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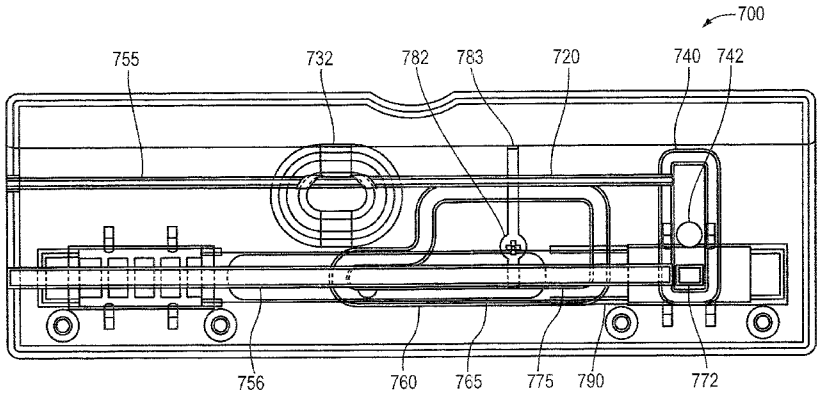

FIG. 7A

(57) Abstract: An apparatus for preparing a test sample includes a container for receiving a volume of a fluid sample. The container utilizes capillary channels and one or more chambers with removable separators to measure and commingle specific volumes of each constituent of the test sample, thereby providing a prepared test sample for an immunoassay.

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SAMPLE PREPARATION DEVICE

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/195,282, filed on October 6, 2008, the entire teachings of which are incorporated herein by reference.

BACKGROUND

Quantitative analysis of cells and analytes in fluid samples, particularly bodily fluid samples, often provides critical diagnostic and treatment information for physicians and patients. Immunoassays, such as qualitative, quantitative and semi-quantitative immunoassays, utilize the specificity of the antigen (Ag)-antibody (Ab) reaction to detect and quantitate the amount or presence of an Ag or Ab in a sample. In solid phase immunoassays, for example, one reagent (e.g., the Ag or Ab) is attached to a solid surface, facilitating separation of bound reagents or analytes from free reagents or analytes. The solid phase is exposed to a sample containing the analyte, which binds to its Ag or Ab; the extent of this binding is quantitated to provide a measure of the analyte concentration in the sample.

In preparing a fluid sample for analysis, a test sample is made. The test sample is a homogenous mixture comprising the fluid sample, a conjugate and a diluent. The conjugate includes a solid-phase reagent corresponding to the analyte of the fluid sample to be quantitated. Exposing the fluid sample to the conjugate in the presence of the diluent facilitates binding between the reagent and the analyte, resulting in a prepared test sample for analysis by an immunoassay reader.

Typical methods for preparing a test sample for a quantitative immunoassay require precise measurements of each constituent and a time-consuming process of

combining those constituents by trained personnel. These methods are subject to human error, thereby leading to incorrect results in a subsequent immunoassay.

SUMMARY

Example embodiments of an apparatus of the invention provide an inlet for receiving a fluid sample, and an entry capillary channel coupled to the inlet at one end for receiving the fluid sample into the entry capillary channel. The entry capillary channel may include a vent and may be configured to draw a specific volume of the fluid sample. The entry capillary channel is coupled, via a separator, to a chamber having a section containing a dry conjugate and another section containing a diluent, the sections separated by another separator. Upon removal or opening of the separators, the fluid sample, conjugate and diluent may be mixed in the chamber to form a homogeneous mixture, resulting in a prepared test sample. A terminal capillary channel draws a specific volume of the prepared test sample upon removal of an additional separator. From this terminal capillary channel, the volume of prepared test sample can be dispensed into a test cartridge for analysis by an immunoassay reader.

In further embodiments of the invention, the chamber may be adapted to enable an applied mechanical force against a wall of the chamber to facilitate the mixing. The capillary channels may also be adapted to enable an applied mechanical force to dispense the fluid sample or test sample from the respective capillary channel. The entry capillary channel, upon being filled with the fluid sample, may be isolated from the inlet, thereby maintaining the specific volume of fluid sample. The separators may form a valve, a removable or breakable wall or other barrier that can be removed by external mechanical means. Separators may include shear valve mechanisms, poppet valves, ball check valves, flap valves or other mechanisms to open or close the separator by mechanical, electrical or electro-mechanical means. An additional separator, located at the terminal capillary channel, may be removed so as to enable the test sample to flow into a test cartridge. The additional separator may include a vent to facilitate drawing the prepared test sample into the terminal capillary channel.

Methods and apparatus of the present invention provide means to facilitate preparation of samples and conductions of assays, while eliminating the need for multiple precise measurements or for specially trained personnel.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

FIG. 1 is a block diagram of an immunoassay reader and test sample in which embodiments of the present invention may be implemented.

FIG. 2 is a block diagram of a device for preparing a test sample for an immunoassay.

FIG. 3 is a block diagram of a container implemented by the device of FIG. 2 for preparing an immunoassay test sample.

FIG. 4 is a flow diagram illustrating a method of preparing a test sample.

FIG. 5 illustrates a container for preparing a test sample.

FIG. 6 is a block diagram of a container for preparing an immunoassay test sample.

FIG. 7A illustrates a test cartridge having a device for preparing a test sample.

FIG. 7B illustrates a diluent pouch for dispensing a volume of diluent.

DETAILED DESCRIPTION

A description of example embodiments of the invention follows.

Embodiments of the present invention provide methods and apparatus for preparing a test sample for testing by an immunoassay processor-reader. In some embodiments, a device accepts a specific volume of a sample fluid into a structured container. The container may be, for example, a pouch made of aluminum foil or other material and having one or more chambers. The chambers contain a predetermined amount of dry conjugate and diluent. The container is configured

such that, upon manipulation by the encompassing device, the specific volume of the sample fluid is mixed with the conjugate and diluent to form a homogeneous mixture, being the prepared test sample. Upon further manipulation, a specific volume of the test sample is extracted from the container and dispensed into a test cartridge. As a result, the test cartridge contains a prepared test sample for an immunoassay. In contrast to prior art procedures, embodiments of the present invention provide an accurate, prepared test sample for an immunoassay quickly and in an automated manner.

The devices described here in can be utilized for assessment of presence, absence, or quantity of analytes in a sample. The terms, analyte or analyte of interest, as used herein, refer to a first member of a binding pair. The analyte is a molecule or compound for which the amount will be measured. The analyte can be in the form of a solid, such as a dry substance (e.g., a powder, a particulate; spore; or other particle), or can be in the form of a fluid (e.g., a solid as described above that has been dissolved or suspended in a fluid; or other liquid sample). Examples of analytes include bacteria; spores; proteins, such as hormones or enzymes; glycoproteins; peptides; small molecules; polysaccharides; antibodies; nucleic acids; drugs; toxins (e.g., environmental toxins); viruses or virus particles; portions of a cell wall; and other compounds. In a preferred embodiment, each analyte is "immunogenic," which indicates that antibodies (as described below) can be raised to that analyte, or to analyte that is bound to a carrier (e.g., a hapten-carrier conjugate, for which antibodies can be raised to the hapten). The analytes of interest can be in a liquid sample; alternatively, the analytes of interest can be in a dry (non-fluid) sample (e.g., a solid, such as a particulate sample, powder sample, food sample, or soil sample). Each analyte of interest is a first member of a binding pair – e.g., a specific binding pair, in which a first member of the binding pair (e.g., analyte) reacts specifically with a second member (e.g., the binding agent). One or both members of the binding pair can be an antibody. For example, a first member of the binding pair (e.g., an analyte of interest) can be an antibody, and a second member of the binding pair (e.g., a binding agent) can be anti-immunoglobulin antibody; alternatively, the first member of the binding pair (e.g., the analyte) can be

an antigen, and the second member of the binding pair (e.g., the binding agent) can be an antibody.

The devices of the inventions utilize a "conjugate". The conjugate comprises a population of particles. The population(s) of particles varies, depending on the size and composition of the particles, the composition of the components of the device, and the level of sensitivity of the assay. In certain embodiments, the conjugate comprises particles such as liposomes, colloidal gold, organic polymer latex particles, inorganic fluorescent particles or phosphorescent particles. In a particularly preferred embodiment, the particles are polystyrene latex beads, and most particularly, polystyrene latex beads that have been prepared in the absence of surfactant, such as surfactant free Superactive Uniform Aldehyde/Sulfate Latexes (Interfacial Dynamics Corp., Portland, OR).

In one embodiment, the conjugate comprises a population of analyte binding particles which are coated with an analyte binding agent for each analyte of interest: for example, a first analyte binding agent for a first analyte of interest; a second analyte binding agent for a second analyte of interest; etc., such that there is an analyte binding agent corresponding to each analyte of interest. Alternatively, the sample collection apparatus can contain a population of analyte binding particles for each analyte binding agent; that is, a population of analyte binding particles for a first analyte of interest; a population of analyte binding particles for a second analyte of interest; etc., such that there is a population of analyte binding particles corresponding to each analyte of interest. If desired, a combination of different types of populations of analyte binding particles can also be used.

