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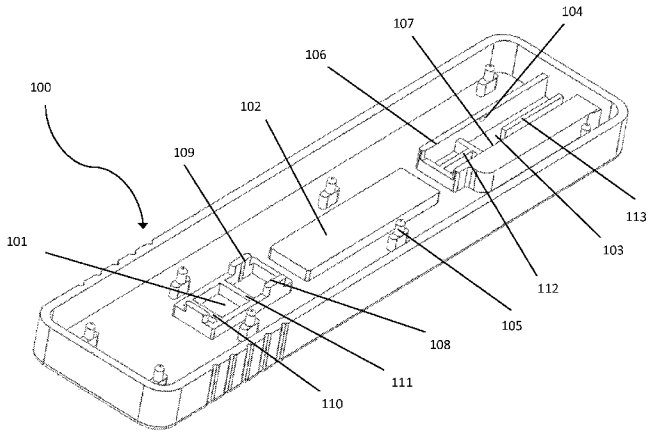
(54) **Title**: LATERAL FLOW ASSAY WITH TEST STRIP RETAINER

FIG. 1A



(57) **Abstract**: It is an object of the present invention to provide improved methods and compositions for manufacture and use of lateral flow test devices. In particular, the present invention provides a molding method which provides one or more features in the housing base configured to retain the test strip within the base. These features are provided as undercuts in the housing base. The test strip is configured as a bibulous lateral flow material disposed on a substantially non-compressible base layer, and the base layer is positioned within the undercut in order to retain the test strip in the housing base. Optionally, one or more features in the housing base which create the undercut are configured to engage the bibulous lateral flow material by compression and/or friction, thereby increasing the ability of the base to maintaining the test strip in its proper position within the device.

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LATERAL FLOW ASSAY WITH TEST STRIP RETAINER

[0001] This application claims the benefit of U.S. Provisional Application No. 61/769,709, filed February 26, 2013, which is hereby incorporated by reference in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

[0002] The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

[0003] Lateral flow assay devices are widely used in many different areas of analytical chemistry and medicine, and have become the format of choice for tests such as immunoassays which are to be performed by relatively untrained users in a rapid testing protocol. Typically, the devices and methods allow for application of a sample to a lateral flow matrix. The sample flows along the lateral flow matrix, and one or more analyte components to be detected in the sample react with at least one reagent which is provided in or added to the lateral flow matrix. At least one reagent is typically immobilized in the device for reaction with the analyte component to be detected or a reagent thereof, and labels are typically employed to measure the extent of reaction with an immobilized reagent. See, e.g., U.S. patents and patent application publications: 5,602,040; 5,622,871; 5,656,503; 6,187,598; 6,228,660; 6,818,455; 2001/0008774; 2005/0244986; 6,352,862; 2003/0207465; 2003/0143755; 2003/0219908; 5,714,389; 5,989,921; 6,485,982; 11/035,047; 5,656,448; 5,559,041; 5,252,496; 5,728,587; 6,027,943; 6,506,612; 6,541,277; 6,737,277 B1; 5,073,484; 5,654,162; 6,020,147; 4,956,302; 5,120,643; 6,534,320; 4,942,522; 4,703,017; 4,743,560; 5,591,645; and RE 38,430.

[0004] Lateral flow assay devices may comprise a housing having a sample port and a result window downstream of the sample port, and, optionally, a control window downstream of the result window. The sample port is adapted to receive a quantity of liquid buffer or sample applied thereto which traverses a lateral flow path via a lateral flow matrix within the housing, extending from the sample port to a downstream location. The housing may be formed of any suitable material, an example of which comprises molded plastic, and is preferably sufficiently rigid to provide support and stability for the

lateral flow path or paths housed therein adhesive may be is assembled on a housing surface with the adhesive facing the lateral flow matrix to assist in maintaining the lateral flow matrix in position within the housing.

[0005] WO2007/063423 discloses a lateral flow device in which the housing also comprises one or more pressure bars, supports and/or locating pegs for arranging the various layers and strips in the housing and maintaining them in position in the assembled device. For example, the housing top may be provided with a pressure bar for maintaining the upstream portion of the lower wicks in place at the buffer well and a pressure bar for maintaining the downstream ends of the lower wicks and the upstream ends of the main strips in contact with one another and in place in the assembled device. In one embodiment, these pressure bars may be formed integrally with the housing top, for example when the housing top is formed of molded plastic. Alternatively, one or more of the pressure bars may be provided as separate components.

SUMMARY OF THE INVENTION

[0006] It is an object of the present invention to provide improved methods and compositions for manufacture and use of lateral flow test devices. In particular, the present invention provides a molding method which provides one or more features in the housing base configured to retain the test strip within the base. These features are provided as undercuts (a design feature that obstructs the smooth opening of the mold or part ejection from the mold (without distorting or destroying the nominal design part geometry) in the housing base. The test strip is configured as a bibulous lateral flow material disposed on a substantially non-compressible base layer, and the base layer is positioned within the undercut in order to retain the test strip in the housing base. Optionally, one or more features in the housing base which create the undercut are configured to engage the bibulous lateral flow material by compression and/or friction, thereby increasing the ability of the base to maintaining the test strip in its proper position within the device.

[0007] Thus, in a first aspect, the present invention provides methods for forming a lateral flow test device. These methods comprise:

providing a first mold assembly which forms a test device base, the test device base comprising on a floor thereof a first vertical wall and a second vertical wall forming a recess having a defined width there between, the first vertical wall comprising one or

more first structures and the second vertical wall comprising one or more second structures, wherein the first and second structures define an undercut portion between the first vertical wall and second vertical wall;

introducing a moldable material into the first mold assembly to form the test device base;

removing the test device base from the first mold assembly;

providing a test strip comprising

a substantially uncompressible base layer,

a first bibulous material disposed on the base layer at a proximal end thereof and forming a sample receiving region,

a second bibulous material disposed on the base layer at a distal end thereof and forming an absorbent region, wherein the second bibulous material is compressible, and

a porous membrane disposed on the base layer between the proximal and distal ends thereof and fluidly connected to the first bibulous material and the second bibulous material,

the test strip defining a flow path wherein a sample applied to the sample receiving region flows through the porous membrane to the absorbent region, wherein the porous membrane comprises one or more test zones, each test zone comprising one or more reagents configured to bind for detection one or more analyte of interest; and

inserting the test strip into the test device base such that the base layer inserts into the undercut portion, where the second bibulous material is optionally compressibly and/or frictionally engaged by the first structures and the second structures.

