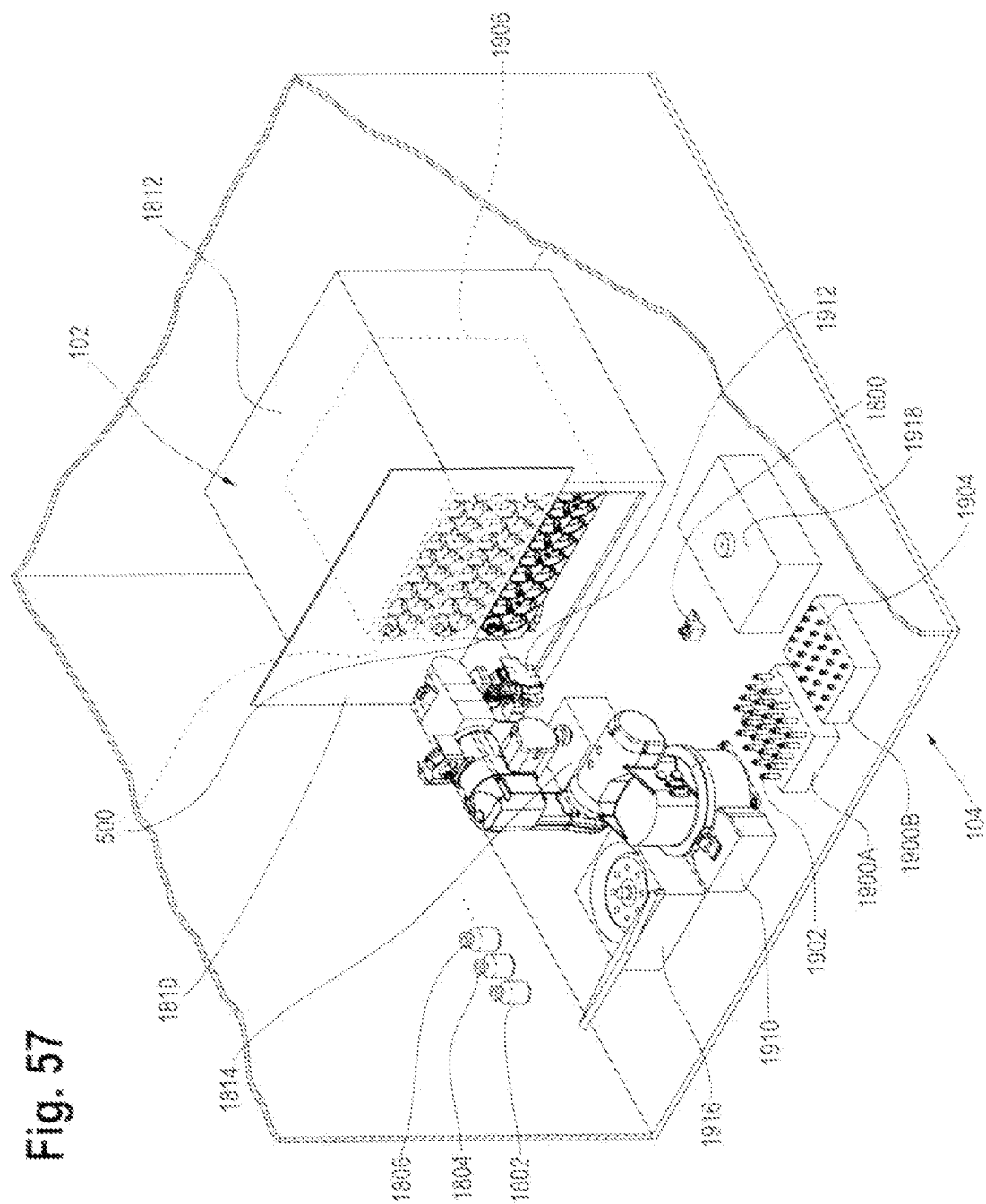


Fig. 56C

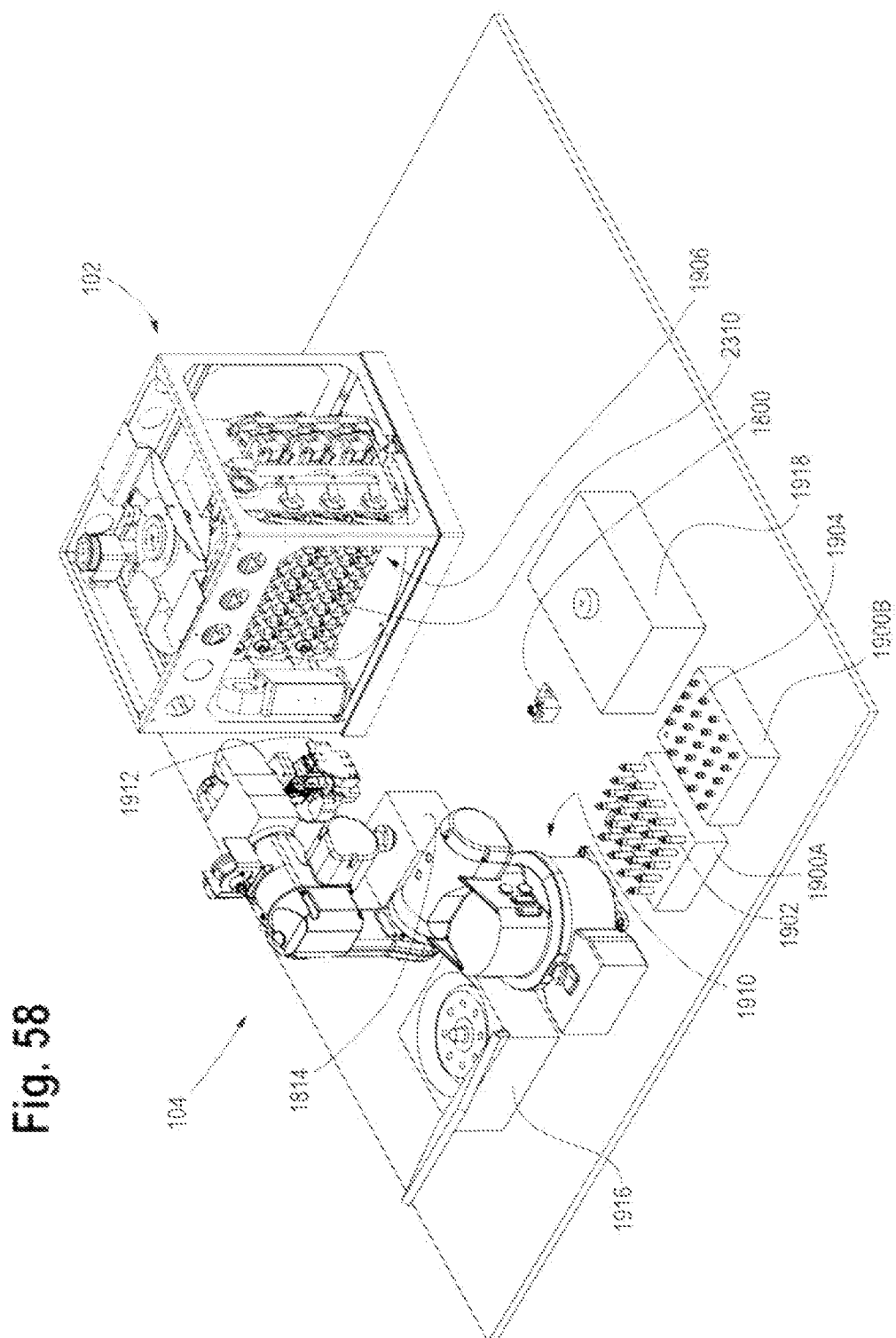


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Fig. 60

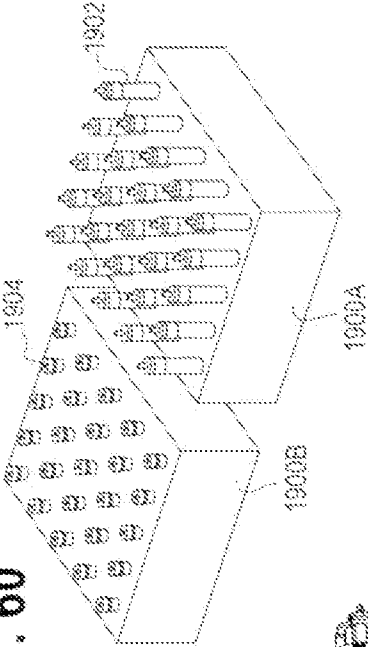


Fig. 59

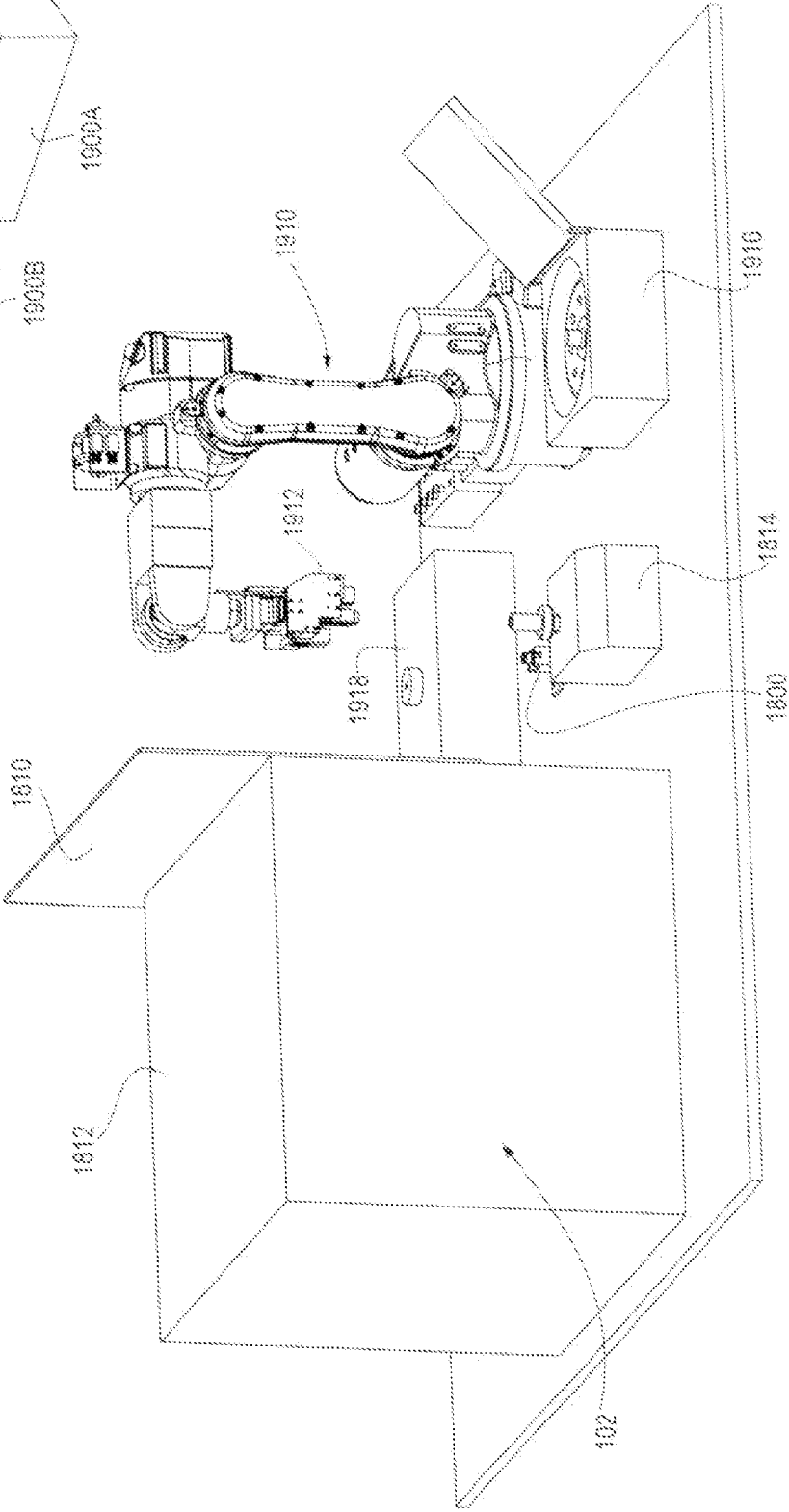


Fig. 61