The size of the particles is related to porosity of the membrane in the device or the size of the capillary channels in the device, and also to the size of the analytes of interest (e.g., for particulate analytes): the particles must be sufficiently small to be transported along the membrane or through the capillary channel by capillary action of fluid, and also (for solid, e.g., particulate analytes) sufficiently small for the complex of contacted analyte binding particles, as described below, to be transported along the membrane or through the capillary channel by capillary action. The particles can be labeled to facilitate detection. The particles are labeled by a means which does not significantly affect the physical properties of the particles; for

example, the particles are labeled internally (that is, the label is included within the particle, such as within the liposome or inside the polystyrene latex bead). Representative labels include luminescent labels; chemiluminescent labels; phosphorescent labels; enzyme-linked labels; chemical labels, such as electroactive agents (e.g., ferrocyanide); and colorimetric labels, such as dyes or fluorescent labels. In one embodiment, a fluorescent label is used. In another embodiment, phosphorescent particles are used, particularly "up-converting" phosphorescent particles, such as those described in U.S. Patent No. 5,043,265, the entirety of which is incorporated herein by reference. If the sample capture zones are separate, for example, the same type of label can be used for each population of analyte binding particles (e.g., for both the population of particles for the first analyte of interest, and the population of particles for the second analyte of interest). Alternatively, different types of labels (distinctive labels) can be used, e.g., if the sample capture zones over lap or occupy the same area. Immunoassays based on fluorescence utilize a fluorescently labeled solid phase. An immunoassay reader employing a fluorescence analyzer may therefore measure the quantity or presence of an antibody or antigen by optically scanning and detecting fluorescent emissions of the solid phase following contact of the sample containing the analyte with the solid phase. By measuring the level of fluorescence, the immunoassay reader provides quantitative analysis of analyte concentration of a fluid sample.

The particles are coated with an analyte binding agent that is a second member of the binding pair for each analyte of interest (e.g., particles having more than one type of analyte binding agent coated thereon; or different populations of particles, each population having a single type of analyte binding agent for its analyte coated thereon). As described above, an analyte binding agent (second member of a binding pair) specifically and preferentially binds to its analyte of interest (first member of the binding pair). Representative analyte binding agents include antibodies (or fragments thereof); haptens; drug conjugates; receptors; or other binding partners. In one preferred embodiment, the analyte binding agent is an antibody to the analyte of interest. Antibodies can be monoclonal antibodies or polyclonal antibodies. The term "antibody", as used herein, also refers to antibody fragments which are sufficient to bind to the analyte of interest. Alternatively, in

another embodiment, molecules which specifically bind to the analyte of interest, such as engineered proteins having analyte binding sites, can also be used (Holliger, P. and H. R. Hoogenbloom, Trends in Biotechnology 13:7 9 (1995); Chamow, S. M. and A. Ashkenazi, Trends in Biotechnology 14:52 60:1996)). In still another embodiment, if the analyte of interest is a drug, a hapten or other drug conjugate can be used as the analyte binding agent. Alternatively, in a further embodiment, a receptor which binds to the analyte can be used (e.g., if the analyte of interest is a ligand). If the analyte is an antibody of known specificity, the particles can be coated with the antigen against which the analyte antibody is directed, or can be coated with antibody to the analyte-antibody. Furthermore, because the analyte and the analyte binding agent form a binding pair, compounds or molecules described as representative analytes can also serve as analyte binding agents, and those described as representative analyte binding agents can similarly serve as analytes, as described herein.

The analyte binding particles of the conjugate are stored in a stable form within the device. A "stable form," as the term is used herein, indicates a form in which the particles do not significantly change in chemical makeup or physical state during storage. The stable form can be a liquid, gel, or solid form. In preferred embodiments, the analyte binding particles are evaporatively dried; freeze-dried; and/or vacuum-dried.

In some example embodiments, one or more analyte binding agent(s) corresponds to the analyte(s) of interest. Each analyte of interest and its analyte binding agent are members of a specific binding pair, in which a first member of the binding pair (e.g., analyte) reacts specifically with a second member (e.g., the binding agent). One or both members of the binding pair can be an antibody. For example, a first member of the binding pair (e.g., an analyte of interest) can be an antibody, and a second member of the binding pair (e.g., a binding agent) can be anti-immunoglobulin antibody; alternatively, the first member of the binding pair (e.g., the analyte) can be an antigen, and the second member of the binding pair (e.g., the binding agent) can be an antibody.

In other embodiments of the assays of the invention, neither an analyte nor its binding agent in a specific binding pair are antibodies: for example, the first

member of the binding pair can be a ligand, and the second member of the binding pair can be a receptor; alternatively, the first member of the binding pair can be a lectin, and the second member of the binding pair can be a sugar. In still another embodiment, the first member of the binding pair can be a nucleic acid (e.g., DNA, RNA), and the second member of the binding pair can be a nucleic acid which specifically hybridizes to the first member of the binding pair. Specific hybridization, as used herein, refers to the ability of a first nucleic acid to hybridize to a second nucleic acid in a manner such that the first nucleic acid does not hybridize to any nucleic acid other than to the second nucleic acid (e.g., when the first nucleic acid has a higher similarity to the second nucleic acid than to any other nucleic acid in a sample wherein the hybridization is to be performed). "Stringency conditions" for hybridization is a term of art which refers to the incubation and wash conditions, e.g., conditions of temperature and buffer concentration, which permit hybridization of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (i.e., 100%) complementary to the second, or the first and second may share some degree of complementarity which is less than perfect (e.g., 70%, 75%, 80%, 85%, 90%, 95%). For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions", "moderate stringency conditions" and “low stringency conditions” for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 and pages 6.3.1-6.3.6 in Current Protocols in Molecular Biology (Ausubel, F.M. et al., Current Protocols in Molecular Biology, John Wiley & Sons, (1998), the entire teachings of which are incorporated by reference herein). The exact conditions which determine the stringency of hybridization depend not only on ionic strength (e.g., 0.2XSSC, 0.1XSSC), temperature (e.g., room temperature, 42oC, 68oC) and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, equivalent conditions can be determined by varying one or more of these parameters while maintaining a similar degree of identity or similarity between the two nucleic acid molecules.

Regardless of the composition of an analyte and its binding agent, these two components form a specific binding pair in which the first member reacts specifically with the second member. Specific interaction between the members of the binding pair indicates that the first member of the binding pair preferentially binds or otherwise interacts with the second member of the binding pair. Such interaction may occur to the exclusion of any binding to another compound in the assay.

In example embodiments of the methods of the invention, a fluid sample is assessed for the presence or absence, or quantity, of two or more analytes of interest. The fluid can be a fluid that wets the material of the solid phase, and that supports a reaction between each analyte of interest and its analyte binding agent, such as the antibody/antigen reaction (i.e., does not interfere with antibody/antigen interaction). The fluid may also have a viscosity that is sufficiently low to allow movement of the fluid by capillary action. In an example embodiment, the fluid may be an aqueous solution, such as a bodily fluid. The fluid sample can be a fluid having relatively few components, for example, an aqueous solution containing the analyte of interest. Alternatively, the fluid sample can be a fluid having many components, such as a complex environmental sample (e.g., sewage, waste water, groundwater, or other water sample), or a complex biological fluid (e.g., whole blood, plasma, serum, urine, cerebrospinal fluid, saliva, semen, vitreous fluid, synovial fluid, or other biological fluid). In an embodiment in which the fluid is a biological fluid, the fluid is whole blood, plasma, or serum. In another embodiment in which the fluid is a biological fluid, the fluid is a mucosal fluid. If desired, the fluid sample can be diluted; for example, if a complex biological fluid is used as the fluid sample, it can be diluted with a solution (e.g., an aqueous solution).

A diluent (also referred to as a "buffer") can be an aqueous fluid that supports a reaction between the analyte of interest and the analyte binding agent (e.g., does not interfere with antibody/antigen interaction); and that has a viscosity that is sufficiently low to allow movement of the fluid by capillary action. In one embodiment, the diluent contains one or more of the following components: a buffering agent (e.g., phosphate); a salt (e.g., NaCl); a protein stabilizer (e.g., BSA, casein, serum); and/or a detergent such as a nonionic detergent or a surfactant (e.g.,

one or more of the following agents commonly available in surfactant tool kits: NINATE 411, Zonyl FSN 100, Aerosol OT 100%, GEROPON T 77, BIO TERGE AS 40, STANDAPOL ES 1, Tetronic 1307, Surfnyol 465, Surfynol 485, Surfynol 104PG 50, IGEPAL CA210, TRITON X 45, TRITON X 100, TRITON X305, SILWET L7600, RHODASURF ON 870, Cremophor EL, TWEEN 20, TWEEN 80, BRIJ 35, CHEMAL LA 9, Pluronic L64, SURFACTANT 10G, SPAN 60, CREL). Optionally, if desired, the diluent can contain a thickening agent. Such components for diluents are commercially available. Representative diluents include, for example, saline, or 50 mM Tris HCl, pH 7.2. Alternatively, in some embodiments, water may be used in lieu of a buffered solution; as used herein, the term "diluent" may refer to a buffered solution or to water. In another embodiment, the components of the diluent may be lyophilized and included in the sample collection apparatus.