[0008] In a related aspect, the present invention provides methods of assembling a lateral flow test device. These methods comprise:

providing a test strip comprising

a substantially uncompressible base layer,

a first bibulous material disposed on the base layer at a proximal end thereof and forming a sample receiving region,

a second bibulous material disposed on the base layer at a distal end thereof and forming an absorbent region, wherein the second bibulous material is compressible, and

a porous membrane disposed on the base layer between the proximal and distal ends thereof and fluidly connected to the first bibulous material and the second bibulous material,

the test strip defining a flow path wherein a sample applied to the sample receiving region flows through the porous membrane to the absorbent region, wherein the porous membrane comprises one or more test zones, each test zone comprising one or more reagents configured to bind for detection one or more analyte of interest;

providing a generally rigid base which supports the test strip, the base comprising a first vertical wall and a second vertical wall forming a recess which receives the base layer therewithin at the distal end of the base layer, the width of the recess being approximately equal to the width dimension of the base layer,

the first vertical wall comprising one or more first structures formed thereon above the level of the base layer, and the second vertical wall comprising one or more second structures formed thereon above the level of the base layer, wherein the first and second structures are configured to retain the test strip within the base; and

inserting the test strip into the test device base such that the base layer inserts into the undercut portion, where the second bibulous material is optionally engaged by the first compression structures and the second compression structures.

[0009] In another related aspect, the present invention provides lateral flow analyte test devices, comprising:

(a) a test strip comprising

a substantially uncompressible base layer,

a first bibulous material disposed on the base layer at a proximal end thereof and forming a sample receiving region,

a second bibulous material disposed on the base layer at a distal end thereof and forming an absorbent region, wherein the second bibulous material is compressible, and

a porous membrane disposed on the base layer between the proximal and distal ends thereof and fluidly connected to the first bibulous material and the second bibulous material,

the test strip defining a flow path wherein a sample applied to the sample receiving region flows through the porous membrane to the absorbent region, wherein the porous membrane comprises one or more test zones, each test zone comprising one or more reagents configured to bind for detection one or more analyte of interest;

(b) a generally rigid base which supports the test strip, the base comprising a first vertical wall and a second vertical wall forming a recess which receives the base layer therewithin at the distal end of the base layer, the width of the recess being approximately equal to the width dimension of the base layer,

the first vertical wall comprising one or more first structures formed thereon above the level of the base layer, and the second vertical wall comprising one or more second structures formed thereon above the level of the base layer, wherein the first and second structures are configured to retain the test strip within the base, and wherein the second bibulous material is optionally engaged by the first compression structures and the second compression structures.

[0010] The term "undercut" as used herein refers to a portion of a part's geometry that would prevent the part from being ejected from a straight-pull mold without a portion of the mold damaging the part. The simplest example of an undercut feature on a part would be a through-hole aligned perpendicular to the direction of part ejection. In certain embodiments, the undercut feature of the present invention is provided by one or more structures formed on an internal wall of the base; in these embodiments, the undercut is the space lying beneath these structures.

[0011] For purposes of the present invention, these features which establish the undercut will be referred to as "undercut structures." Such structures may be in the form of ribs, bars, spherical caps, frustums, etc. Preferably the features are radiused in profile to assist in assembly of the test device and removal of the device from the mold. In certain embodiments the undercut structures on opposing walls may be offset from one

another to further aid in insertion of the test strip. In certain embodiments, once the base layer of the test strip is seated beneath the undercut structures and into the undercut, the undercut structures engage the bibulous material disposed on the base layer. This engagement can be compressive, as the bibulous material is often a compressible material, or frictional, or a combination of these forces. This engagement can assist in accurately positioning the test strip within the housing base.

[0012] As noted above, the test strip is preferably formed as a lamination of one or more bibulous materials on a substantially uncompressible and nonabsorbent base layer. The term "substantially uncompressible" as used herein refers to a material that substantially maintains its original thickness when subjected to compressive forces experienced during the insertion of the material into the undercut portion as described herein. In preferred embodiments, the base layer is also substantially nonabsorbent. The term "substantially nonabsorbent" as used herein refers to a material which is not sufficiently hydrophilic and porous as to support lateral flow of an aqueous sample.

[0013] The term "bibulous" as used herein refers to a material which is sufficiently hydrophilic and porous to support lateral flow of an aqueous sample. Such materials include cellulose papers, nitrocellulose membranes, polyvinylidine fluoride, charge modified nylon, polyethersulfone, porous polyethylene sheets, glass fiber mats, etc. This list is not meant to be limiting.

[0014] The term "test zone" as used herein refers to a discrete location on a lateral flow test strip which is interrogated in order to generate a signal related to the presence or amount of an analyte of interest. Such interrogation may be performed visually as in an over-the-counter pregnancy test, or in an instrumented fashion as through the detection of reflectance, absorption, fluorescence, luminescence, etc. by a suitably configured meter.

[0015] The term "generally rigid" as used herein in reference to the housing refers to a material which is sufficiently rigid to maintain the test strip in position relative to the other features of the device and signal detection system during use of the test device in a lateral flow assay method.

[0016] The presence and shape of internal features may influence and define the flow path through the lateral flow material. By way of example, liquid can move across the top or bottom of the lateral flow material and pool on the surface thereof. Such flow may reduce the flow through the detection region of the device, thereby reducing sensitivity.

Additionally, because such aberrant flow is unpredictable, failure to control such flow contributes substantially to assay imprecision as measured by a coefficient of variation (CV). Preferably, the test devices of the present invention exhibit a CV of less than 10%.