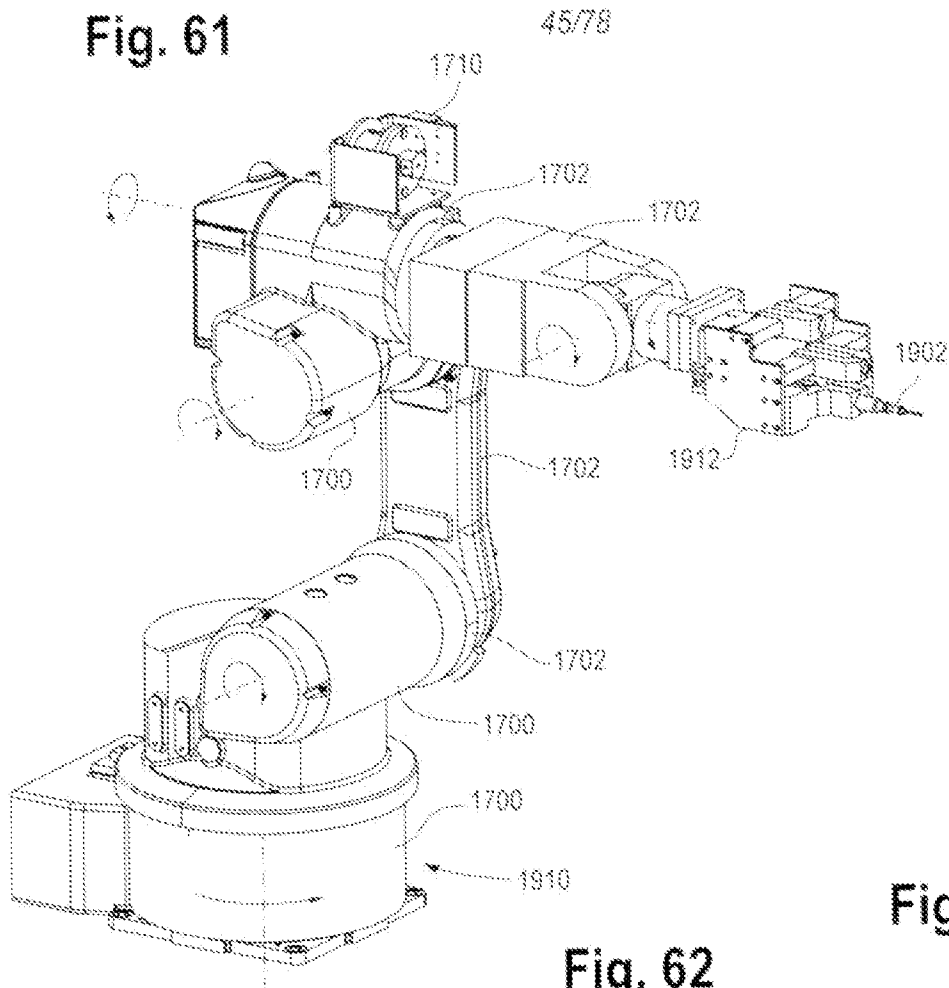


Fig. 62

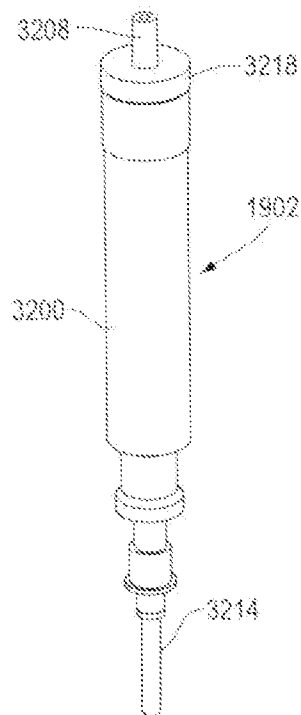
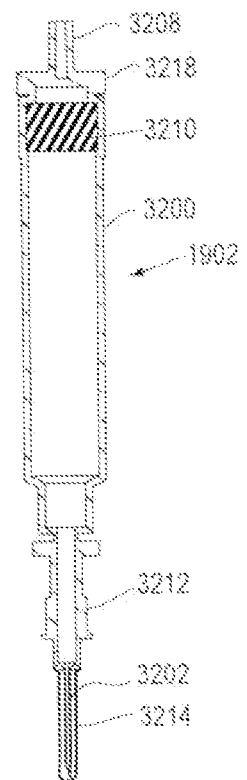
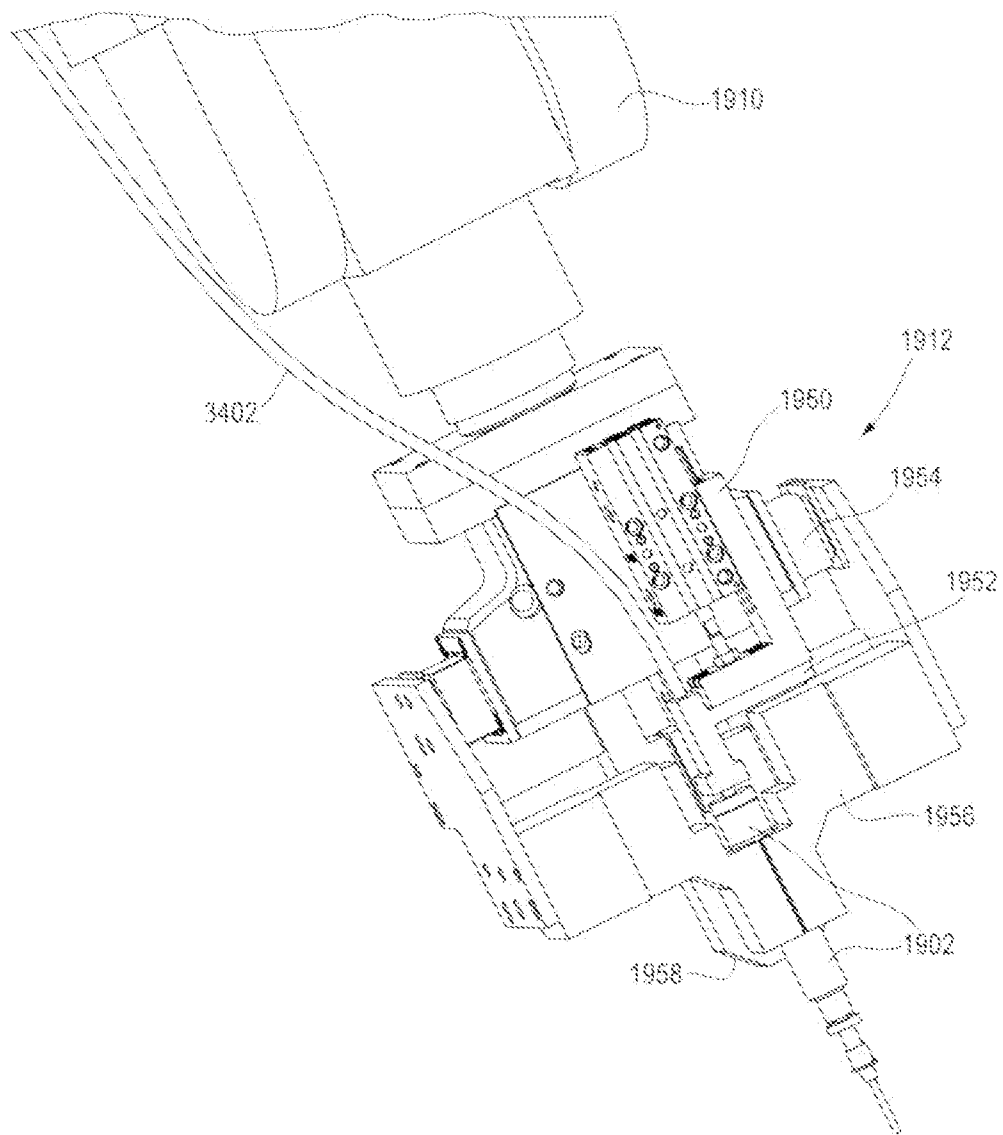


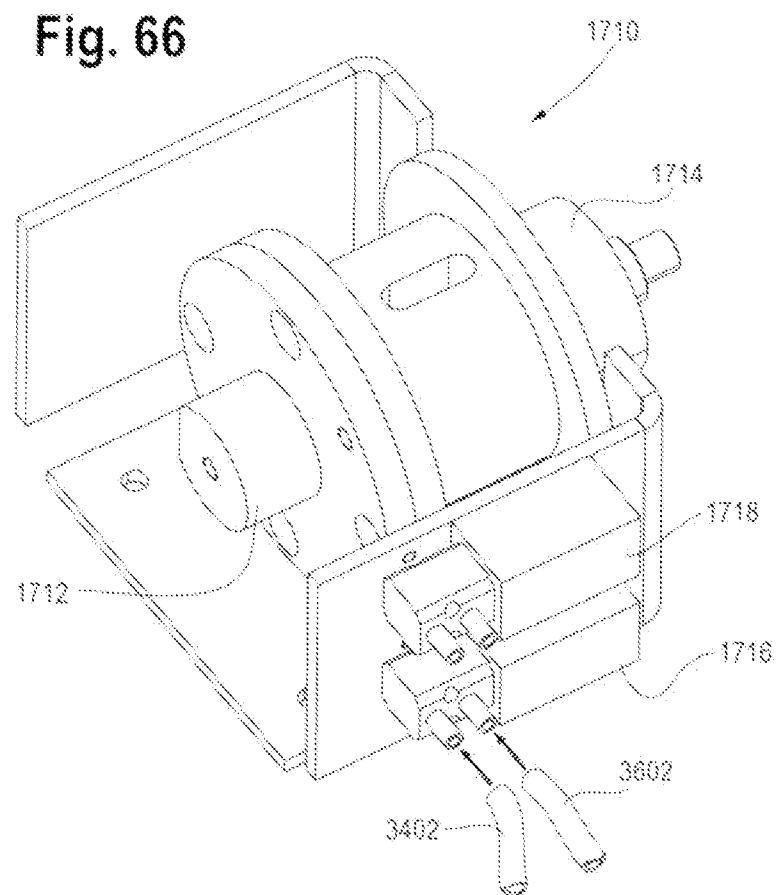
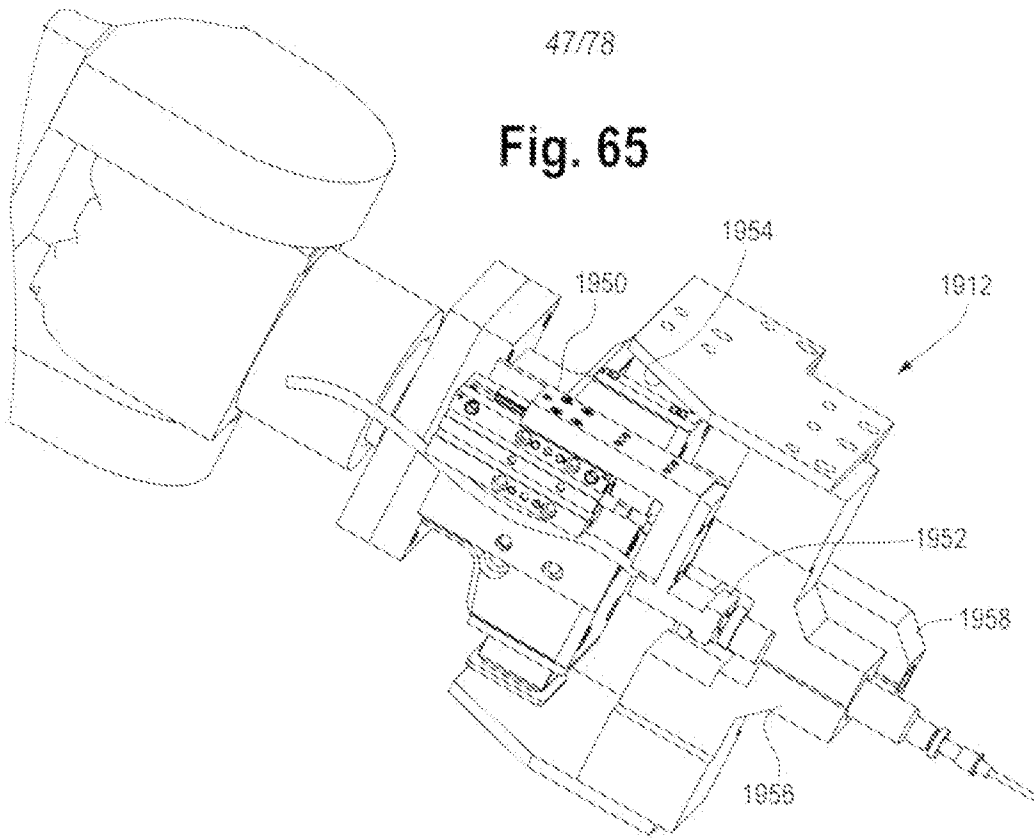
Fig. 63