A representative embodiment of the invention is shown in Fig. 1, which illustrates an immunoassay reader 110 and test cartridge 190 containing a test sample. A test sample is prepared and inserted into the test cartridge 190, which in turn is inserted into a port 125a-b of the immunoassay reader 110 for reading and analysis of the test sample. The immunoassay reader 110 can be configured to perform one or more of fluorescence detection, optical absorption sensing, electrochemical sensing, oxygen sensing, conductivity sensing, and chemiluminescent detection on the test sample. An example system is the RAMP® Medial Diagnostic Device, available from Response Biomedical Corporation, Vancouver, British Columbia, Canada. An example immunoassay reader is described in U.S. Patent Application No. 12/077,529, entitled "Modular Assay Reader System And Apparatus," the entirety of which is incorporated herein by reference. Once the test cartridge 190 is inserted into a port 125a-b, a user may initiate a test of the test sample, the immunoassay reader 110 performing a selected reading of the test sample. Upon completion of the test, the immunoassay reader 110 processes results of the reading and displays the results on a reader interface 115 or transmits the results to another device. In performing a fluorescence immunoassay, for example, the immunoassay reader 110 optically scans the test

cartridge 190 to detect and measure the extent of an antibody or antigen in the test sample, and produces the results indicating this measure on the interface 115.

Fig. 2 is a block diagram of a device 200 for preparing a test sample for an immunoassay. With reference to Fig. 1, the device 200 may be employed to prepare a test sample in a test cartridge 190 for analysis by the immunoassay reader 110. The device 200 itself may be a self-contained module, or may be coupled to the immunoassay reader 110 (preferably at one of the ports 125a-b), or may be integral to (i.e., encompassed within) the immunoassay reader 110.

The device 200 accepts a preparation container 230 into a support structure 250. An example preparation container is described in further detail below with reference to Fig. 3. The container 230 is aligned within the support structure 250 such that the inlet 232 and outlet 234 of the container matches corresponding ports of the device 200 and support structure 250. In particular, the inlet 232 may have a portion extending above the device 200, enabling the inlet to receive a volume of fluid sample from outside the device 200. The outlet 234 extends below the support structure 250 and proximate to a test cartridge 290, such that the outlet 234 may dispense a volume of a prepared test sample into the test cartridge 290.

Coupled to the support structure 250 are a plurality of mechanical fingers 255a-g. These fingers may be configured to provide a number of functions, including holding the container 230 in a fixed position within the device 200, applying pressure to specific areas of the container 230, breaking or cutting the container 230 at particular points, and manipulating separators or valves within the container 230. Such functions are described in further detail below with regard to Fig. 3 and the structure of the container 230. Although the fingers 255a-g are shown at fixed locations at one side of the container 230, the fingers 255a-g may also be located at any other side of the container as required by a process for preparing a test sample. The device 200 may include more or fewer fingers 255a-g as required by such a process, and the fingers 255a-g may have other shapes adopted to specific functions for that process. The fingers 255a-g may be fixed to the support structure 250, or may be configured to relocate. For example, finger 255d may apply pressure to the container 230 while moving along the length of the support structure, thereby applying pressure along the wall of the container 230. The fingers 255a-g may also

include electromagnetic actuators (coils) that can act by magnetic coupling to: a) cause a steel (or other material) ball to move and thus result in mixing of constituents or b) induce a metallic material (not shown) to heat and thus melt or seal the separators 270-272 between adjacent chambers to control fluid flow.

A controller unit 290 is configured to control operation of the fingers 255a-g during a process of preparing a test sample. The controller 290 may comprise a number of components configured to exercise this control, including a computer processor, programmable memory or storage, motor controllers, and stepping motors (or other motors or power drivers) coupled to the fingers 255a-g. The controller 290 can be programmed such that, upon initialization of a process to prepare a test sample, the controller 290 manipulates the fingers 255a-g to perform one or more functions directed to the container 230. For example, the controller 290 may be programmed to control one or more functions described below with reference to the process 400 of Fig. 4.

The test cartridge 290 is positioned so as to collect a prepared test sample from the outlet 234 of the container 230. Alternatively, the test cartridge 290 may be located external to the device 200. A tray 285 secures the test cartridge 290 while receiving the prepared test sample, and may transport the test cartridge 290 out of the device 200 through a loading port 202. If the loading port 202 is coupled to the port of an immunoassay reader (e.g., port 125a of the reader 110 in Fig. 1), then the tray 285 may load the prepared test cartridge directly into the port of the immunoassay reader. Otherwise, the tray 285 may provide the provide the prepared test cartridge 290 to a user for loading into an immunoassay reader.

Fig. 3 is a block diagram of a container 330 for preparing a test sample for an immunoassay. The container 330 may be implemented in the device 200 as described above with reference to Fig. 2. The container 330 may be formed of foil, such as cold-formed aluminum foil, a formed plastic, or other semi-rigid material configured to maintain a predefined structure and change shape in response to external manipulation. Some or all walls of the container 330 may also include a plastic or other rigid backing to maintain the shape of the container 330. A container comparable to this container 330 is shown in Fig. 5, described below.

An inlet 332 extends to the top (or above the top) of the container 330. The inlet may be shaped as a cone or other shape for receiving a volume of a fluid sample. The inlet 332 leads to an entry capillary channel 320, having a cylindrical or similar shape and being formed of the walls of the container 330 or other material internal to the container 330. The entry capillary channel 320 extends down into the container 330 and terminates at a vent 322. The vent 322 may be located elsewhere at the entry capillary channel 320 to expel air from the entry capillary channel 320 as it fill with the fluid sample. The vent 322 may enable capillary action at the entry capillary channel 320, thereby drawing into the entry capillary channel 320 a portion of the fluid sample from the inlet 332. The entry capillary channel 320 and vent 322 may be formed so as to draw a specific volume of the fluid sample, rejecting any amount of the fluid sample exceeding that specific volume. Although the entry capillary channel 320 is shown to be cylindrical in shape, it may be adapted to any shape to accommodate a specific volume of the fluid sample. Likewise, a terminal capillary channel 334 (described below) may be adapted in a similar manner to accommodate a specific volume of a prepared test sample.

A chamber 340 within the container 330 includes a first section 350 and a second section 360 separated by a first separator 371. The walls of the container 340 may be formed of the walls of the container 330 and/or other material, and may hold any shape sufficient to accommodate preparation of the test sample as described below. The entry capillary channel 320 may be coupled to the chamber 340 via a second separator 370. The first section 350 contains a dry conjugate 355, which corresponds to the particular fluid sample received and the immunoassay to be conducted on the prepared test sample. The second section 360 contains a diluent for mixing with the fluid sample and the conjugate. The conjugate 355 and diluent are maintained in separate sections 350, 360 of the chamber 340 until shortly before mixing with the test sample, thereby preserving the integrity of the conjugate.

The chamber 340 its constituent sections 350, 360 and separators 370-372 may be configured in a number of different ways. For example, the chamber 340 may be formed with a greater degree of separation between the sections 350, 360, which in turn may be connected via one or more conduits (not shown). The chamber may also comprise additional sections (not shown), which may be

configured to contain other constituents of a prepared test sample, or may be utilized for combining constituents from other sections (e.g., sections 350, 360). For such configurations, additional separators (not shown) may be placed within the chamber 340, isolating constituents as required prior to preparing the test sample. One skilled in the art will appreciate that the chamber 340 can occupy any number of different shapes and configurations of sections and separators to enable the constituents of a prepared test sample to commingle in a common space. The separators 370-372 isolate sections of the chamber 340 from one another and the chamber 340 from the capillary channels 320, 334. The separators 370-372 may be configured to be removed in response to an applied external force, enabling constituents to pass through a space previously occupied by the separators 370-372. A number of devices or materials may be configured to function as the separators 370-372, such as a valve, a removable wall or a breakable seal.

The chamber 340 is coupled to a terminal capillary channel 334 via a third separator 372. A vent 336 terminates the terminal capillary channel 334 at an opposite end extending outside of the container 330, the vent 336 facilitating capillary action of the terminal capillary channel 334 to draw a given volume of a prepared test sample (i.e., a homogeneous mixture of fluid sample, conjugate 355 and diluent). The terminal capillary channel 334 may be formed so as to draw only a specific volume of the prepared test sample as required to supply a test cartridge 390 with the prepared test sample. In order to supply the test sample to the test cartridge 390, the terminal capillary channel 334 may be severed or the vent 336 may be removed, thereby allowing the prepared test sample to flow into the test cartridge 390. Alternatively, a fourth separator (not shown) may be located at the terminal capillary channel 334, allowing the test sample to be dispensed upon removal of the fourth separator. To facilitate expelling the prepared test sample from the terminal capillary channel 334, an additional vent (not shown) at the top of the channel 334 may allow the test sample to drain, or a mechanical force may be applied to force the test sample out of the channel 334.

In further embodiments of the invention, a container (e.g., container 330) may be adapted for preparing a test fluid for detecting or quantitating more than one analyte in a fluid sample. With reference to Fig. 3, for example, a chamber section

350 enclosing a conjugate 355 may also enclose one or more additional conjugate (not shown) corresponding to the additional analyte(s) to be detected or quantitated. Alternatively, the container 330 could include one or more additional chambers or chamber sections (not shown) that include one or more additional conjugates, and are configured to enable combination of the additional conjugate(s) with the fluid sample and diluent as described above, thereby resulting in a prepared test sample including the fluid sample, diluent, and more than one conjugates.

Example procedures for preparing a test sample using the container 330 are described below with reference to Fig. 4.