[0017] In certain embodiments, the base and test strip form the entire test device. In certain other embodiments, a second mold assembly may be used to form a test device lid comprising a sample receiving aperture and a test aperture which can be mated to the base and enclose regions of the test strip which are not accessed (either fluidly or optically) during a test. By way of example only, a test device may be formed by introducing a moldable material into the second mold assembly to form the test device lid; removing the test device lid from the second mold assembly; and mating the test device lid to the test device base such that the sample receiving aperture overlies the first bibulous material and the test aperture overlies the one or more test zones. While the lid and base may be formed with discrete molds, the first mold assembly and the second mold assembly may be configured as a single assembly, wherein the test device base and the test device lid are formed as a unitary part. To facilitate fit of the lid and base, the test device base and the test device lid may be formed as a unitary part connected by one or more flexible hinge regions (e.g. living hinges) configured to allow the test device lid to mate to the test device base.

[0018] The foregoing description of the test devices is not intended to preclude the provision of additional zones, apertures, features, etc. commonly used in such lateral flow devices. By way of example, a conjugate pad comprising a detectable label for use in the assay can be included. When sample flows into the conjugate pad, the detector reagent solubilizes, lifts off the pad material, and moves with the sample front into the membrane. In the case of a sample such as whole blood which contains cells and other particulates, a filter matrix can be provided to

[0019] The presence and shape of internal features may influence and define the flow path through the lateral flow material. By way of example, liquid can move across the top or bottom of the lateral flow material and pool on the surface thereof. Such flow may reduce the flow through the detection region of the device, thereby reducing sensitivity. In certain embodiments, the mold assembly may be configured to form a raised platform portion of the test device base. This platform is preferably configured to underlie the base layer of the test strip between the proximal and distal ends thereof and to support the test strip without contacting the porous membrane. Such a platform can be used to position

the test strip away from the sidewalls and floor of the test device base to prevent capillary flow of aqueous sample along the edges of the test strip.

[0020] To further manage this unproductive flow, the mold assembly may be configured to form one or more features to retain (e.g., ribs, bumps, pins, or bars) in the test device lid and/or base, where these features are configured to engage the surface of the bibulous material and promote desired flow through the bibulous material and impede undesired flow. In certain embodiments, these retaining features contact, but do not substantially compress the bibulous material, as overcompression can reduce flow rate through the device. In particularly preferred embodiments, the retaining features may be configured to account for swelling of the lateral flow structures due to absorption of the liquid components during a test such that these retaining features contact, but do not substantially compress the bibulous material during performance of a test with the test device.

[0021] It is to be understood that the invention is not limited in its application to the details of construction and to the arrangements of the components set forth in the following description or illustrated in the drawings. The invention is capable of embodiments in addition to those described and of being practiced and carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein, as well as the abstract, are for the purpose of description and should not be regarded as limiting.

[0022] As such, those skilled in the art will appreciate that the conception upon which this disclosure is based may readily be utilized as a basis for the designing of other structures, methods and systems for carrying out the several purposes of the present invention. It is important, therefore, that the claims be regarded as including such equivalent constructions insofar as they do not depart from the spirit and scope of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

[0023] Fig. 1A depicts a perspective view of a test device base of the present invention.

[0024] Fig. 1B depicts a top view of a test device base of the present invention.

[0025] Fig. 2 depicts an exploded view of a test device of the present invention, showing the test device base, test strip, and test device lid.

[0026] Fig. 3 depicts a cutaway view of an assembled test device of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0027] Figures 1A and 1B show two views of a generally rigid base **100** configured to support a test strip. The test device base may be formed using a number of methods known to those of skill in the art, including but not limited to injection molding, blow molding, machining, etching, etc. In preferred embodiments, the test device base is injection molded, a process for forming thermoplastic and thermoset materials into molded products of intricate shapes, at high production rates and with good dimensional accuracy. The process typically involves the injection, under high pressure, of a metered quantity of heated and plasticized material into a relatively cool mold--in which the plastic material solidifies. Resin pellets are fed through a heated screw and barrel under high pressure. The liquefied material moves through a runner system and into the mold. The cavity of the mold determines the external shape of the product while the core shapes the interior. When the material enters the chilled cavities, it starts to re-plasticize and return to a solid state and the configuration of the finished part. The machine then ejects the finished parts or products.

[0028] The skilled artisan will understand that a number of polymers may be used to form the test device base, including thermoplastics, some thermosets, and elastomers. Common thermoplastics include PMMA, cyclic olefin copolymer, ethylene vinyl acetate, polyacrylate, polyaryletherketone, polybutadiene, polycarbonate, polyester, polyetherimide, polysulfone, nylon, polyethylene, and polystyrene. Common thermosets include polyesters, polyurethanes, duroplast, epoxy resins, and polyimides. This list is not meant to be limiting. Functional filler materials such as talc and carbon fibers can be included for purposes of improving stiffness, working temperatures, and part shrinkage.

[0029] As noted herein, the test device base of the present invention is formed to provide an undercut into which one or more components of the test strip are inserted for purposes of retaining the test strip during manufacture and use of the test device. Undercuts on molded parts are features that prevent the part from being directly ejected from the injection molding machine. Undercuts can be molded as an integral, unitary part of the test device base, but typically require a “side action,” “lifter” or “collapsible core” mold component that moves separately from the two halves. In the case that the plastic

material of the test device base is sufficiently flexible, a side action or other similar mold component is not always required. In these cases the undercut is stripped or snapped out of the mold. When this is done usually a stripping plate or ring is used instead of stripper pins so that the features forming the undercut are not damaged in the process of removing the part from the mold. The skilled artisan will recognize that the features forming the undercut need not be molded into the part, but may be formed by machining or etching of the part after the part is formed.