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Fig. 64





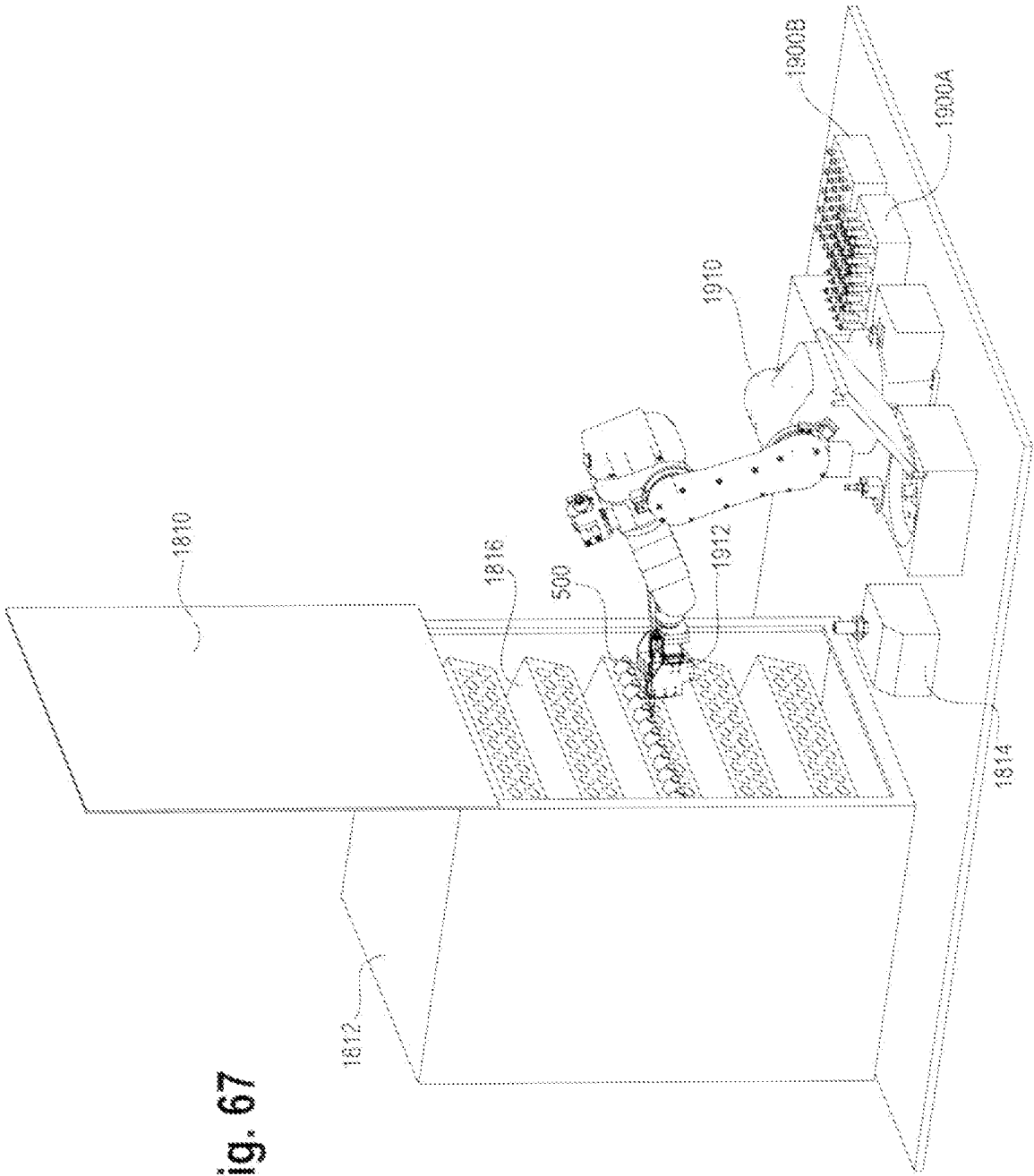


Fig. 67

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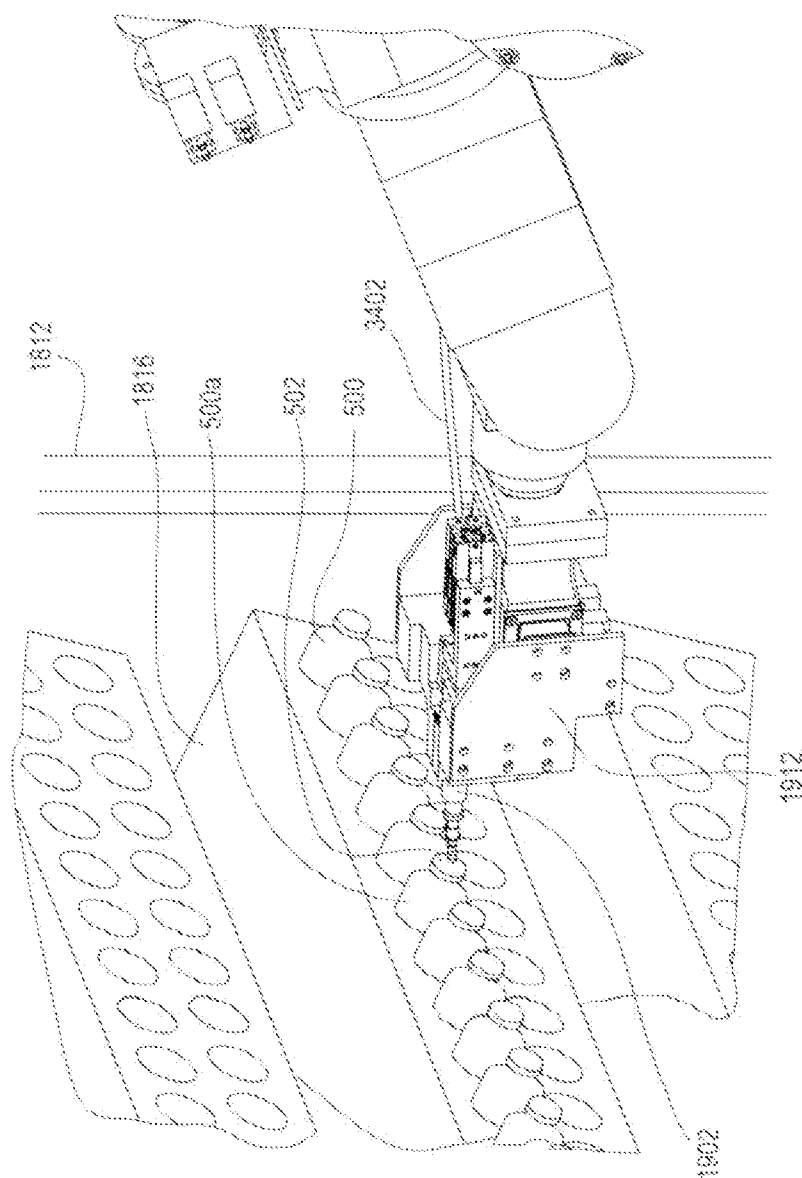


Fig. 69

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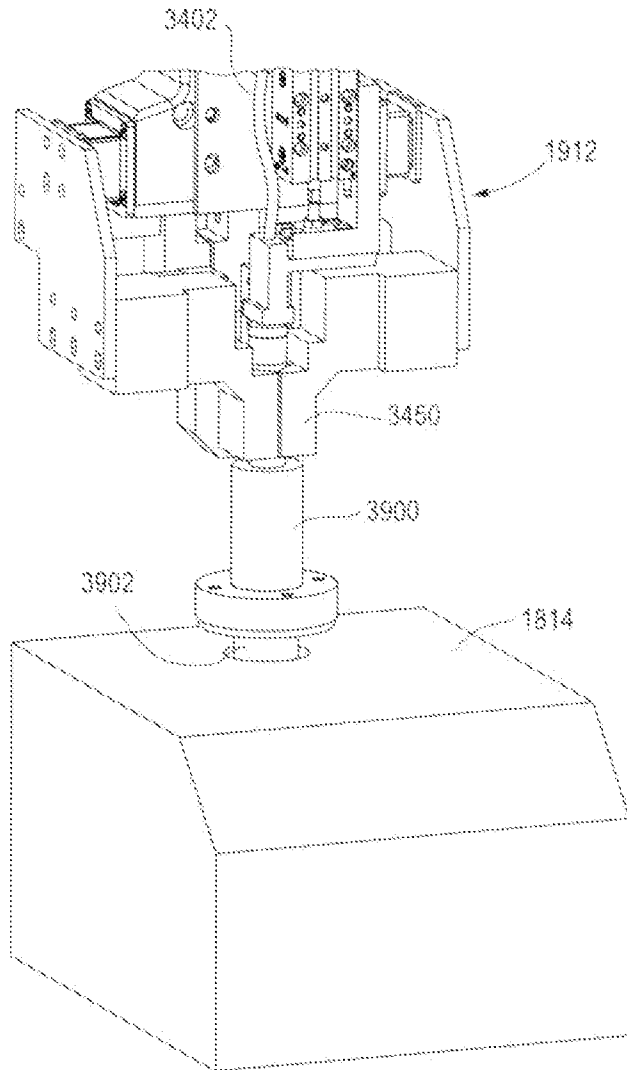


Fig. 69A

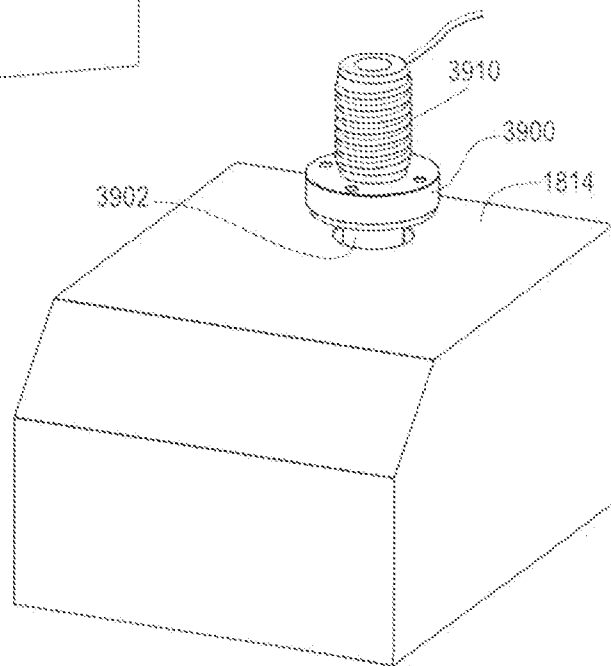
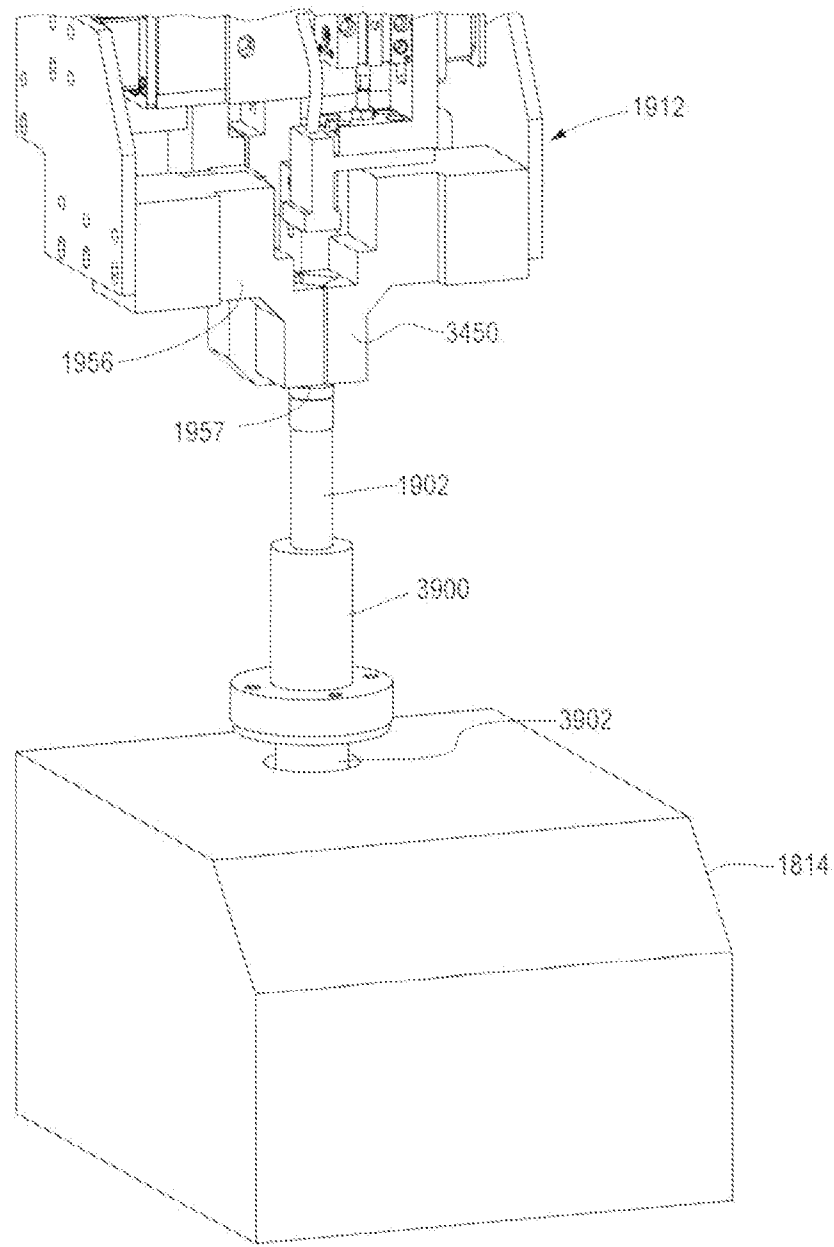


Fig. 70



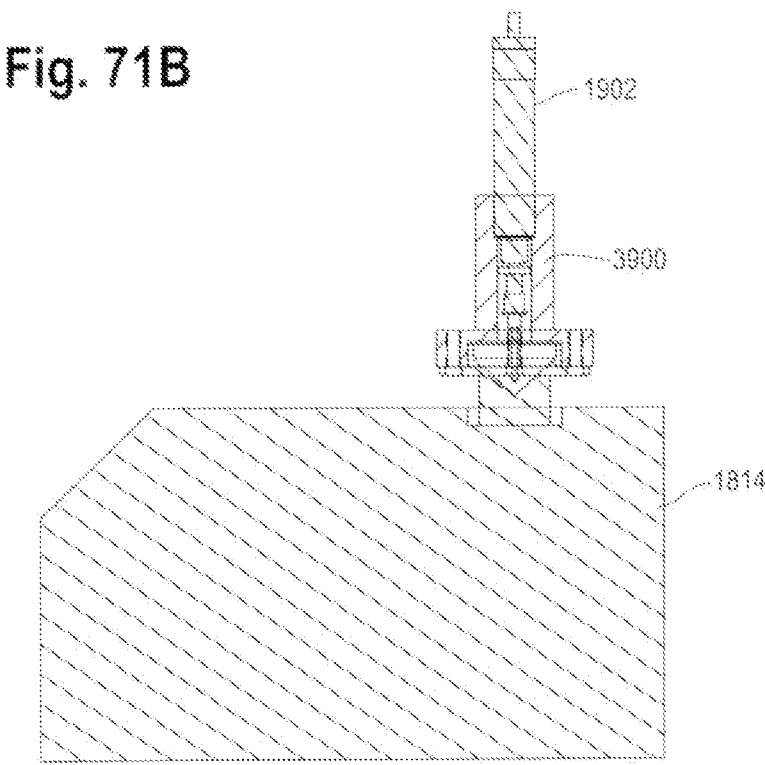
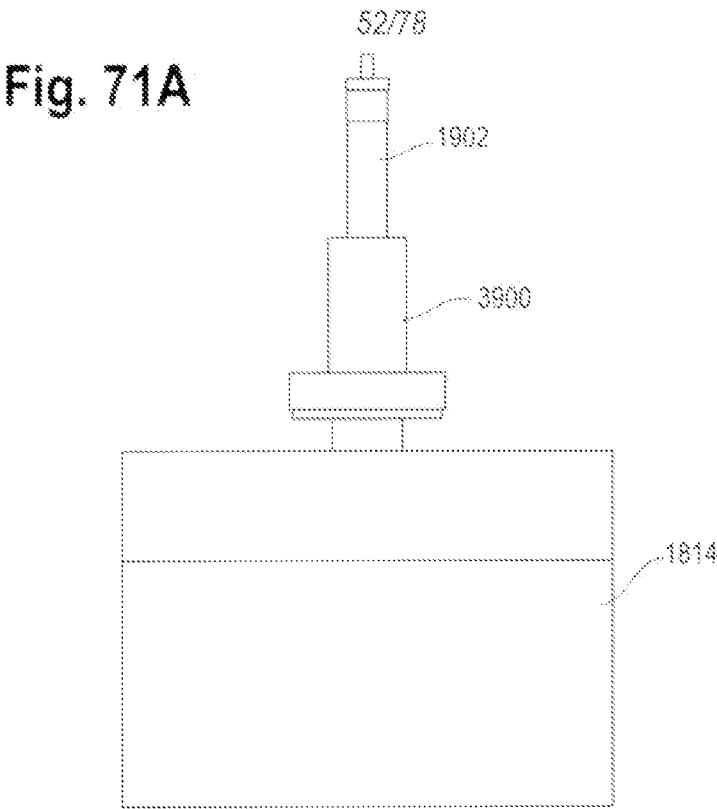