Fig. 4 is a flow diagram illustrating a method 400 of preparing a test sample for an immunoassay. The method 400 is described below with reference to the container 330 of Fig. 3, but may alternatively be applied to other embodiments of the invention. A number of the functions of the method 400 may be performed by a device utilizing a mechanical, electrical or electro-mechanical apparatus, such as the mechanical fingers 255a-g in Fig. 2. A programmable controller, such as the controller unit 290 in Fig. 2, may control the apparatus in performing these functions, and so may be programmed according to one or more functions of the method 400. The method 400, as well as each function of the method 400, may be initiated and controlled by a programmable controller, a user, or both.

A user places a fluid sample into the inlet 332 (410). Through capillary action or passive filling, the entry capillary channel 320 draws a specific volume of the fluid sample (415). Because the entry capillary channel 320 draws a specific volume as required for preparing the test sample, a user need not measure a precise volume for placing in the inlet 332. Once the entry capillary channel 320 is filled with the fluid sample, the top of the entry capillary channel 320 may be isolated from the inlet 332, for example by applying pressure or heat to the entry capillary channel at the point connecting to the inlet 332 (417). A mechanical device encompassing the container 330 (e.g., mechanical fingers 255a-g in Fig. 2) may be configured to apply this pressure or heat to the entry capillary channel 320, thereby causing the walls of the entry capillary channel 320 at this point to fuse together, forming a separator (not shown) that isolates the entry capillary channel 320 from the inlet 332.

Upon filling the entry capillary channel 320, the first separator 371 and the second separator 370 are removed, enabling the fluid sample to flow into the chamber 340, and the diluent to flow into the first section 350 of the chamber (420). To facilitate this step, external mechanical means (e.g., mechanical fingers 255a-g in Fig. 2) may remove the separators 370-371, as well as apply pressure to the entry capillary channel 320 to force the fluid sample into the chamber 340. As a result, each of the constituents of the test sample (fluid sample, conjugate 355 and diluent) occupy a common volume within the chamber 340 (425). The chamber 340 may then be isolated from the entry capillary channel 320 in a manner similar to isolating the entry capillary channel 320 from the inlet 332, for example by replacing the second separator 370 or applying heat or pressure to the relevant edge of the chamber 340 (427).

The fluid sample, conjugate 355 and diluent are then mixed to form a homogeneous mixture within the chamber 340 (430). This mixing may be facilitated by external mechanical means, for example by applying pressure to the chamber 340 to agitate the constituents. In a particular example, one or more mechanical fingers may apply pressure to the chamber 340 while moving along the wall of the chamber 340, thereby forcing the constituents within the chamber 340 to commingle.

Once a homogeneous mixture of the constituents is formed, the resulting prepared test sample is contained within the chamber 340. The third separator 372 is removed (435), enabling a given volume of the test sample to flow into the terminal capillary 334. Mechanical means as described above may facilitate this flow, until the terminal capillary 334 is filled with the given volume of the test sample (440). A test cartridge 390 may be positioned to receive the test sample from the terminal capillary 334. To extract the test sample, the terminal capillary 334 may be opened by mechanical means, such as by removing or breaking the vent 336 or severing a portion of the terminal capillary 334 (445). Pressure may also be applied to the terminal capillary 334 to force the test sample out of the container 330 and into the test cartridge 390. As a result, the test cartridge 390 contains a specific volume of a test sample that is prepared for analysis by an immunoassay reader.

Fig. 5 illustrates a 3-dimensional external view of a container 530 for preparing a test sample, which may be comparable to the containers 230, 330 described above with reference to Figs. 2 and 3. The container 530 includes a top wall 531a, from which a conical inlet 532 extends upward, and a side wall 531b. The top and side walls 531a-b, along with a bottom wall and opposite side wall (not shown), form a rigid "frame" supporting the remaining structure of the container 530. This frame extends inwards to meet the front wall 531c and a rear wall (not shown). The front wall 531c may be comprised of a foil or other semi-rigid or deformable material, and may be adhered to the back wall (not shown), comprised of the same or a different material.

The front wall 531c may further comprise one or more features present in the container 330 of Fig. 3, such as the entry capillary channel 520, vent 522, chamber 540, and terminal capillary channel 534. The front wall 531c may form these or other features by forming corresponding protrusions, perforations or other features in the wall 531c. The separators 570-572 may appear at the surface of the front wall 531c as a plurality of tabs, valves or other features that may be manipulated by external mechanical or electrical means in order to open or close conduit occupied by the separators 570-572. The appearance of the capillaries 520, 534, chamber 540 and separators 571-572 at the surface of the front wall 531c enables these features to be manipulated by mechanical means as described above, for example, with respect to Figs 2-4. Thus, the container 530 may be implemented to accept a fluid sample, prepare a test sample, and provide the test sample to a test cartridge for an immunoassay reader.

Fig. 6 is a block diagram of a container 630 for preparing a test sample for an immunoassay. The container 630 may be implemented in the device 200 as described above with reference to Fig. 2. The container 630 may be constructed and configured in a manner comparable to the containers 330, 530 described above with reference to Figs. 3 and 5. For example, the container 630 includes an inlet 632 for receiving a volume of a fluid sample, which extends to an entry capillary channel 620 with a vent 622 for receiving a specific volume of the test sample.

In contrast to the container 330 in Fig. 3, the container 630 includes a chamber 640 having a single section containing a dry conjugate 655. A separate

diluent chamber 660 contains a quantity of diluent meeting or exceeding the volume of diluent required for preparing the test sample. The diluent chamber 660 is coupled to a diluent capillary channel 675, which may be comparable to the entry capillary channel 620, and which is formed to draw a specific volume of diluent from the diluent chamber 660. The diluent capillary channel 675 may also include a vent 623 to facilitate filling the diluent capillary channel 675. First, second and third separators 670, 671, 672 separate the chamber 640 from the entry capillary channel 620, a terminal capillary channel 634, and the diluent capillary channel 675, respectively, while a fourth separator 673 couples the diluent chamber 660 to the diluent capillary channel 675.

A process for preparing a test sample in the container 630 may be similar to the process 400 described above with reference to Fig. 4, with an exception of manipulating the diluent. Prior to – or concurrently with – drawing a fluid sample into the entry capillary channel 620, the fourth separator 673 is removed to allow a specific volume of the diluent to flow into the diluent capillary channel 675. Once both the entry capillary channel 620 and the diluent capillary channel 675 are filled with their respective constituents, the first and second separators 670, 671 may be removed, allowing the volumes of fluid sample and diluent to flow into the chamber 640. Because the entry capillary channel 620 and the diluent capillary channel 675 are in close proximity and parallel to one another, a single mechanical apparatus (e.g., a mechanical finger) may apply pressure to both capillary channels 620, 675 simultaneously, facilitating moving the diluent and fluid sample into the chamber 640.

Once all constituents of the test sample (i.e., fluid sample, diluent and conjugate) are located within the chamber 640, a test sample may be prepared by one or more processes described above with reference to Figs. 2-4. A specific volume of the resulting prepared test sample may be drawn into the terminal capillary channel 634 upon removal of the third separator 672. The terminal capillary channel 634 may include a vent 636 to facilitate filling the channel 634. The terminal capillary channel 634 is then opened to enable the volume of prepared test sample to flow into a test cartridge 690 for analysis by an immunoassay reader.

Fig. 7A illustrates a test cartridge 700 configured for preparing a test sample in an embodiment of the present invention. In contrast to the device 200 and containers 230, 330, 530, 630 described above, a configuration for test sample preparation is integrated into the test cartridge 700 itself. The test cartridge 700 may further include elements or functionality described above with respect to Figs. 2-6 in addition to, or in place of, the configuration as shown in Fig. 7.

The test cartridge 700, depicted in a top-down view, may include an external enclosure, the top of which is removed to show elements within the cartridge 700. A test fluid chamber 732 and a diluent chamber 760 open through the top cover, and are accessible to receive volumes of test fluid and diluent, respectively. The test fluid chamber 732 may function as an inlet for receiving the test fluid, as described above. The diluent chamber 760 may receive a volume of diluent by way of a diluent pouch 765. The diluent pouch 765 may be composed of foil or other material containing a given volume of diluent, which is received by the diluent chamber 760 upon opening (via, e.g., puncturing, tearing) the diluent pouch 765. The material of the diluent pouch 765 may have low water vapor permeability, such that materials outside of the diluent may remain desiccated and liquid loss from the diluent chamber 760 is minimized.

The test fluid chamber 732 connects to a test fluid capillary channel 720 through an inlet at the bottom of the chamber 732, thereby allowing the test fluid to flow from the chamber 732 into the channel 720. Likewise, the diluent chamber 760 connects to a diluent capillary channel 775 through an inlet at the bottom of the chamber 760, allowing the diluent to flow from the chamber 760 into the channel 775. A spike 782, located at the bottom of the diluent chamber 760, ruptures the diluent pouch 765 as it is placed in the diluent chamber 760. Once ruptured, the diluent pouch 765 allows a volume of diluent to flow, by gravitational force, into the diluent chamber 760 and into the diluent capillary channel 775 by capillary force. A vent 783, running along a portion of the bottom and side of the diluent chamber 760 and the top of the test cartridge 700, facilitates this flow of diluent. Each of the channels 720, 775 may be configured, such as by occupying a given volume, to permit only a given volume of respective test fluid or diluent to flow into the channels 720, 775. The channels 720, 775 connect to a mixing chamber 740.