[0030] As shown in Figs. 1A and B, the features forming the undercut are provided as small protrusions **104** in vertical walls **106** and **107**. These "undercut structures." may be in the form of ribs, spherical caps, frustums, etc. Preferably the features are radiused in profile to assist in releasing the nominal geometry from the mold, and in assembly of the test device, as the radiused profile can allow the test strip to more easily slide past these structures when it is inserted from the top into recess **103**. In certain embodiments, this radiused profile is only on the top surface of the structures, while the bottom surface is somewhat flat. In this way, once the test strip is inserted, it can "snap" into the undercut and fit against the flat bottom surface of the structures. In certain embodiments the undercut structures on opposing walls may be offset from one another longitudinally on vertical walls **106** and **107** as depicted in Fig. 1B to further aid in insertion of the test strip.

[0031] Fig. 2 depicts the relative orientations of the various components of the test device. In this embodiment, a test strip **200** is mated to the test device base **100**. When inserted, the proximal end **201** of the test strip lies within in recess **101** on bars **110** and **111** and between side walls **108** and **109**; the central portion **202** of the test strip lies across raised platform **102**; and the distal end **203** of the test strip lies within recess **103** on bars **112** and **113** and between side walls **106** and **107**. The terms "proximal" and "distal" are not used in any functional sense, but rather simply to distinguish the two ends of the test strip.

[0032] The test strip **200** is configured to perform a lateral flow assay to detect the presence or amount of one or more analytes. Lateral flow assay strips typically comprise series of materials which provide capillary flow spaces. Suitable materials include materials derived from cellulose (e.g. papers), nitrocellulose, cellulose acetate, glass fibers, nylon, dacron, PVC, polyacrylamide, cross-linked dextran, agarose, polyacrylate, ceramic materials, sintered polymers, etc. The material or materials of the test strip may

optionally be treated to modify their capillary flow characteristics or the characteristics of the applied sample. For example, the sample application region of the test strip may be treated with buffers to correct the pH or alter the contact angle to correct the hydrophilic character of the materials. Each of these elements has the capacity to transport fluid. The first bibulous material (sometimes referred to as the sample pad) receives the sample fluid. The fluid migrates to the second element (e.g., a nitrocellulose membrane strip) in which a chemical partner (e.g., antibody) that has been immobilized on the particle's surface participates in a binding event related to the presence or amount of an analyte of interest in the sample. The immobilized materials are provided in areas (often called stripes, reaction zones, or detection zones) which are interrogated for a detectable signal indicative of the binding event(s) of interest. After passing these zones, the fluid enters a final porous material (often referred to as a wick or waste zone) that promotes flow of sufficient sample past the detection zones. Lateral Flow Tests typically operate as either competitive or sandwich assay format, and a single device can simultaneously detect multiple analytes.

[0033] In Fig. 2, test strip **200** is formed as a laminated structure having a substantially uncompressible base layer **206**, a first bibulous material disposed on the base layer at the proximal end **201** which provides a sample receiving region which promotes flow of sample to a lateral flow membrane in central portion **202**. The first bibulous material and the lateral flow membrane contain a region of overlap **204** to aid in transfer of a sample from the first bibulous material to the lateral flow membrane. At the distal end **203**, a second bibulous material provides the wick for the test strip. Again, the second bibulous material and the lateral flow membrane contain a region of overlap **205** to aid in transfer of a sample from the lateral flow membrane to the second bibulous material.

[0034] The substantially uncompressible base layer **206** preferably comprises a hydrophobic material so as to reduce the tendency of sample to flow along the interface between the various flow promoting materials and the base layer. Suitable materials include films made of such hydrophobic polymers such as polypropylene, polystyrene, polymethylmethacrylate, etc. The thickness of this layer is selected to provide a desired level of stiffness so as to support the lateral flow materials, and is preferably between 0.001 and 0.02 inches.

[0035] Optionally, the test device comprises a lid (or cover) **300** which serves to aid in handling of the device without contamination of the test strip. As depicted in Fig. 2, a series of posts **105** in the test device base **100** mate with corresponding posts **303** in the cover **300** to hold the cover in place in the completed test device. A sample aperture **301** provides fluid ingress to the first bibulous material, and a reading aperture or window **302** provides access to interrogate the detection zones on the porous lateral flow membrane.

[0036] In assembling the test strip **200** into the test device base **100**, at least the base layer **206** is inserted into the undercut formed by undercut structures **113**. Because base layer **206** has some residual flexibility, it can be inserted past the undercut structures **113** by pressing in from the top. As noted above, a radiused upper surface and staggered placement of undercut structures **113** can assist in allowing the base layer **206** to slip into the undercut. Alternatively, the test strip can be inserted into recess **103** from the end closest to platform **102** and slid into the undercut.

[0037] In certain embodiments, the second bibulous material is engaged by the undercut structures **113** when the base layer **206** is in place. This can provide compressive and/or frictional forces which assist in proper positioning of test strip **200** in test device base **100**. This engagement can be on the sides of the second bibulous material, or on the upper surface of the second bibulous material.

[0038] As discussed above, lateral flow assays may be configured using a variety of detectable labels known in the art. The most commonly used label materials in visual read tests are colloidal gold particles. Other possible label modalities include enzyme, conjugates, other colloidal metals, fluorescent particles, and magnetic particles. Many label modalities (e.g., optical labels, magnetic labels, etc.) can be interrogated by instruments. Slot **114** depicts a channel which may be configured to mate with a corresponding structure within an instrument. Such elements can serve to both accurately position the test device within the instrument, and to permit only appropriate test devices to be inserted and read by the instrument.

[0039] Fig. 3 shows the fully assembled test device, with a partial cutaway of the cover to show the relative positioning of various internal structures. Sample aperture **301** is positioned over, but not in contact with, the sample receiving region of the test strip **200**. Sample flow is initiated by introduction of sample fluid into aperture **301**, and subsequently occurs through the first bibulous material, aided by ribs **304** which contact

the surface of the test strip and act to inhibit flow across the surface, as well as to aid in accurate position of the test strip within the device. Flow is lateral in a proximal-to-distal direction. Reading aperture **302** is positioned over, but not in contact with, the test zones of the nitrocellulose membrane. Ribs or other structures such as posts, bumps, bars, etc. **305** contact the surface of the test strip at the junction between the membrane and the second bibulous material and act to inhibit flow across the surface, as well as to aid in accurate position of the test strip within the device.