Fig. 72A

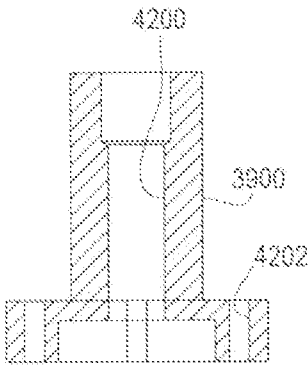
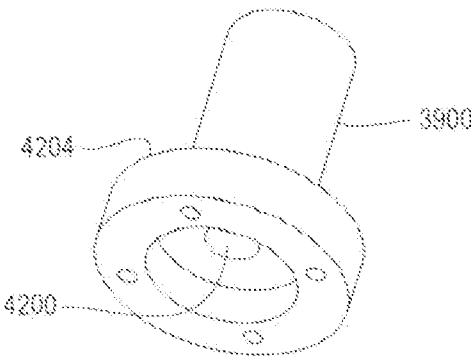


Fig. 72B



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Fig. 73A

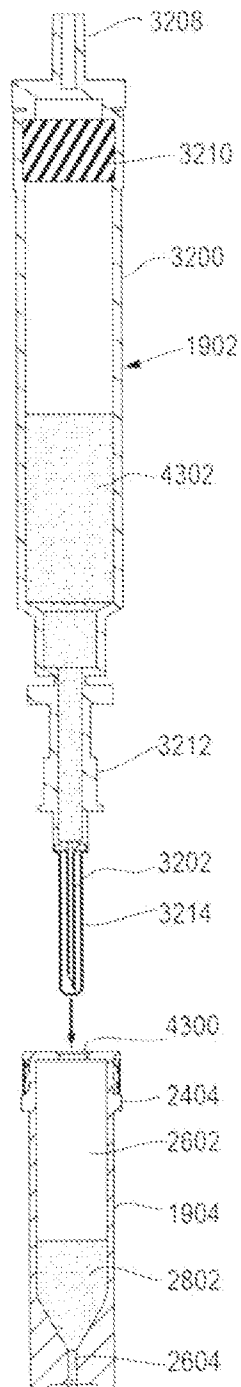
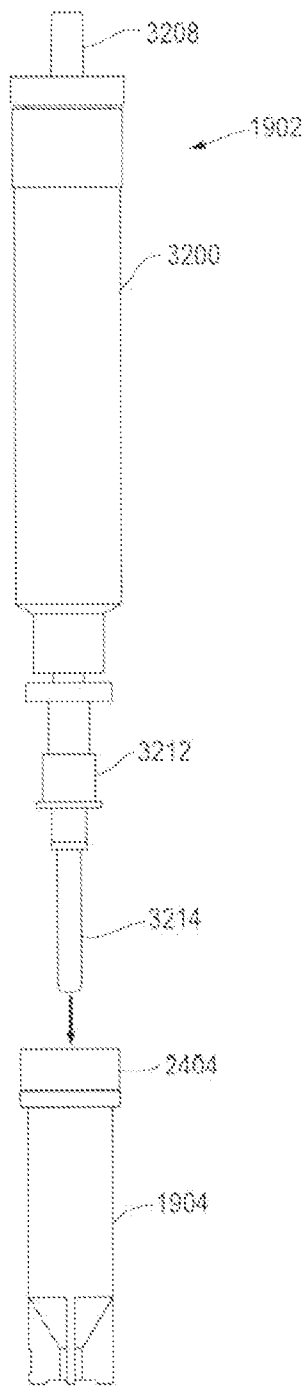


Fig. 73B



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Fig. 73C

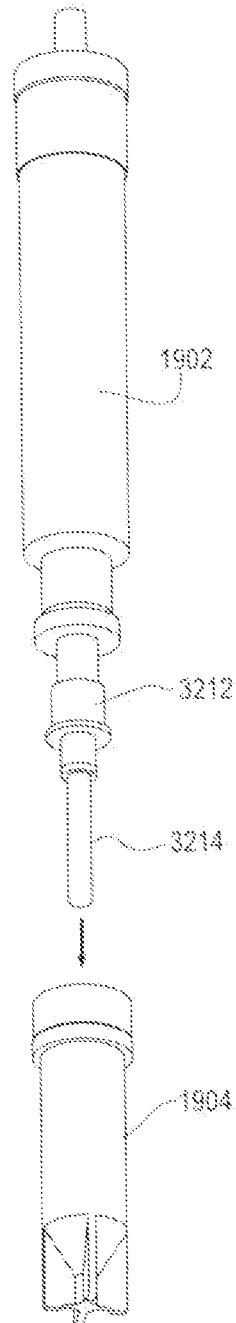


Fig. 73D

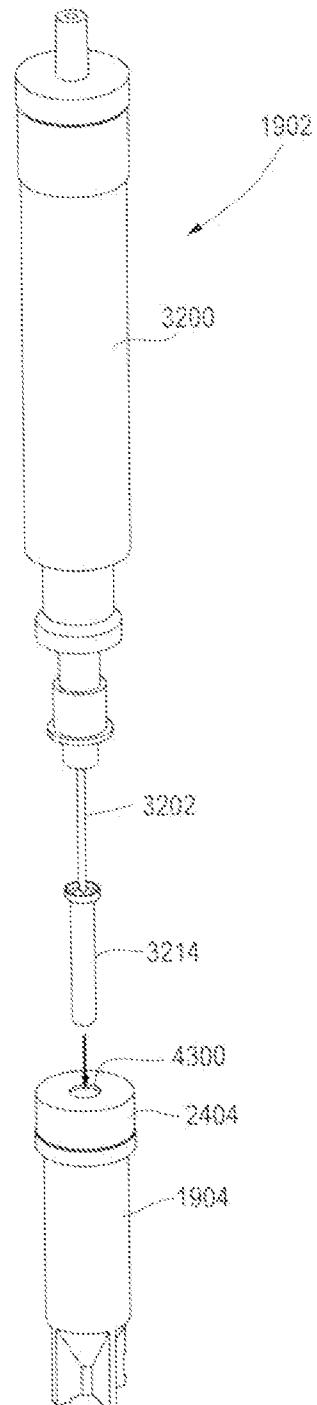


Fig. 74

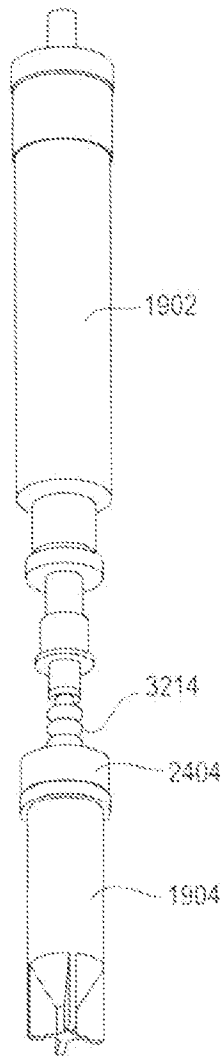
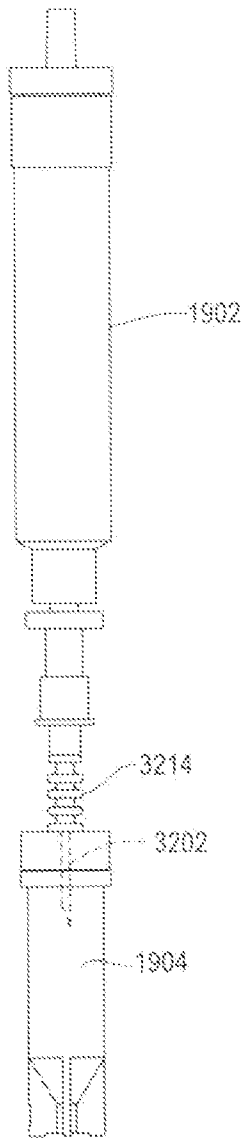


Fig. 75



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Fig. 76

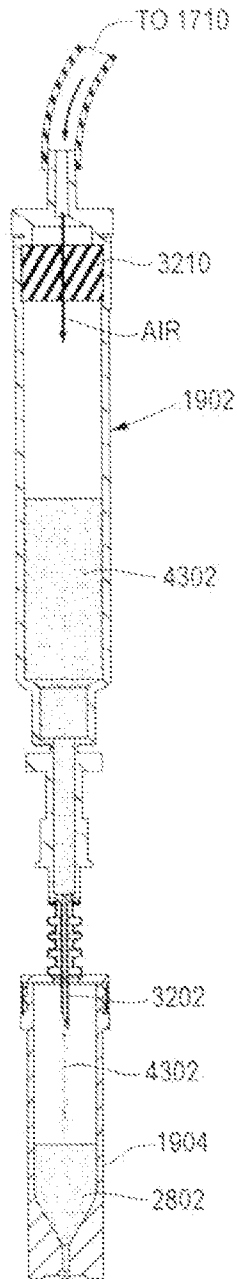


Fig. 76A

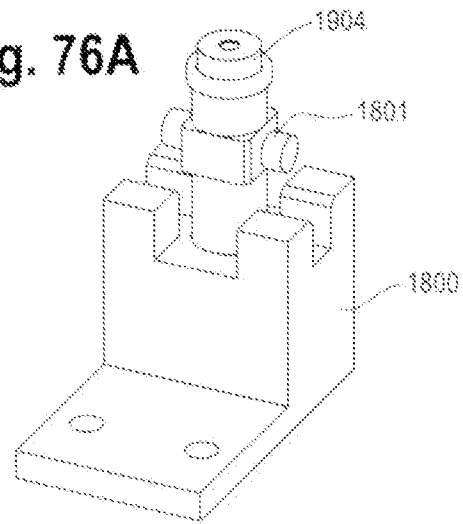


Fig. 76B

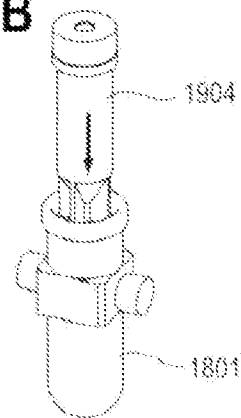
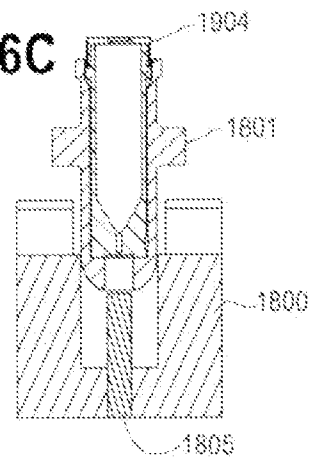


Fig. 76C



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Fig. 77

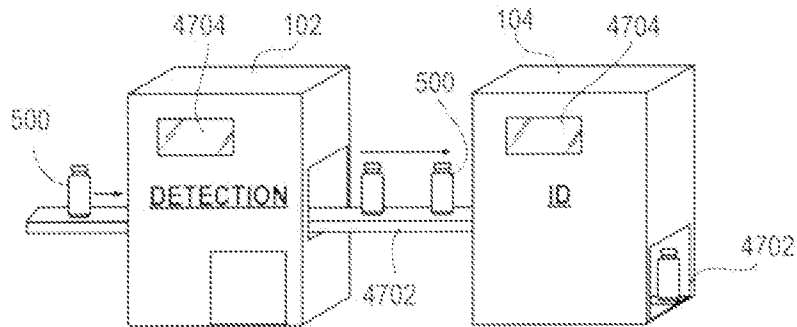


Fig. 78

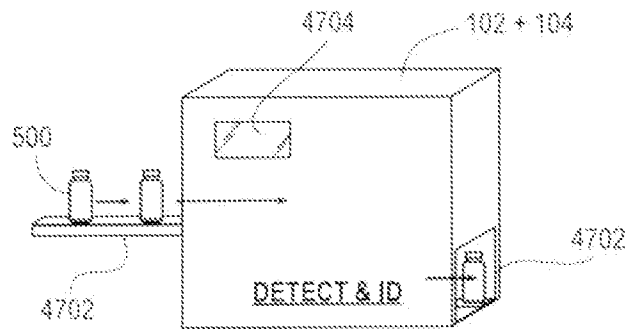
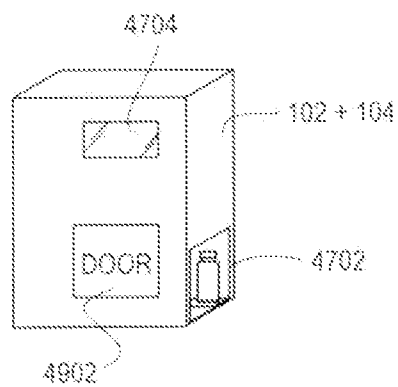
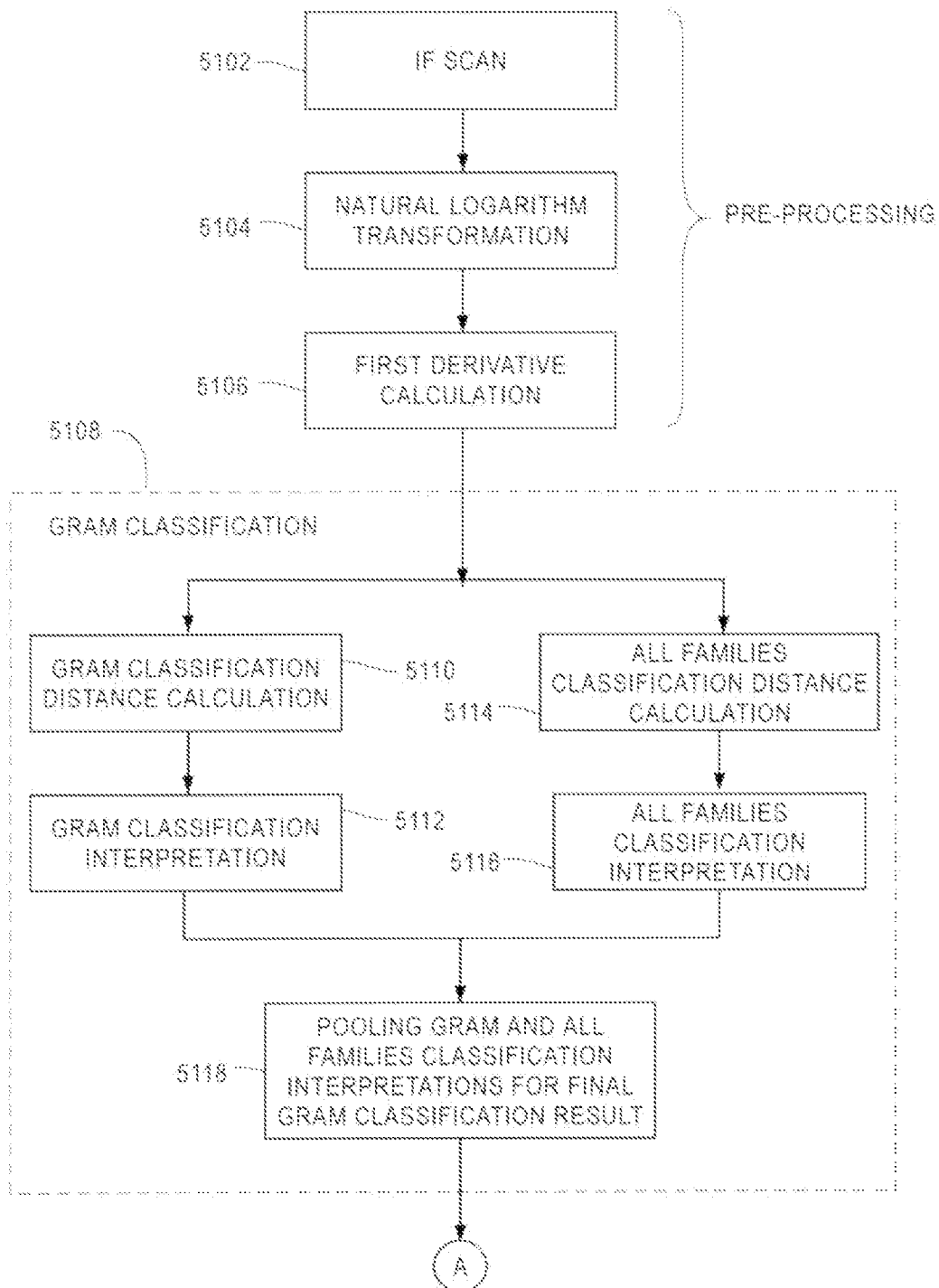