Diluent and test fluid may be prevented from passively flowing into the mixing chamber 740 either by the surface tension of the diluent and test fluid at the ends of each of the channels 720, 775, or by placing separators (not shown) between the channels 720, 775 and the mixing chamber 740. To facilitate such control over the diluent and test fluid, the test fluid capillary channel 720 and the diluent capillary channel 775 may be at least partially composed of a relatively hydrophilic material (e.g., polystyrene, nylon), and the mixing chamber 740 may be at least partially composed of a relatively hydrophobic material (e.g., polypropylene, polyethylene). A test fluid plunger 755 and a diluent plunger 756 may be activated (e.g., by an external mechanical or manual force) to move through respective channels 720, 775, thereby forcing volumes of the test fluid and diluent into the mixing chamber 740.

A conjugate (not shown) may be located within the mixing chamber 740 for combining with the test fluid and diluent. The mixing chamber 740 may include a mixing ball 742 or other movable object to facilitate mixing of the diluent, test fluid and conjugate. The mixing ball 742 may be moved within the mixing chamber 740 by an external force, such as a mechanical arm or magnet (not shown), or by rapid movement of the test cartridge 700, to facilitate the mixing. Thus, the diluent, test fluid and conjugate may be combined in the mixing chamber 740 and agitated, by the mixing ball 742 or other agent, to form a test sample. Once the test sample is formed, it may be transferred to the test strip 790, which includes a portion exposed for analysis by an assay reader (not shown). The test strip 790 connects to the mixing chamber via a test sample outlet 772. A separator at the test sample outlet 772 may be removed (for example, by puncturing the separator), thereby allowing the test sample to be absorbed by the test strip 790. Example procedures for introducing a conjugate, mixing test sample constituents and transferring a test sample onto a test strip are provided in U.S. Patent No. 7,056,473 ("Method and Apparatus for Quantitative Assays"), the entirety of which is incorporated herein by reference.

Fig. 7B illustrates a diluent pouch 765 for use with the test cartridge 700 described above in Fig. 7A. The diluent pouch 765 may be formed in a manner comparable to the container 330 described above with reference to Fig. 3, and includes a flat surface having a rigid or semi-rigid backing and a pouch coupled

below the surface, the pouch containing a volume of diluent. With reference to Fig. 7A, the diluent pouch 765 may be adapted to be coupled to the top surface of the test cartridge 700. Thus, the flat surface of the diluent pouch 765 corresponds with the top surface of the test cartridge 700. In particular, the flat surface of the diluent pouch 765 is shaped to accommodate the test fluid chamber 732, and the pouch portion is positioned so as to occupy a volume in the diluent chamber 760. As a result, the diluent pouch 765 may be placed onto the test cartridge 700, enabling a volume of diluent to flow into the diluent chamber 760 upon opening of the diluent pouch 765.

Advantages of the Invention

Typical procedures for preparing a test sample for an immunoassay involve a number of steps performed by a lab technician or other operator. A volume of the fluid sample, the fluid to be analyzed, is first measured and transferred to a dilution vial containing a pre-measured amount of diluent. The volume is transferred using a pipette tip containing a dry conjugate. The sample-diluent mixture is pipetted in and out of the tip several times until the conjugate is rehydrated and suspended in the liquid and the sample-diluent-conjugate mixture is homogenous. Once mixed, a portion of the mixture, being the prepared test sample, is transferred to the test cartridge for analysis.

The procedure described above for preparing a test sample suffers from a number of drawbacks. The process requires a number of precise measurements to be made, as well as a specific technique that must be followed to obtain a correct mixture of sample fluid, conjugate and diluent. As a result, the process is time-consuming, requires substantial training to perform properly, and is subject to human error, leading to inaccurate results in a subsequent immunoassay.

In contrast to typical procedures, embodiments of the present invention provide means to prepare an accurate test sample for an assay quickly and in an automated manner. The need for multiple precise measurements or for specially trained personnel is eliminated. Thus, embodiments of the invention improve reliability, accessibility, speed and accuracy in preparation of a test sample.

Although the present invention has been described in relation to immunoassays, it is also applicable to similar assays which do not utilize antibodies, such as ligand binding assays and hybridization probe assays.

While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. An apparatus comprising:

an inlet for receiving a fluid sample;

an entry capillary channel coupled to the inlet at one end for receiving the fluid sample into the entry capillary channel, the entry capillary channel being formed to draw a specific volume of the fluid sample;

a chamber for preparing a test sample from the specific volume of the fluid sample, a dry conjugate and a diluent, the chamber having a first section enclosing the dry conjugate and a second section enclosing the diluent, the first and second sections separated by a first separator, the chamber receiving the fluid sample from the entry capillary channel upon removal of a second separator; and

a terminal capillary channel adapted to receive the prepared test sample upon removal of a third separator.

2. The apparatus of claim 1, further comprising a vent at the entry capillary channel, the vent facilitating capillary action at the entry capillary channel.

3. The apparatus of claim 1, wherein the first and second separators are adapted to be removable to enable the fluid sample, conjugate and diluent to commingle.

4. The apparatus of claim 3, wherein the chamber is adapted to receive an applied mechanical force to the chamber to facilitate the commingling, thereby producing the prepared test sample.

5. The apparatus of claim 1, wherein the chamber is adapted to receive an applied mechanical force to the entry capillary channel to facilitate transferring the fluid sample into the test chamber.

6. The apparatus of claim 1, wherein at least one of the first, second and third separators is removable by breaking the at least one separator.

7. The apparatus of claim 1, wherein the entry capillary channel is adapted to reject any quantity of fluid sample in excess of the volume required by the test sample.

8. The apparatus of claim 1, wherein the terminal channel is formed to accept a volume of the test sample coinciding with a volume required by a test cartridge.

9. The apparatus of claim 1, further comprising a fourth separator at the terminal channel, the fourth separator being formed so as to enable the test sample to flow into a test cartridge upon removal of the fourth separator.

10. The apparatus of claim 9, wherein the fourth separator includes a vent.

11. The apparatus of claim 1, wherein the entry capillary channel, upon receiving the fluid sample, is isolated from the inlet.

12. The apparatus of claim 1, wherein the chamber includes at least one wall that is deformable in response to an applied force.

13. The apparatus of claim 12, wherein the at least one wall includes at least one foil layer.

14. The apparatus of claim 1, wherein the entry capillary channel and terminal capillary channel include at least one wall that is deformable in response to an applied force.

15. A method of preparing a test sample, comprising:

receiving a fluid sample into an entry capillary channel via an inlet, the entry capillary channel being formed to draw a specific volume of the fluid sample;

opening the entry capillary channel to at least one chamber having a conjugate and a diluent, enabling the fluid sample to commingle with the conjugate and the diluent to produce a prepared test sample; and

transferring at least a portion of the prepared test sample into a terminal capillary channel.

16. The method of claim 15, further comprising venting the entry capillary channel to facilitate capillary action at the entry capillary channel.

17. The method of claim 15, wherein opening the entry capillary channel includes removing at least one separator.

18. The method of claim 15, further comprising applying a mechanical force to the chamber to facilitate commingling, thereby producing the prepared test sample.

19. The method of claim 15, further comprising applying a mechanical force to the entry capillary channel to facilitate transferring the fluid sample into the test chamber.

20. The method of claim 15, wherein at least one of opening and transferring includes breaking a separator.

21. The method of claim 15, further comprising rejecting any quantity of fluid sample in excess of the volume required by the test sample.

22. The method of claim 15, further comprising accepting a volume of the test sample coinciding with a volume required by a test cartridge.

23. The method of claim 15, further comprising dispensing the test sample into a test cartridge.

24. The method of claim 23, wherein dispensing includes removing a separator at the terminal capillary channel.

25. The method of claim 15, further comprising isolating the entry capillary channel from the inlet upon receiving the fluid sample.

26. An apparatus for preparing a test sample, comprising:

means for receiving a fluid sample into an entry capillary channel via an inlet, the entry capillary channel being formed to draw a specific volume of the fluid sample;

means for opening the entry capillary channel to at least one chamber having a conjugate and a diluent, enabling the fluid sample to commingle with the conjugate and the diluent to produce a prepared test sample; and

means for transferring at least a portion of the prepared test sample into a terminal capillary channel.

27. An apparatus comprising:

an inlet for receiving a fluid sample;

an entry capillary channel coupled to the inlet at one end for receiving the fluid sample into the entry capillary channel, the entry capillary channel being formed to draw a specific volume of the fluid sample;

a diluent chamber containing a diluent;

a chamber for preparing a test sample from the specific volume of the fluid sample, a dry conjugate and the diluent, the chamber receiving the diluent upon removal of a first separator and the fluid sample from the entry capillary channel upon removal of a second separator; and

a terminal capillary channel adapted to receive the prepared test sample upon removal of a third separator.

28. The apparatus of claim 27, further comprising a diluent capillary channel coupled to the chamber via the first separator and coupled to the diluent chamber, the diluent capillary chamber adapted to receive a volume of the diluent from the diluent chamber.

29. The apparatus of claim 27, further comprising a vent at the entry capillary channel, the vent facilitating capillary action at the entry capillary channel.

30. The apparatus of claim 27, wherein the first and second separators are adapted to be removable to enable the fluid sample, conjugate and diluent to commingle.

31. The apparatus of claim 27, wherein the chamber is adapted to receive an applied mechanical force to the chamber to facilitate the commingling, thereby producing the prepared test sample.