[0040] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0041] While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements should be apparent without departing from the spirit and scope of the invention. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention and are defined by the scope of the claims.

[0042] It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[0043] All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[0044] The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either

of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0045] Other embodiments are set forth within the following claims.

We claim:

1. A lateral flow analyte test device, comprising:

(a) a test strip comprising

a substantially uncompressible and nonabsorbent base layer,

a first bibulous material disposed on the base layer at a proximal end thereof and forming a sample receiving region,

a second bibulous material disposed on the base layer at a distal end thereof and forming an absorbent region, wherein the second bibulous material is compressible, and

a porous membrane disposed on the base layer between the proximal and distal ends thereof and fluidly connected to the first bibulous material and the second bibulous material,

the test strip defining a flow path wherein a sample applied to the sample receiving region flows through the porous membrane to the absorbent region, wherein the porous membrane comprises one or more test zones, each test zone comprising one or more reagents configured to bind for detection one or more analyte of interest;

(b) a generally rigid base which supports the test strip, the base comprising a first vertical wall and a second vertical wall forming a recess which receives the base layer therewithin at the distal end of the base layer, the width of the recess being approximately equal to the width dimension of the base layer,

the first vertical wall comprising one or more first structures formed thereon above the level of the base layer, and the second vertical wall comprising one or more second structures formed thereon above the level of the base layer, wherein the first and second structures are configured to retain the test strip within the base.

2. The test device according to claim 1, wherein the housing further comprises a lid which mates with the base, the lid comprising a sample receiving aperture overlying the first bibulous material and a test aperture overlying one or more test zones.

3. The test device according to claim 2, wherein the lid is connected to the base by a hinge.

4. The test device according to one of claims 1-3, wherein the first structures and the second structures engage the second bibulous material to retain the test strip within the base.

5. The test device according to one of claims 1-4, wherein the first bibulous material at least partially overlies the porous membrane.

6. The test device according to one of claims 1-5, wherein the second bibulous material at least partially overlies the porous membrane.

7. The test device according to one of claims 1-6, wherein the porous membrane is a nitrocellulose membrane laminated to a mylar support film.

8. The test device according to one of claims 1-7, wherein the first bibulous material is configured to define a predetermined sample volume to be analyzed by the test device.

9. The test device according to one of claims 1-8, wherein the base further comprises a raised platform underlying base layer between the proximal and distal ends thereof and configured to support the test strip without contacting the porous membrane.

10. The test device according to one of claims 1-9, wherein the lid comprises one or more first ribs which engage the upper surface of the first bibulous material, and one or more second ribs which engage the upper surface of the second bibulous material.

11. The test device according to one of claims 1-10, wherein the first structures are offset from the second structures.

12. The test device according to one of claims 1-1, wherein the first structures and the second structures are rounded in profile.

13. The test device according to claim 12, wherein the first structures and the second compression are in the form of a spherical cap.

14. A method of forming a lateral flow test device, comprising:

providing a first mold assembly which forms a test device base as a non-strippable article, the test device base comprising on a floor thereof a first vertical wall and a second vertical wall forming a recess having a defined width therebetween, the first vertical wall comprising one or more first compression structures and the second vertical wall comprising one or more second compression structures, wherein the first and second compression structures define an undercut portion between the first vertical wall and second vertical wall;

introducing a moldable material into the first mold assembly to form the test device base;

removing the test device base from the first mold assembly;

providing a test strip comprising

a substantially uncompressible and nonabsorbent base layer,

a first bibulous material disposed on the base layer at a proximal end thereof and forming a sample receiving region,

a second bibulous material disposed on the base layer at a distal end thereof and forming an absorbent region, wherein the second bibulous material is compressible, and

a porous membrane disposed on the base layer between the proximal and distal ends thereof and fluidly connected to the first bibulous material and the second bibulous material,

the test strip defining a flow path wherein a sample applied to the sample receiving region flows through the porous membrane to the absorbent region, wherein the porous membrane comprises one or more test zones, each test zone comprising one or more reagents configured to bind for detection one or more analyte of interest; and

inserting the test strip into the test device base such that the base layer inserts into the undercut portion and the second bibulous material is engaged by the first compression structures and the second compression structures.

15. A method according to claim 14, further comprising providing a second mold assembly which forms a test device lid comprising a sample receiving aperture and a test aperture;

introducing a moldable material into the second mold assembly to form the test device lid;

removing the test device lid from the second mold assembly; and

mating the test device lid to the test device base such that the sample receiving aperture overlies the first bibulous material and the test aperture overlies the one or more test zones.

16. A method according to claim 15, wherein the first mold assembly and the second mold assembly are configured as a single assembly, wherein the test device base and the test device lid are formed as a unitary part.

17. A method according to claim 16, wherein the test device base and the test device lid are formed as a unitary part connected by a hinge configured to allow the test device lid to mate to the test device base.

18. The method according to one of claims 15-17, wherein the first mold assembly further forms a raised platform portion of the test device base configured to underlie the base layer between the proximal and distal ends thereof and to support the test strip without contacting the porous membrane.

19. The method according to one of claims 15-18, wherein the second mold assembly further forms one or more first ribs and one or more second ribs in the in the test device lid, the first ribs configured to engage the upper surface of the first bibulous material, and the second ribs configured to engage the upper surface of the second bibulous material.

20. The method according to one of claims 15-19, wherein the first structures are offset from the second structures.

21. The method according to one of claims 15-20, wherein the first compression structures and the second compression structures are rounded in profile.

22. The method according to claim 21, wherein the first compression structures and the second compression structures are in the form of a spherical cap.