Fig. 79

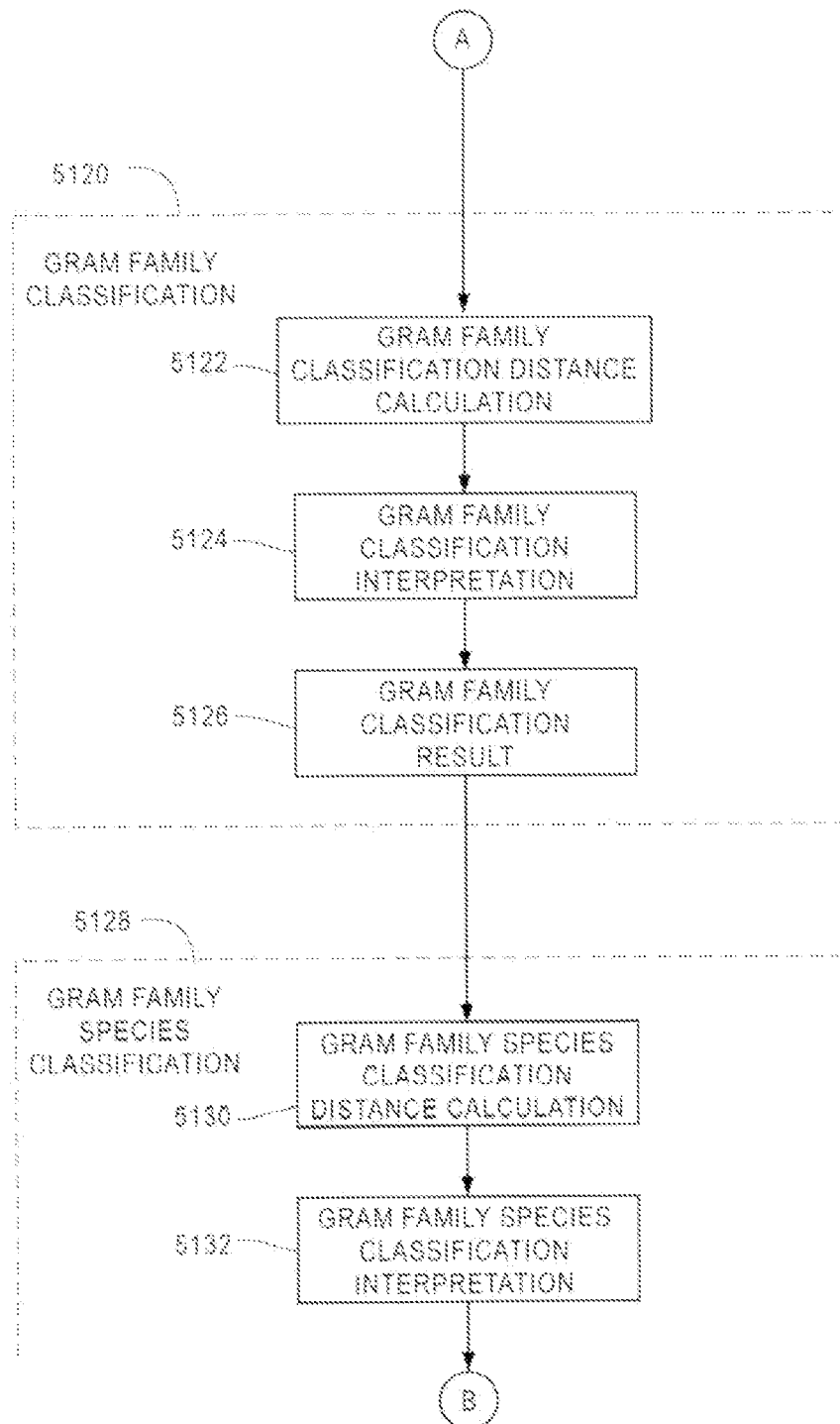


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Fig. 81A

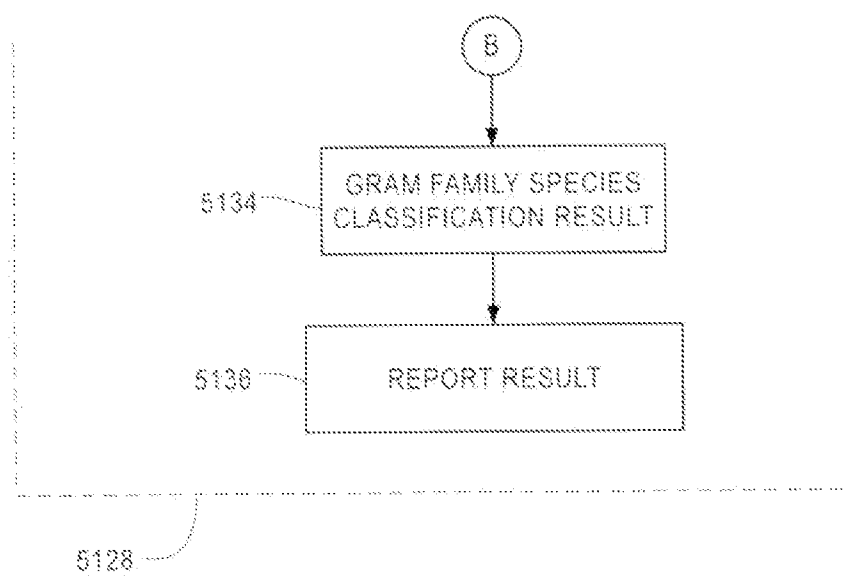
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Fig. 81B

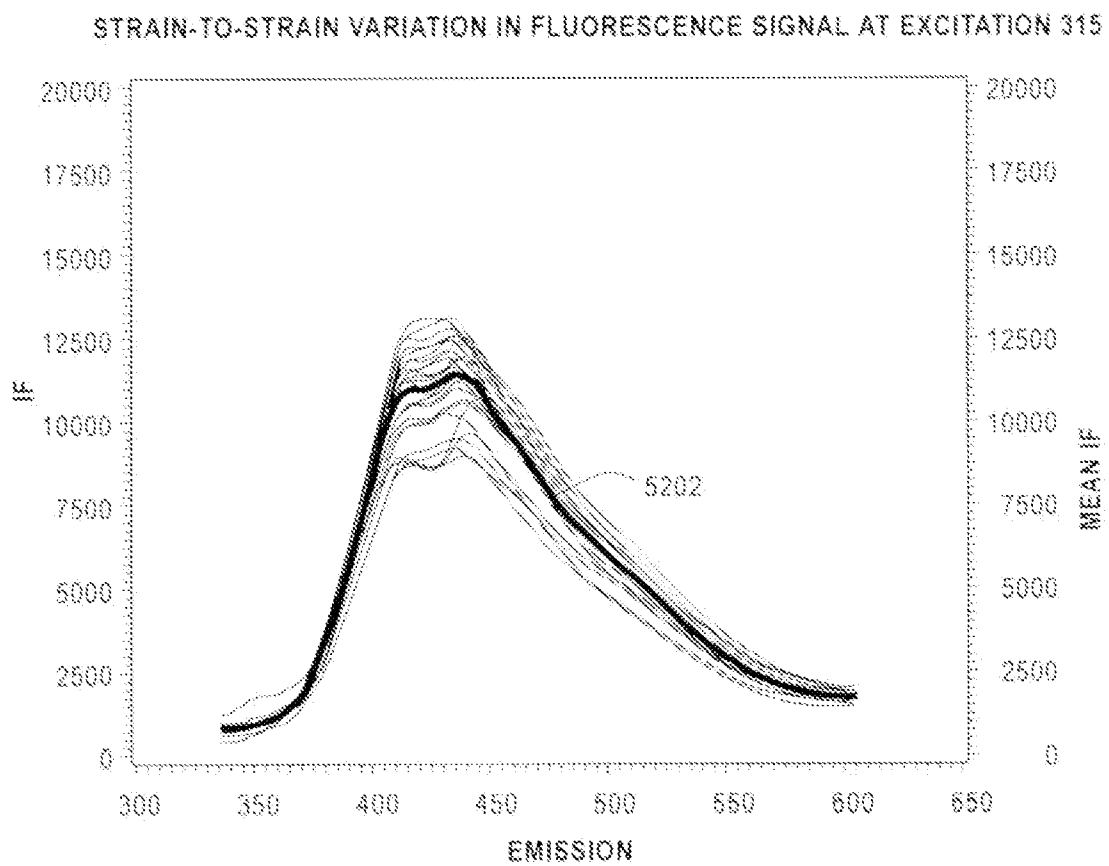


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Fig. 81C



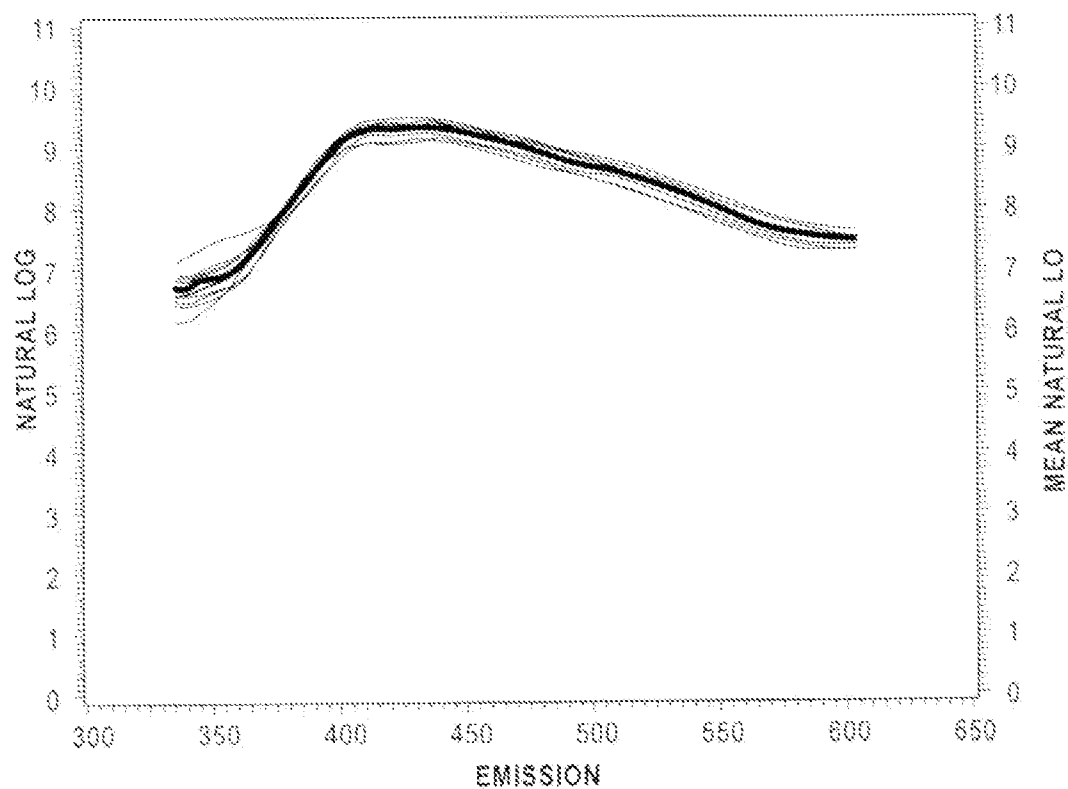
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Fig. 82