32. A apparatus for analyzing a test sample, comprising:

an inlet for receiving a fluid sample;

a test fluid capillary channel coupled to the inlet at one end for receiving the fluid sample into the test fluid capillary channel, the test fluid capillary channel being formed to draw a specific volume of the fluid sample;

a diluent capillary channel for receiving a volume of a diluent; and

a chamber for preparing a test sample from the specific volume of the fluid sample, a dry conjugate and the diluent.

33. The apparatus of Claim 32, further comprising a test strip adapted to receive the test sample.

34. The apparatus of Claim 32, further comprising a diluent chamber for receiving the volume of diluent, the diluent chamber coupled to the diluent capillary channel.

35. The apparatus of Claim 34, wherein the diluent chamber is adapted to receive a pouch containing the volume of diluent.

36. The apparatus of Claim 32, further comprising a first plunger configured to force the test fluid from the test fluid sample into the chamber, and a second plunger configured to force the diluent from the diluent capillary channel into the chamber.

37. The apparatus of Claim 32, wherein at least one of the test fluid capillary channel and the diluent capillary channel includes a hydrophilic material, and the chamber includes a hydrophobic material.

38. The apparatus of Claim 32, wherein the inlet includes a test fluid chamber.

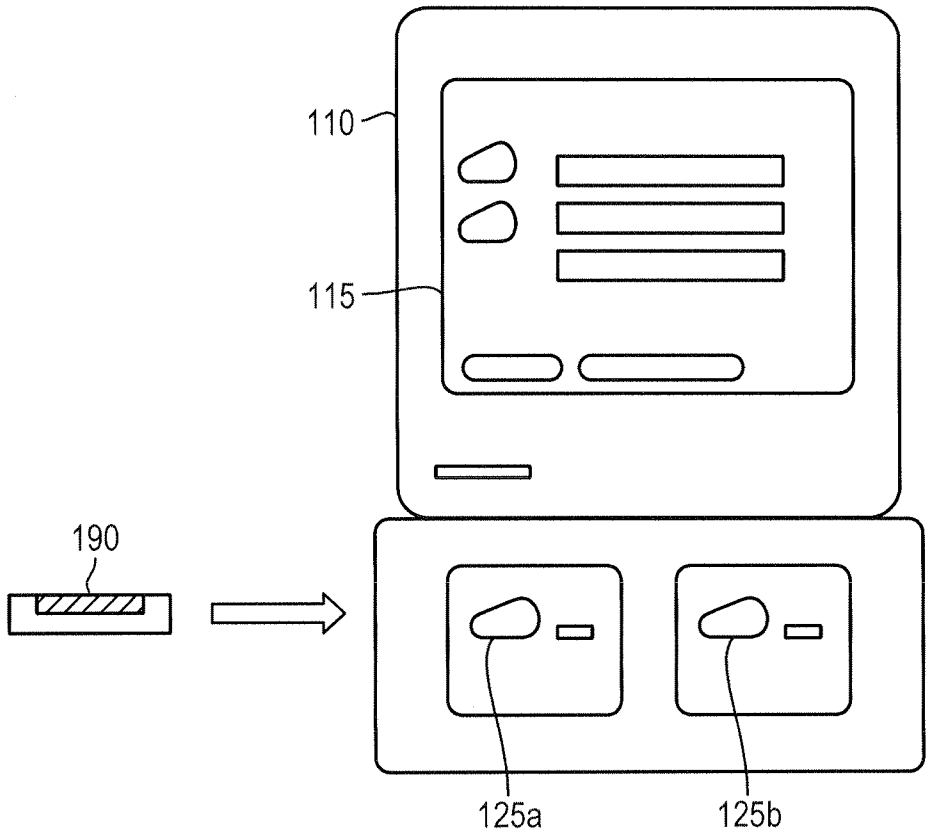
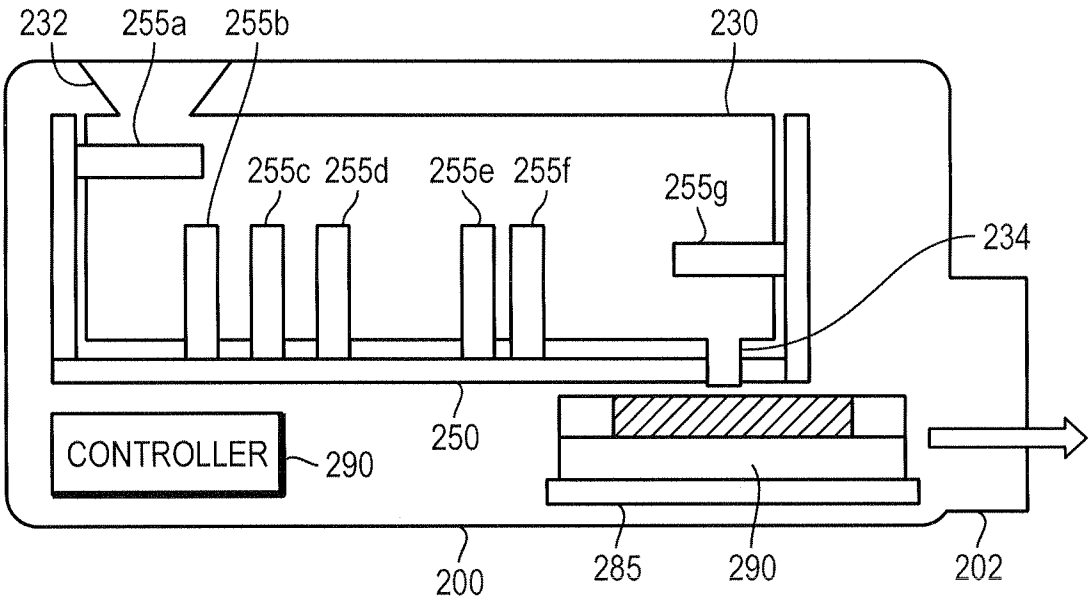


FIG. 1


232
255a
255b
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255d
255e
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255g
234
CONTROLLER
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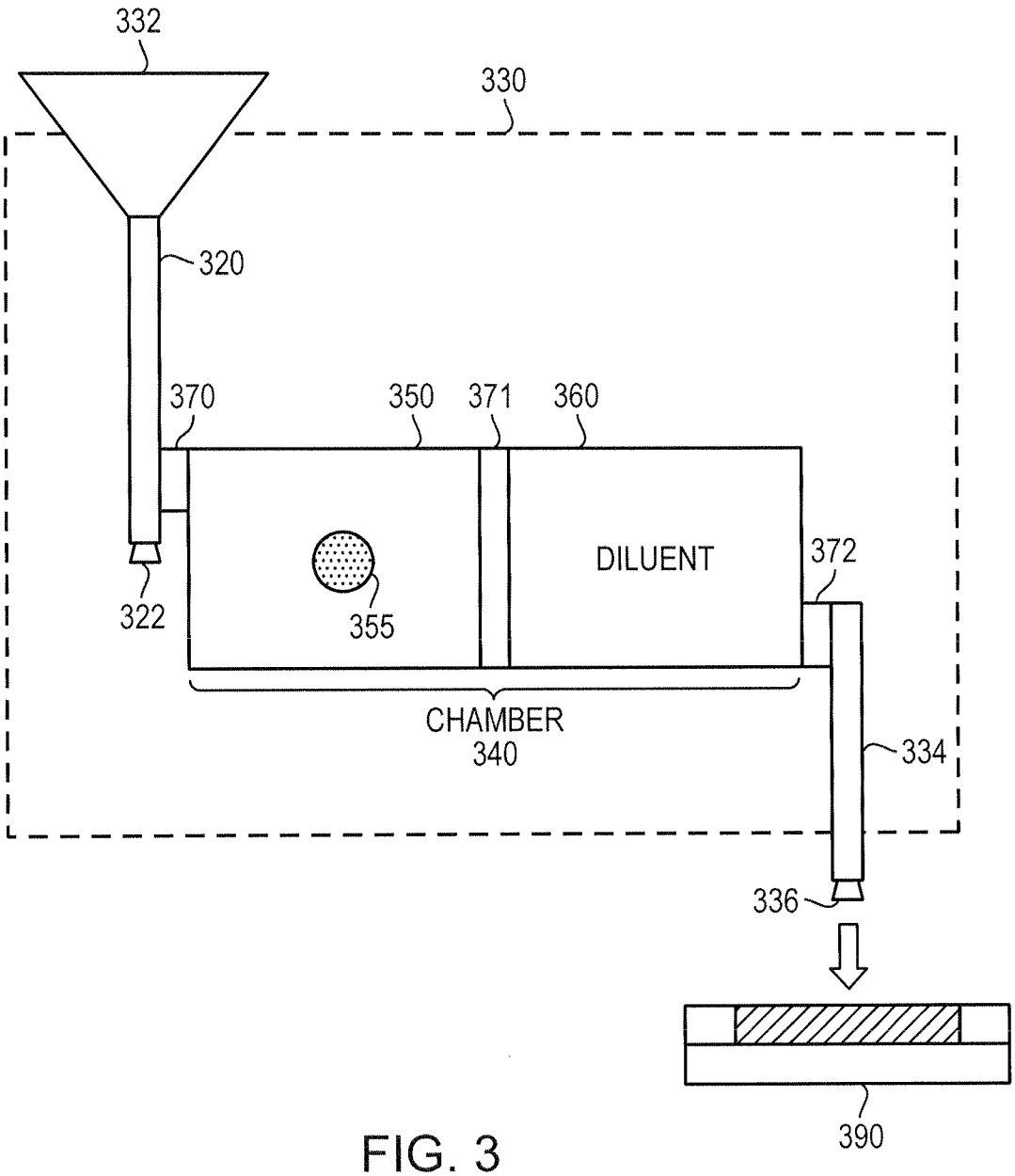
250
200
285
290
202