FIG. 1A

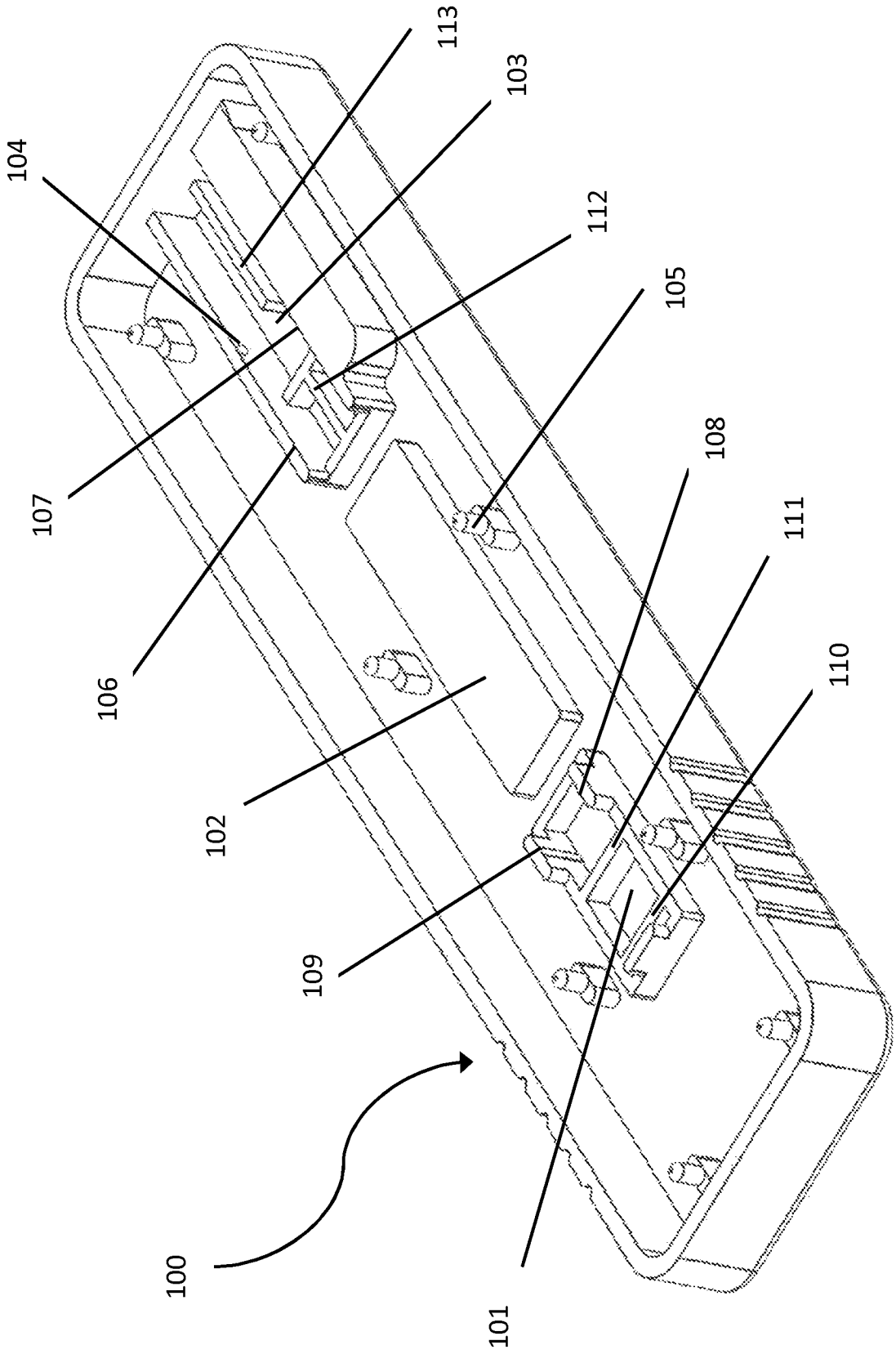

FIG. 1B

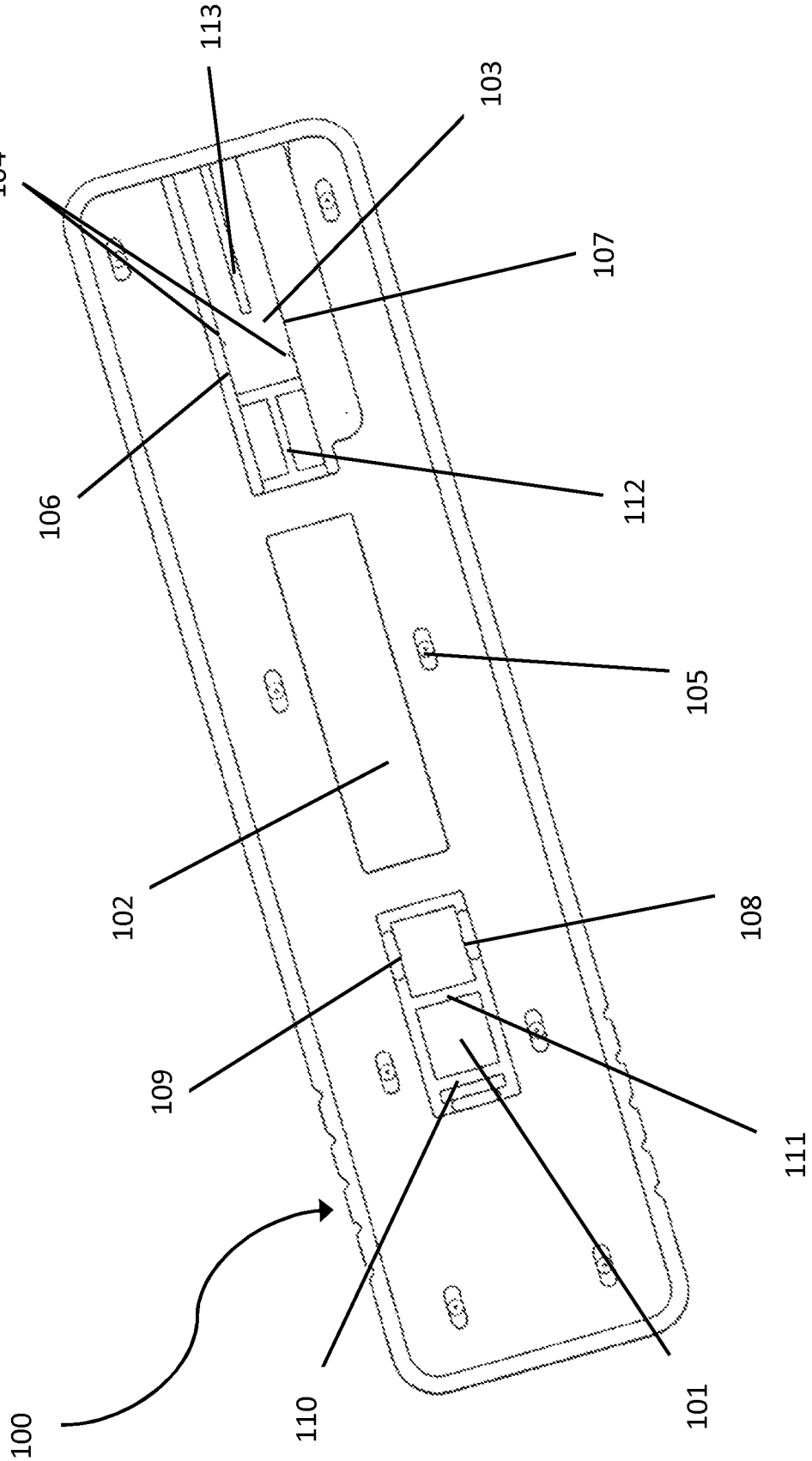

FIG. 2

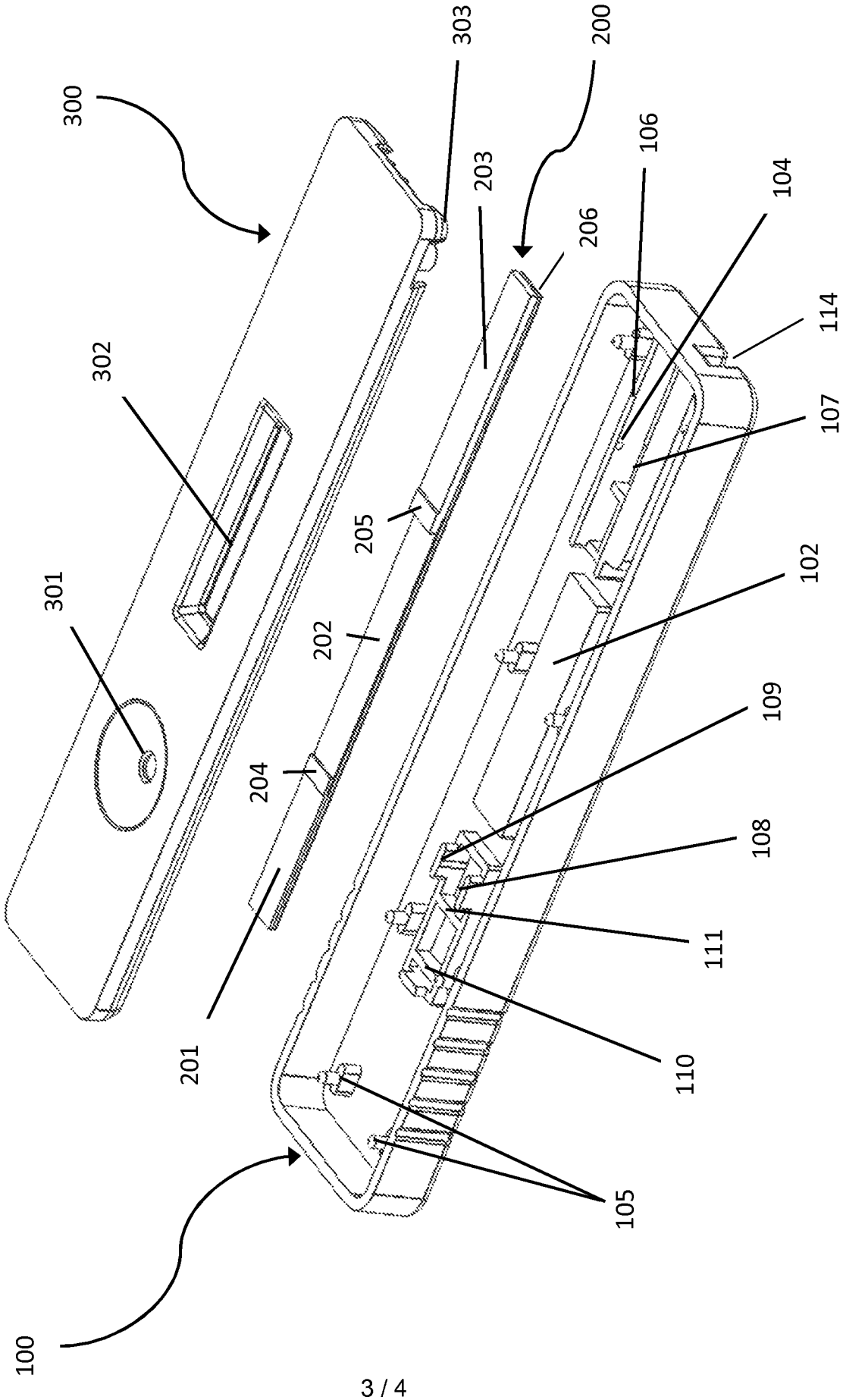