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Fig. 83

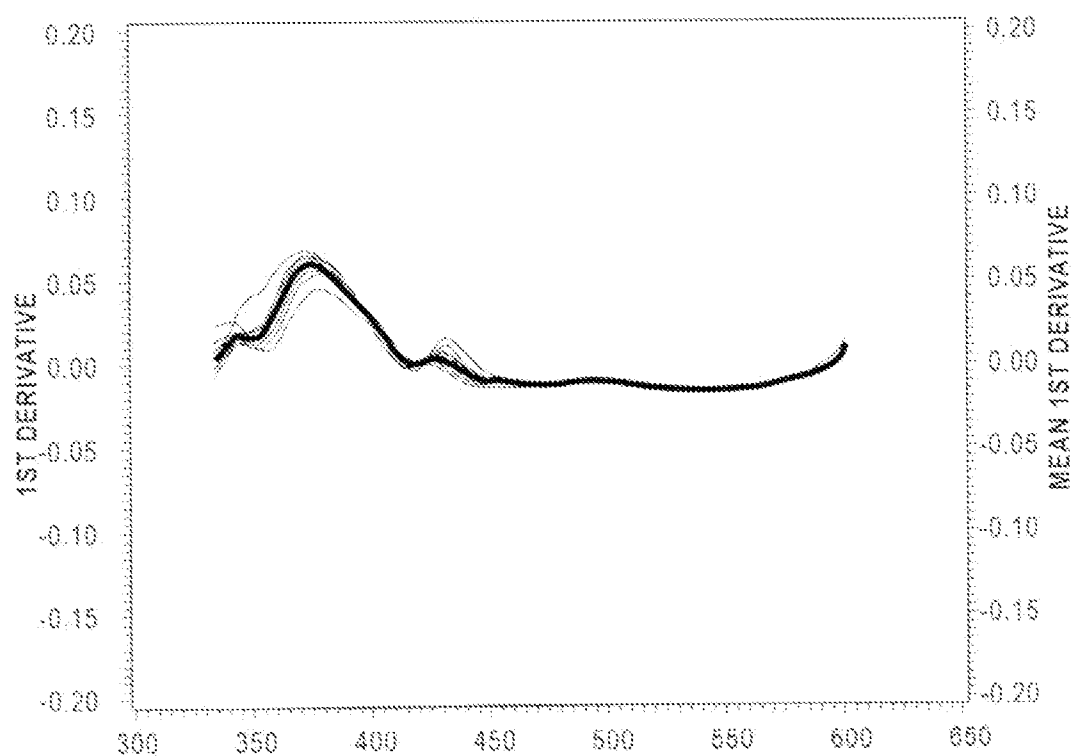
STRAIN-TO-STRAIN VARIATION IN FLUORESCENCE SIGNAL AT EXCITATION 315
AFTER APPLICATION OF THE NATURAL LOGARITHM TRANSFORMATION



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Fig. 84

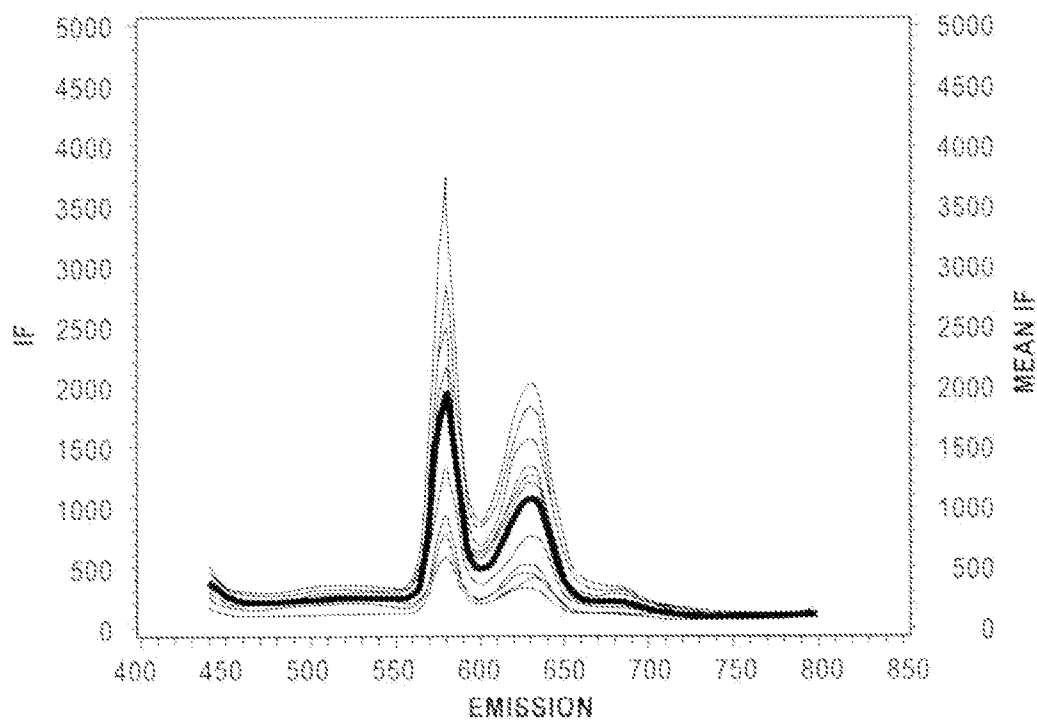
STRAIN-TO-STRAIN VARIATION IN FLUORESCENCE SIGNAL AT EXCITATION 315
AFTER CALCULATING THE 1ST DERIVATIVE



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Fig. 85

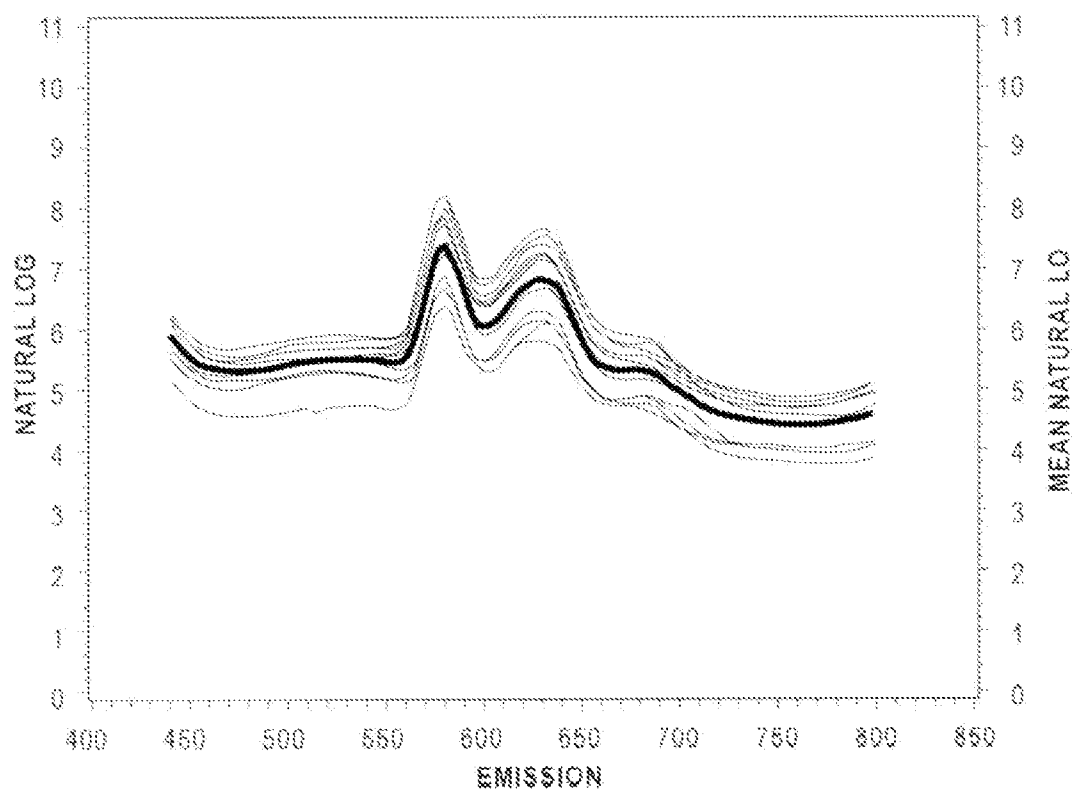
STRAIN-TO-STRAIN VARIATION IN FLUORESCENCE SIGNAL AT EXCITATION 415



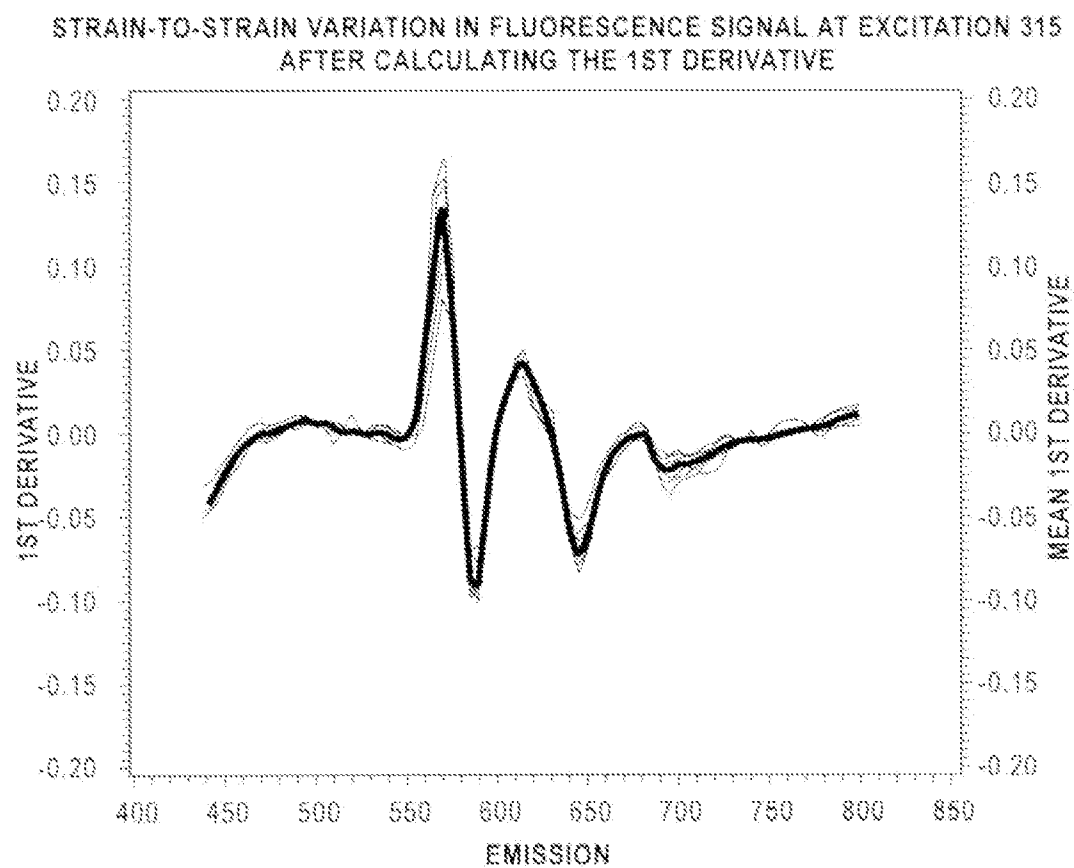
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Fig. 86

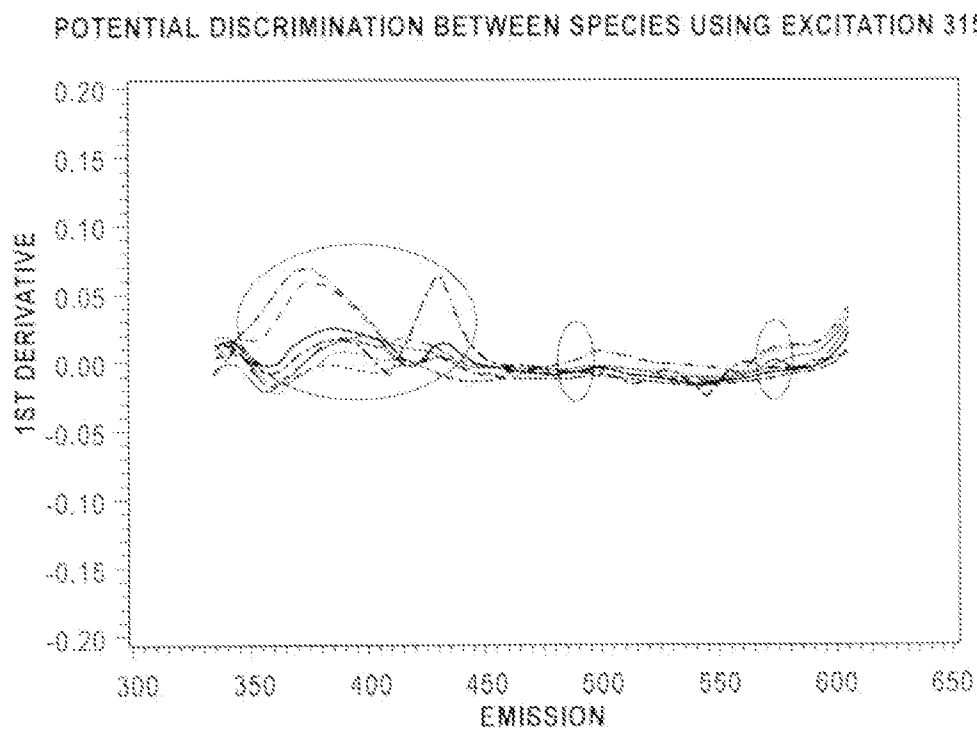
STRAIN-TO-STRAIN VARIATION IN FLUORESCENCE SIGNAL AT EXCITATION 415
AFTER APPLICATION OF THE NATURAL LOGARITHM TRANSFORMATION



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Fig. 87

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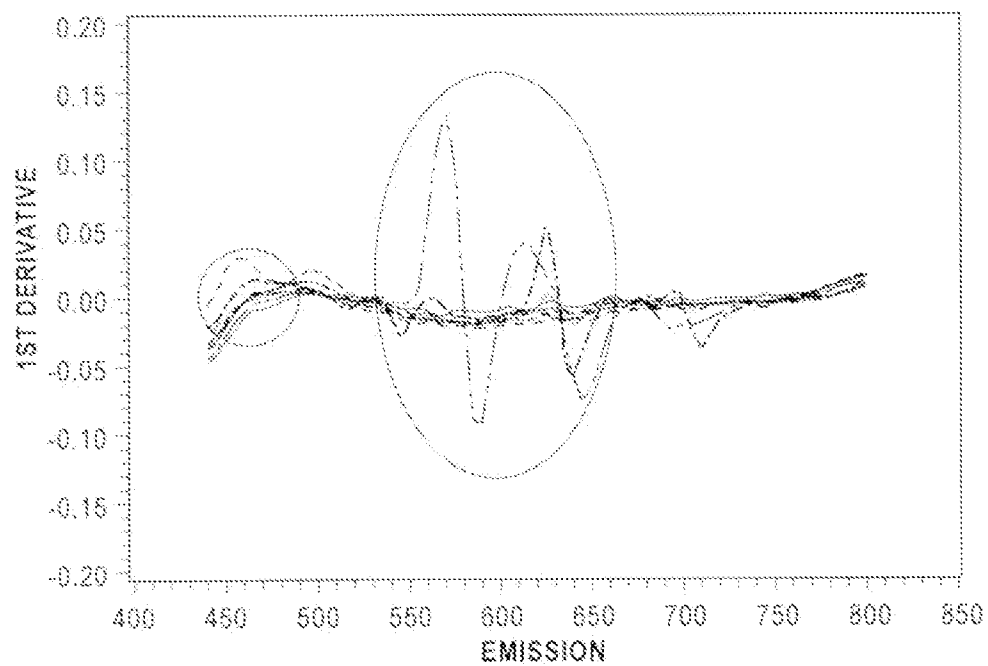
Fig. 88