FIG. 2


332
330
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370
350
371
360
DILUENT
372
322
355
CHAMBER
340
334
336
390

FIG. 3

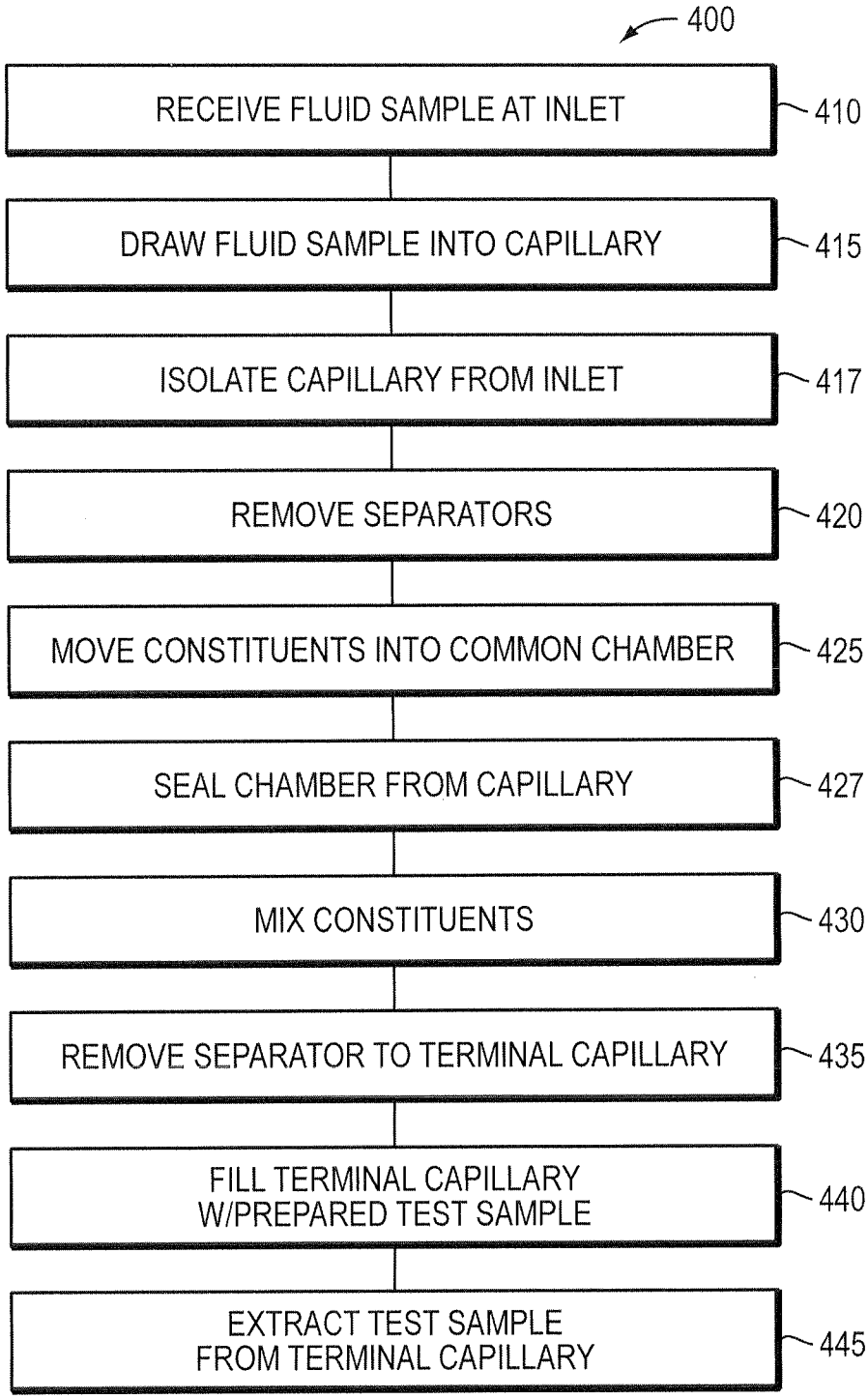

400
RECEIVE FLUID SAMPLE AT INLET
410
DRAW FLUID SAMPLE INTO CAPILLARY
415
ISOLATE CAPILLARY FROM INLET
417
REMOVE SEPARATORS
420
MOVE CONSTITUENTS INTO COMMON CHAMBER
425
SEAL CHAMBER FROM CAPILLARY
427
MIX CONSTITUENTS
430
REMOVE SEPARATOR TO TERMINAL CAPILLARY
435
FILL TERMINAL CAPILLARY W/PREPARED TEST SAMPLE
440
EXTRACT TEST SAMPLE FROM TERMINAL CAPILLARY
445

FIG. 4

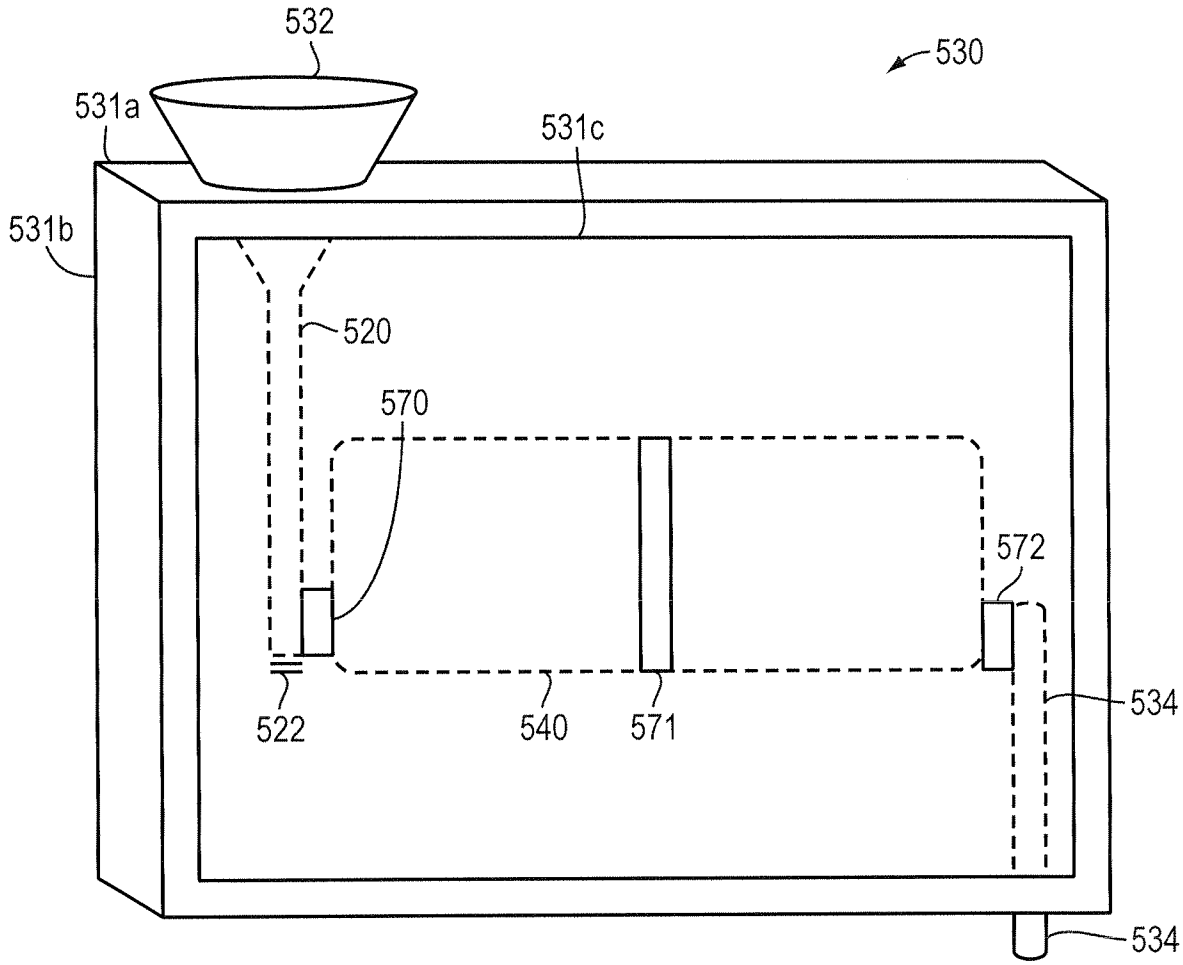
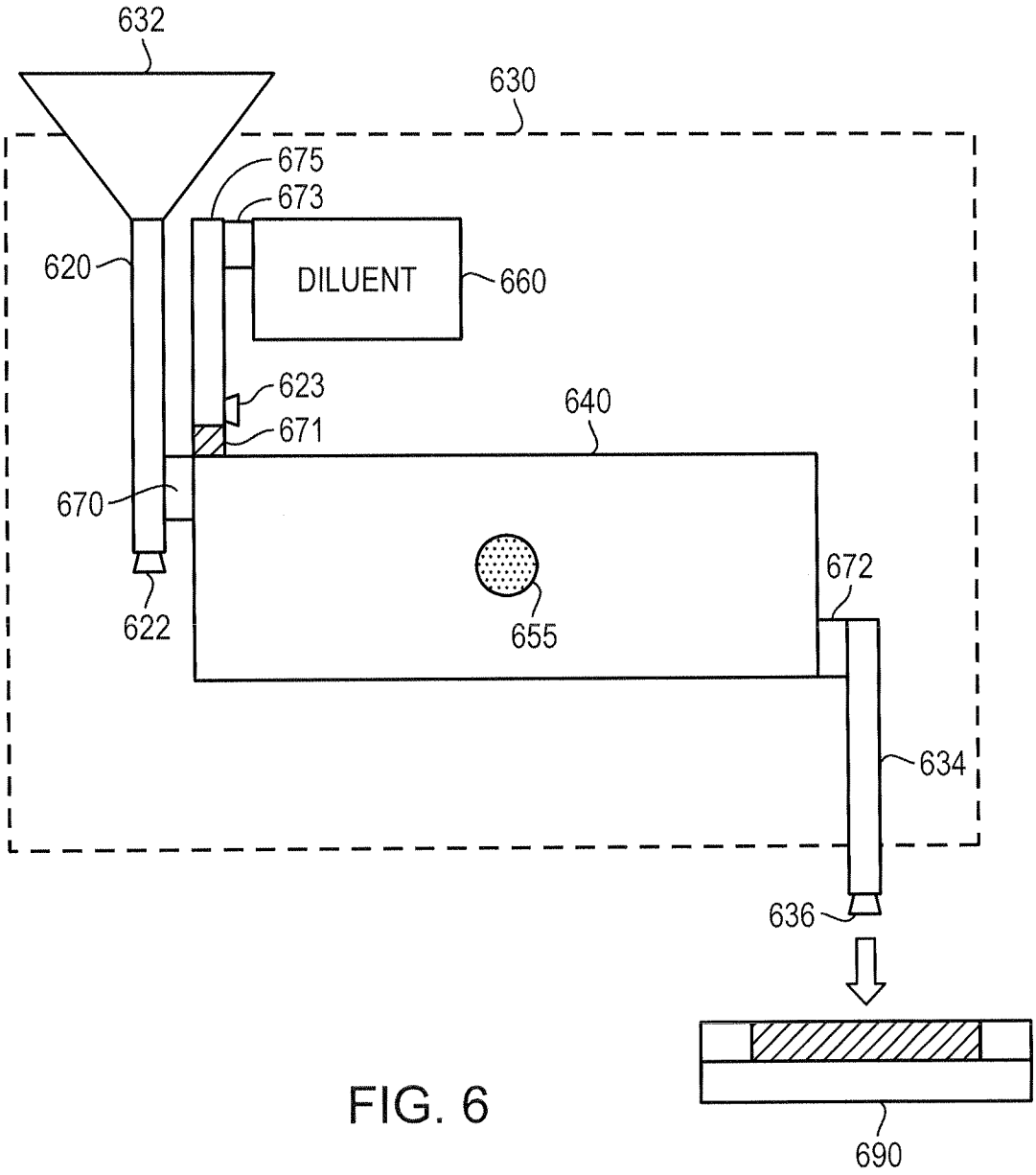