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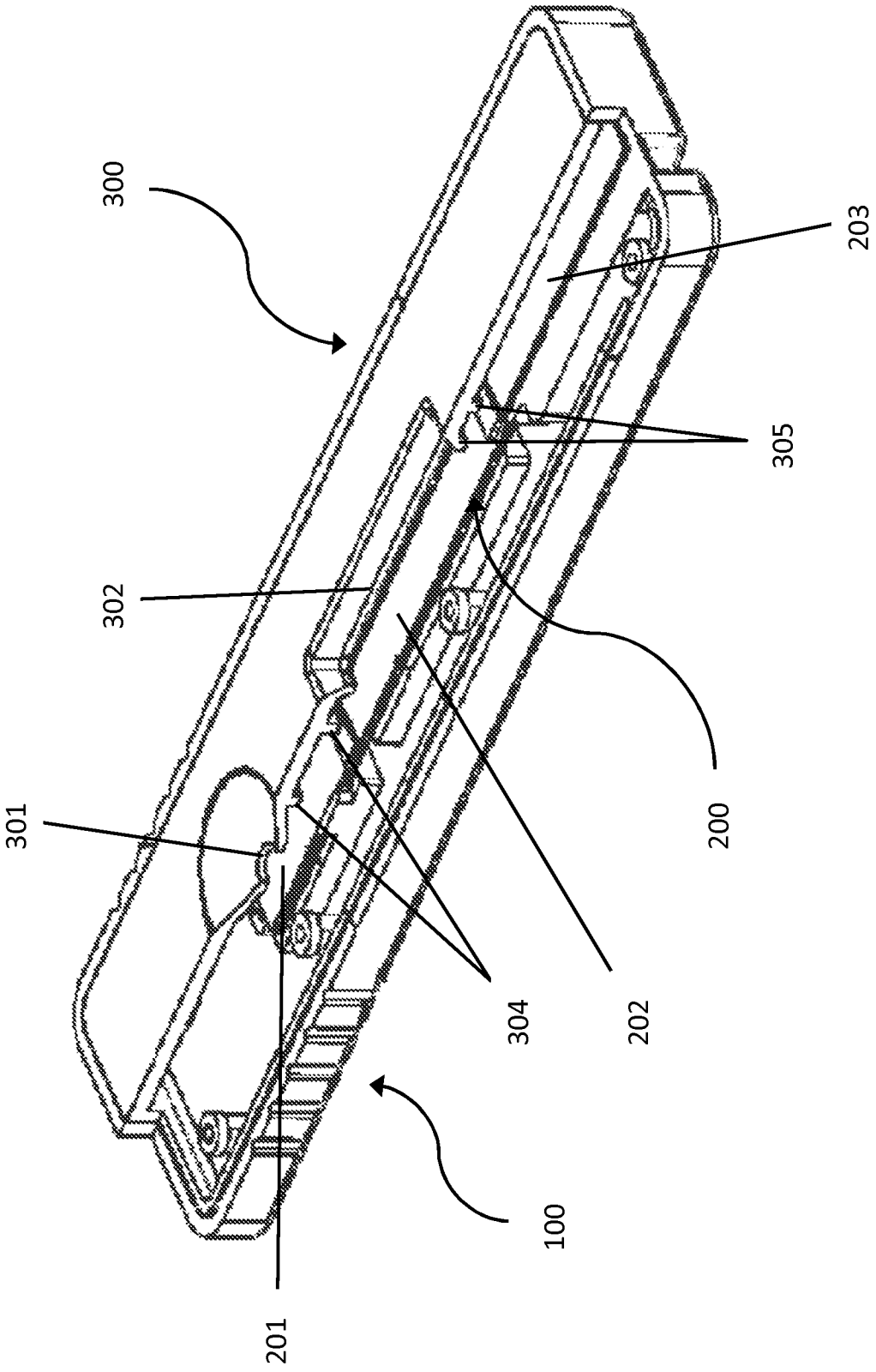
FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/18303