SPECIES	 C.GLABRATA	———— E.COLI
	----- P.AERUGINOSA	- - - - S.EPIDEMIDIS
	-- --- C.PARAPSILOSIS	- - - - H.INFLUENZAE
	- - - - S.AUREUS	 S.PNEUMONIAE

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Fig. 89

POTENTIAL DISCRIMINATION BETWEEN SPECIES USING EXCITATION 415



SPECIES	 C.GLABRATA	 E.COLI
	 PAERUGINOSA	 S.EPIDEMIDIS
	 C.PARAPSILOSIS	 H.INFLUENZAE
	 S.AUREUS	 S.PNEUMONIAE

Fig. 91

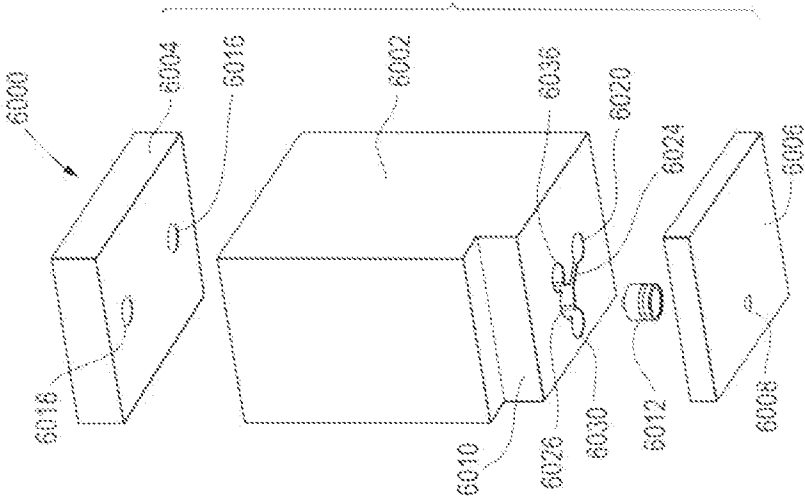
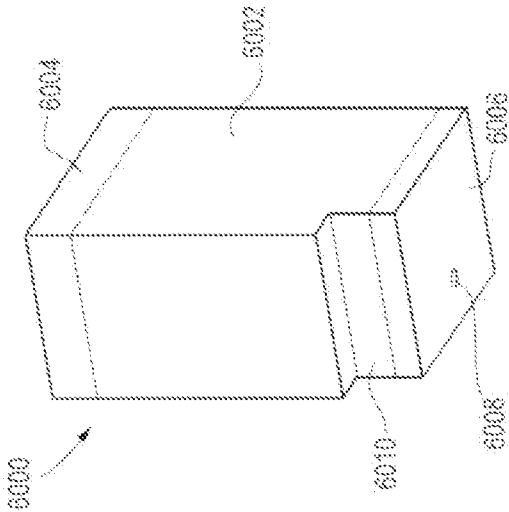
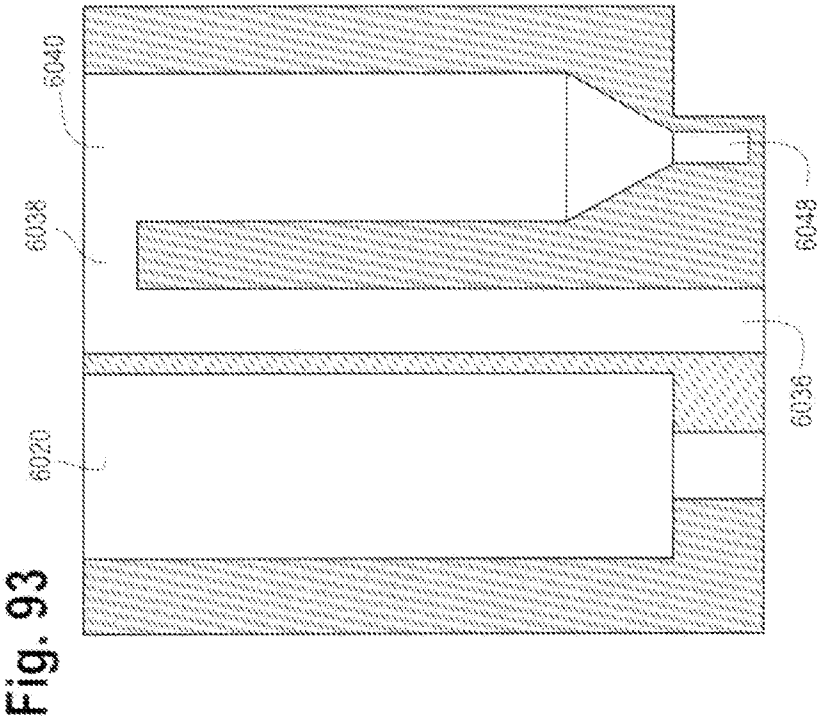
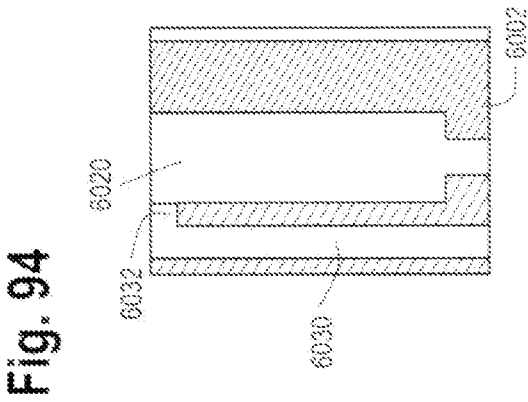
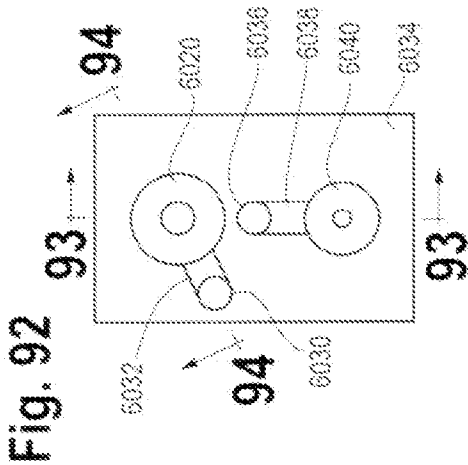


Fig. 90





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Fig. 95

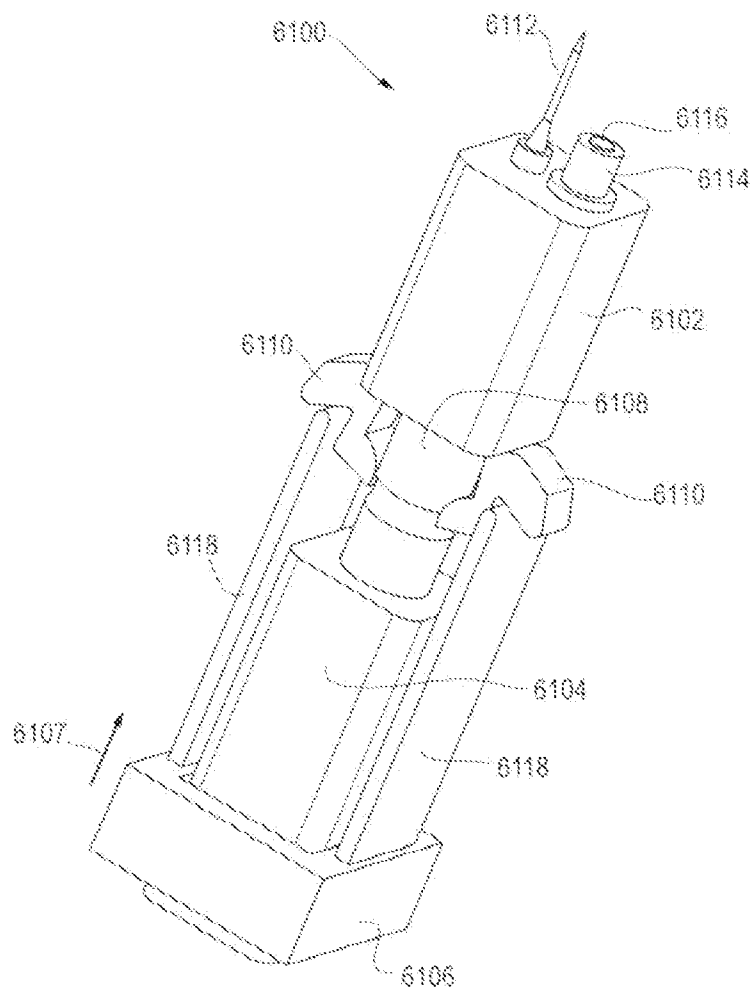


Fig. 96

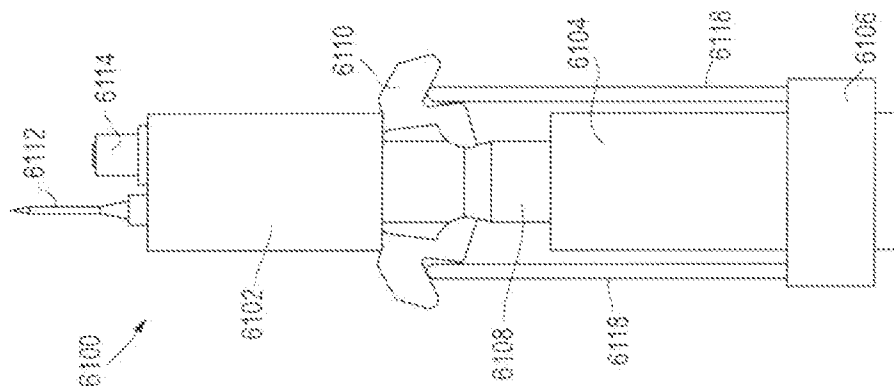


Fig. 97

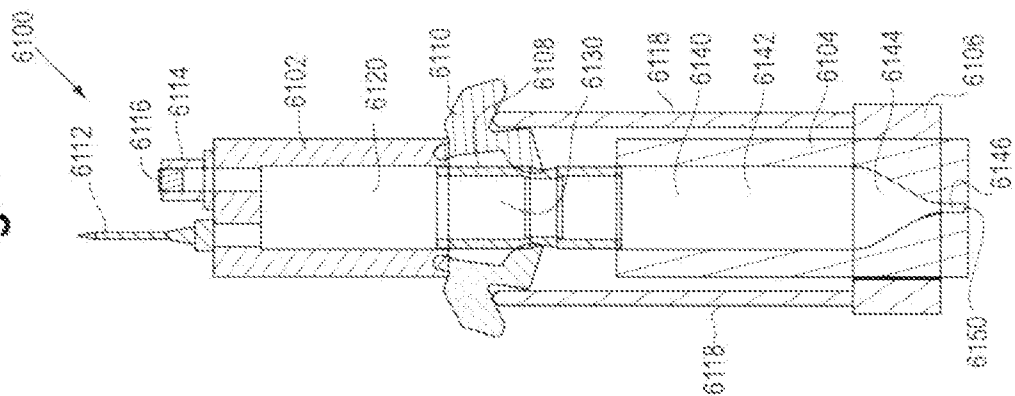


Fig. 98

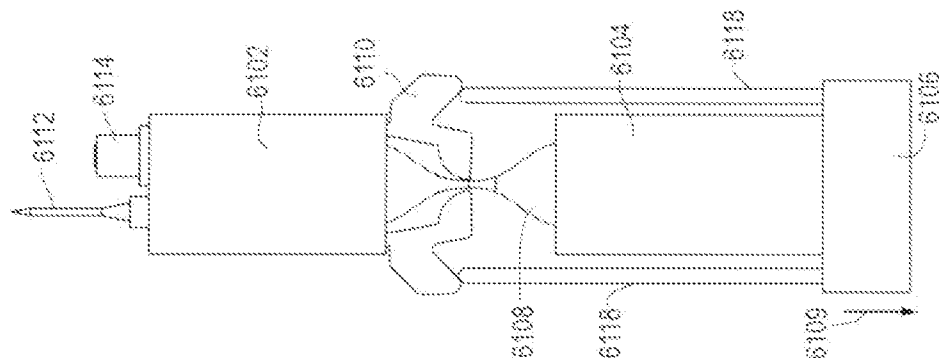
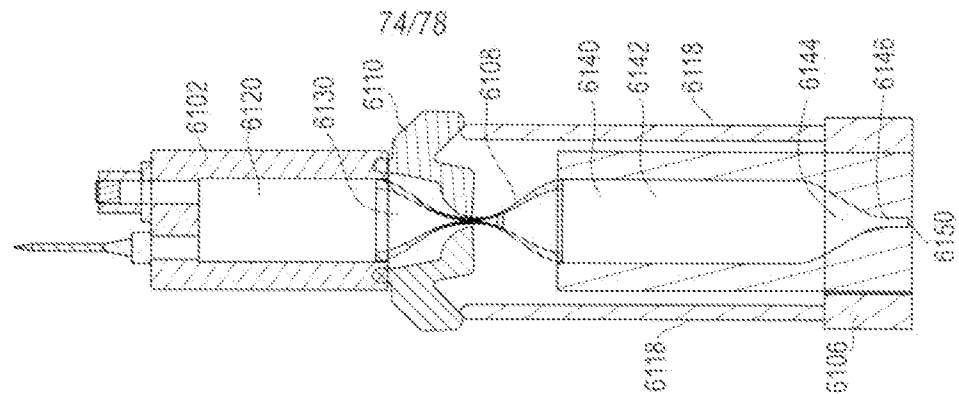
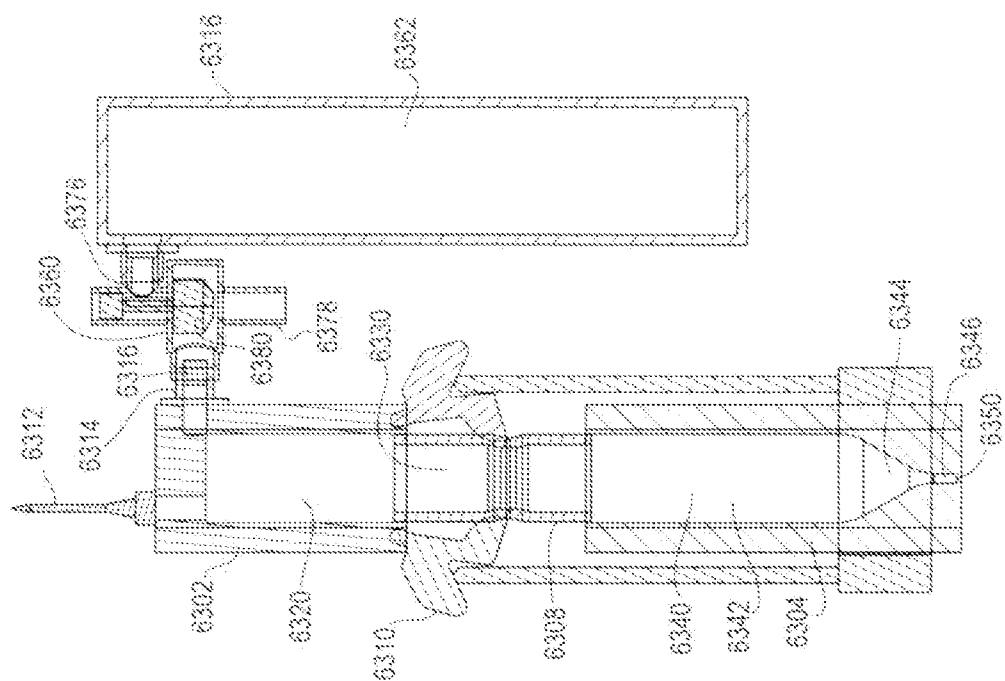