FIG. 5


632
630
675
673
620
DILUENT
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623
640
671
670
672
622
655
634
636
690

FIG. 6

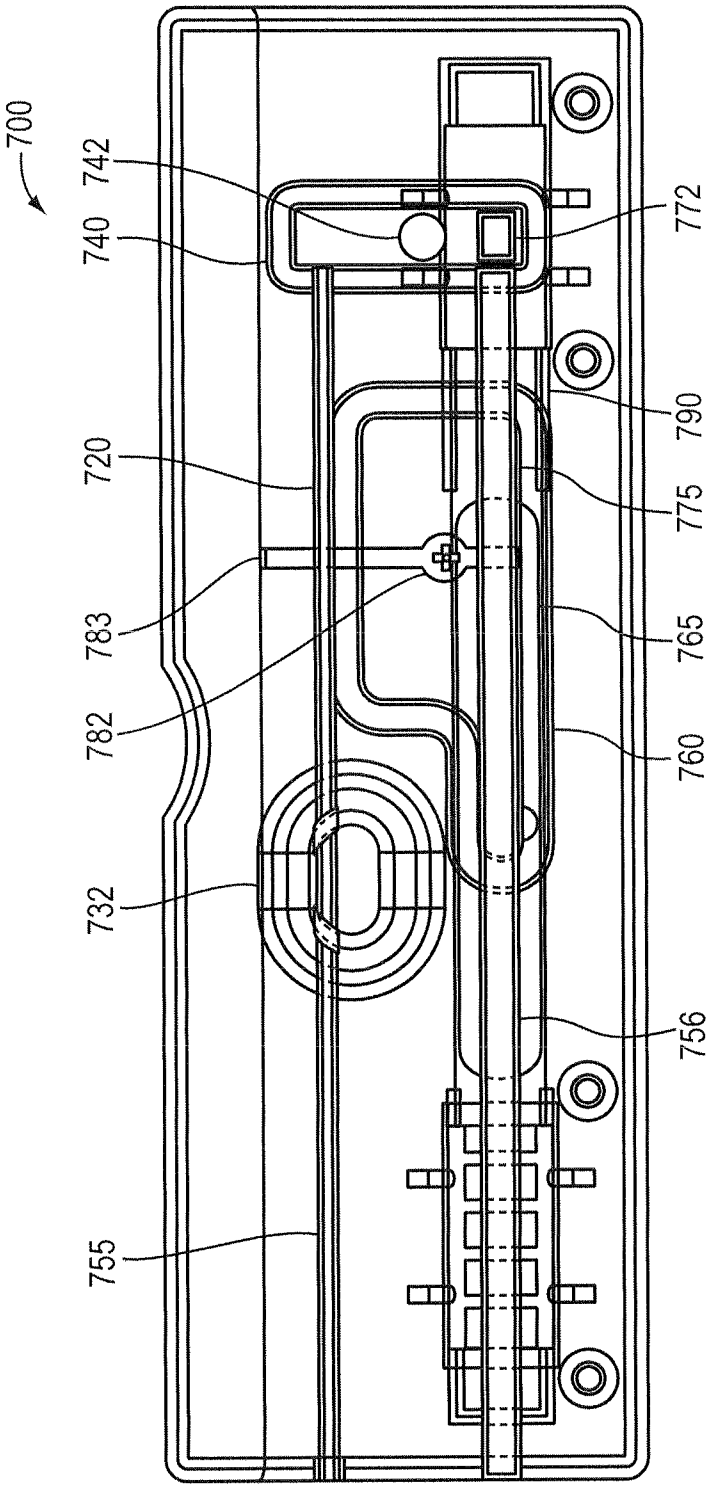

FIG. 7A

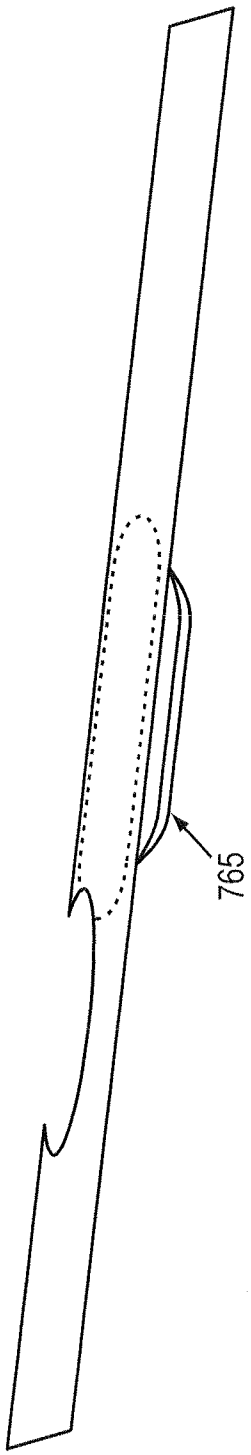

FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/058922

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 063 589 A (KELLOGG GREGORY [US] ET AL) 16 May 2000 (2000-05-16) column 9, lines 2-8 column 27, line 38 - column 28, line 13 column 30, lines 6-60; figures 25-27	1-38
X	WO 2006/056787 A (NORCHIP AS [NO]; SETNA ROHAN PILOO [GB]; GULLIKSEN ANJA [NO]; SOLLI LA) 1 June 2006 (2006-06-01) page 6, lines 4-17; figures 1-3 page 7, line 24 - page 8, line 2 page 9, line 19 - page 10, line 2 page 25, line 26 - page 27, line 29	1-38
X	US 2004/053268 A1 (KARLSEN FRANK [NO]) 18 March 2004 (2004-03-18) paragraphs [0109] - [0113]; figures 2,3	15-27

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2010	17/03/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Viskanic, Martino

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/058922

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 587 128 A (WILDING PETER [US] ET AL) 24 December 1996 (1996-12-24) column 4, line 37 - column 5, line 7 column 7, lines 49-56 column 23, line 29 - column 24, line 61; figures 10,11	1-5, 15-19, 26-29,32
A	WO 2008/083446 A (ENVIRONMENTAL BIOTECHNOLOGY CR [AU]; DITCHAM WILLIAM GRAHAM FOX [AU];) 17 July 2008 (2008-07-17) page 19, lines 3-24; figures 2-5	1-38
A	US 4 963 498 A (HILLMAN ROBERT S [US] ET AL) 16 October 1990 (1990-10-16) abstract	1-38

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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