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/538, 33/52, 33/92 (2014.01)
USPC - 436/810; 435/970, 287.1
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)
IPC(8): C12Q 1/00, 1/54, 1/60; G01N 33/52, 33/92, 33/538 (2014.01)
USPC: 422/401, 402, 408, 420, 423, 424; 435/287.1, 970; 436/169, 807, 810

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 practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; test strip, dipstick, reactant strip, base, bottom, stand, support, platform, rack, layer, film sheet, level, material, porous, permeable, penetrable, membrane, film, bibulous, absorbent, blotting, test, exam, check, assay, analyte, verify, validate, zone, section, area, region, reagent

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 1998/022824 A1 (ZER, A et al.) 28 May 1998; page 2, lines 20-26; page 3, lines 17-20; page 6, lines 6-16; page 7, line 5; page 9, lines 5-25; page 10, lines 2-14; page 11, lines 5-27; page 12, lines 1-21; page 19, lines 25-28; figure 2; claims 1-3, 5, 9, 20, 22, 26	1-3, 12
Y	US 2010/0239460 A1 (NAZARETH, AR et al.) 23 September 2010; paragraphs [0051]-[0059], [0068]-[0069], [0079]; figures 8-9, 14A-14E, 15, 17	1-3, 12
A	US 6,150,178 A (CESARCZYK, EJ et al.) 21 November 2000; column 2, lines 9-41; column 6, lines 41-44; figures 5A-5B; claims 15, 34	1-3, 4/1-3, 12, 14-17, 18/15-17
A	US 2004/0082878 A1 (BALDWIN, D et al.) 29 April 2004; paragraph [0053]	1-3, 4/1-3, 12, 14-17, 18/15-17
A	US 5,597,532 A (CONNOLLY, J) 28 January 1997; column 2, lines 20-23	1-3, 4/1-3, 12, 14-17, 18/15-17
A	US 4,783,056 A (ABRAMS, RS) 08 November 1988; abstract; column 2, lines 7-8; column 4, lines 1-15; column 6, line 9; column 7, lines 12-58; column 8, lines 41-43; claims 1, 3-4, 8	1-3, 4/1-3, 12, 14-17, 18/15-17
A	US 4,689,202 A (KHOJA, MA et al.) 25 August 1987; entire document	1-3, 4/1-3, 12, 14-17, 18/15-17
A	WO 1992/001226 A1 (CARR, AH et al.) 23 January 1992; entire document	1-3, 4/1-3, 12, 14-17, 18/15-17

☐ Further documents are listed in the continuation of Box C. ☐

* 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 application or patent 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
13 May 2014 (13.05.2014)	21 MAY 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/18303

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-11, 13, 19-22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.