Fig. 99



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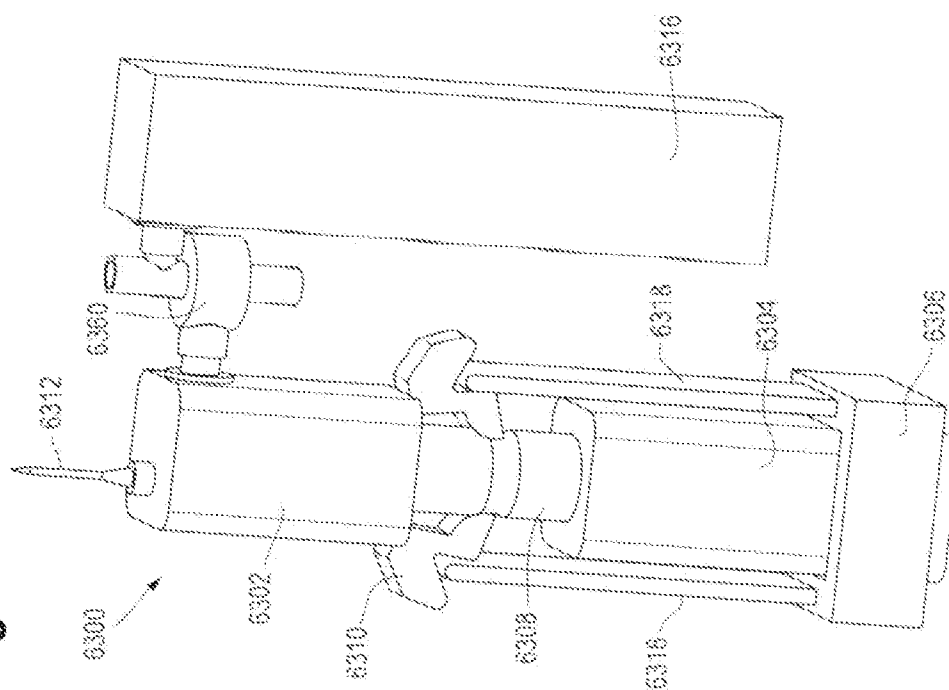


Fig. 102

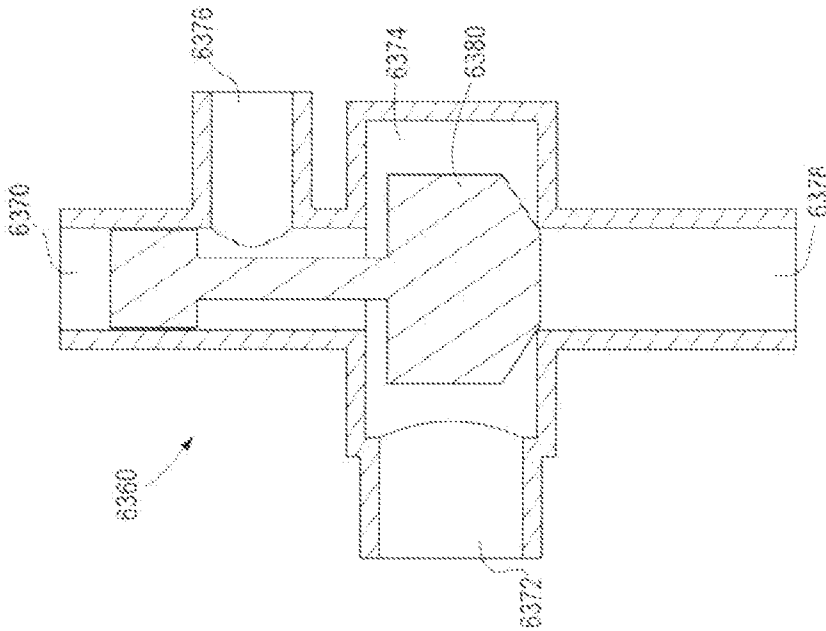


Fig. 103

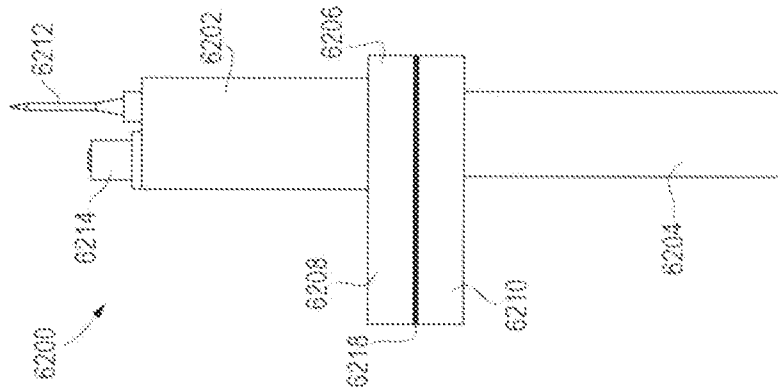


Fig. 104

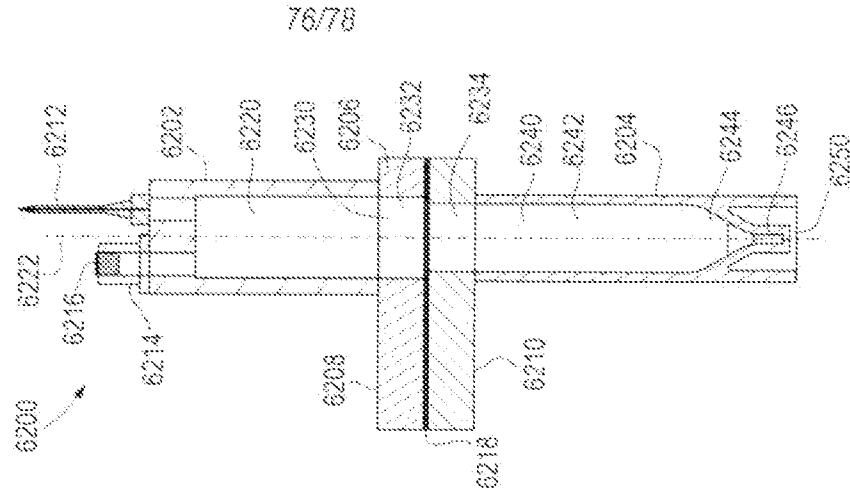


Fig. 106

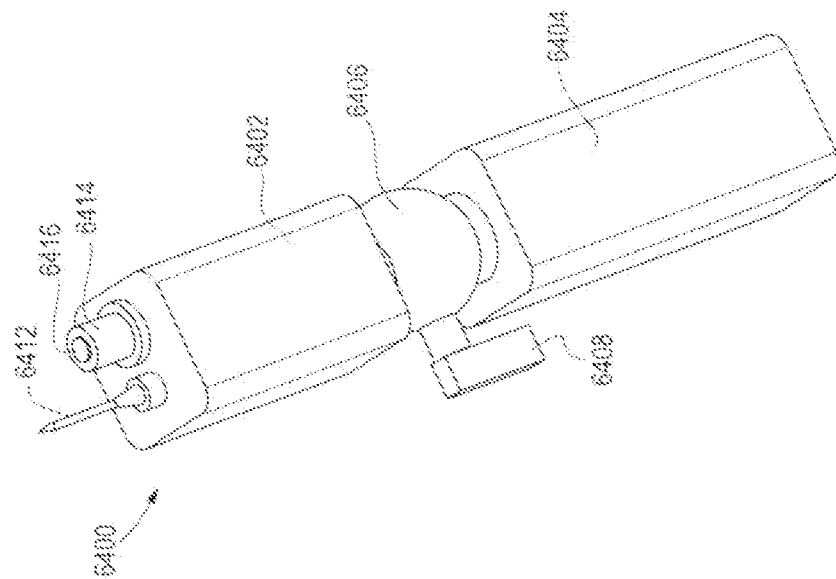


Fig. 105

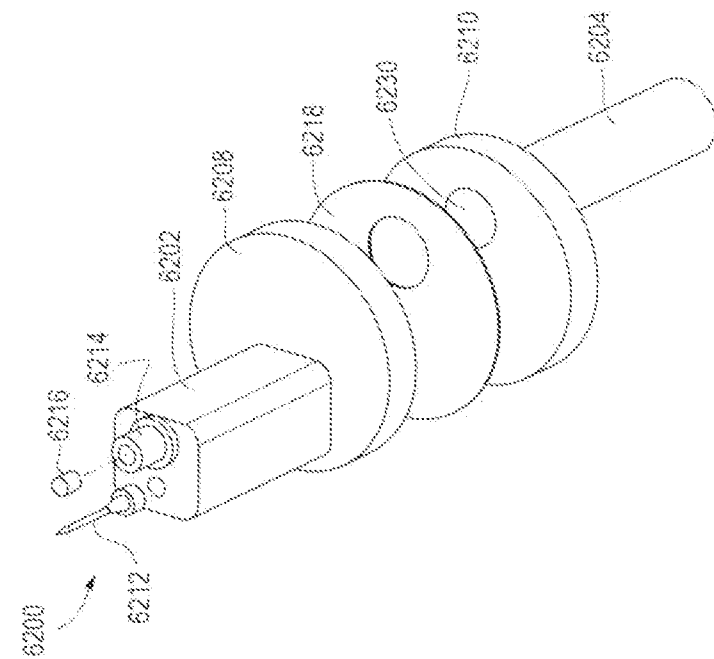


Fig. 107A

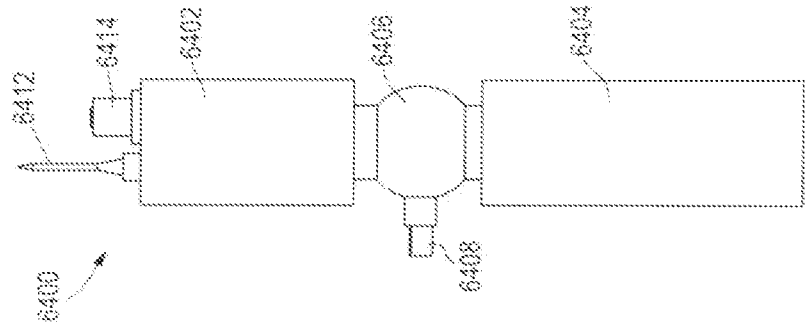


Fig. 107B

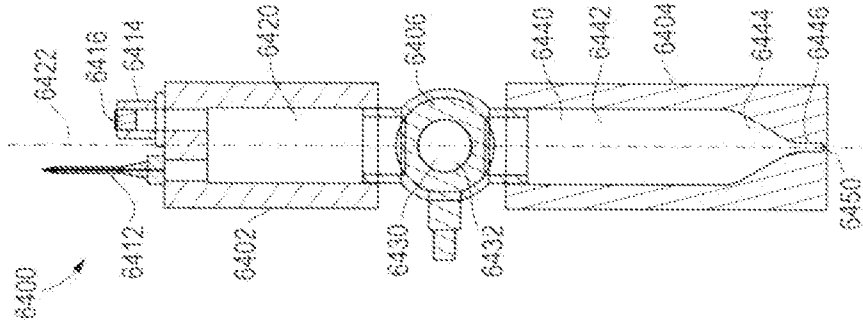


Fig. 108A

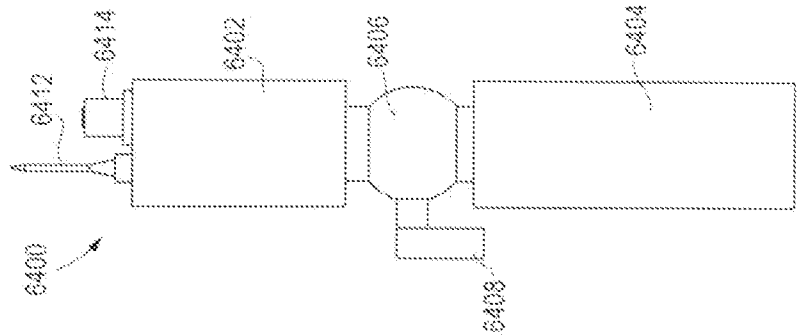


Fig. 108B

