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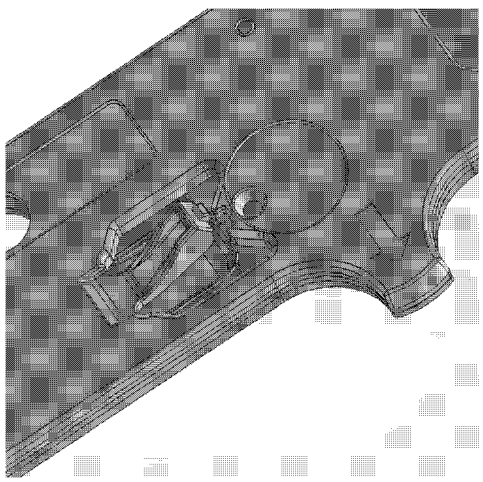
(54) Title: MICRO MECHANICAL METHODS AND SYSTEMS FOR PERFORMING ASSAYS

(57) Abstract: The present invention provides a micro mechanical system for performing assays for determining the presence of one or more selected analytes in a sample. The device comprises of a reader and a test cartridge with at least one reaction well and at least one moveable member capable of moving fluids and parts through the fluids in a defined reaction well. Reagents in the reaction well and or the moveable members, react with the sample to yield a physically detectable change. The moveable parts are capable of executing motions that either mix, move reaction components, exchange or systematically deliver reagents to targets in the cartridge. Sensors in the reader are configured to detect and or quantify the presence of a sample in the reaction well and of analytes in the sample. Signals from the sensors are converted to an output on a visual display window on the external part of the reader.

Figure 1



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MICRO MECHANICAL METHODS AND SYSTEMS FOR PERFORMING ASSAYS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority benefit of the filing date of US Provisional Patent Application Serial No. 62/273,930, filed on December 31, 2015, the disclosure of which application is herein incorporated by reference in its entirety.

FIELD OF INVENTION

The present invention relates to microfluidics systems for performing assays for determining the presence of one or more selected analytes in a sample.

BACKGROUND

Qualitative and quantitative immuno- and chemical assays have gained acceptance as important tools in the medical and food industries. These methods have been used for the diagnosis of disease conditions, detection of analytes, and for the detection of microbes, such as bacteria. These methods of diagnosis have established effectiveness, and the methods have made it easier for physicians to monitor and manage patients undergoing various forms of therapy.

Traditionally, the diagnostic assays have been performed in hospital and clinic settings, and involve the use of sophisticated and expensive equipment, that require specially trained personnel for their operation. Further, the assay results are sometimes not available for days or weeks after the samples from the patients have been obtained. The presently available diagnostic assays are thus costly, time consuming, and not convenient.

Attempts have been made to develop less costly assays. For example, a typical home self-test for detecting blood components requires the patient to prick a finger with a sterilized lancet, apply a drop of blood sample to a sample application area on a disposable strip, and then wait for the results. Assays that use other bodily fluids, such as urine essentially work in a similar manner. These devices are designed such that a typical lay person can perform the assays correctly with very little training. However, these assay systems generally suffer from low accuracy or require a number of preparative steps be performed that could compromise the test results, and are thus not convenient.

U.S. Patent No. 5,580,794 to Allen Michael describes a single use electronic assay device that assays for specific analytes in a given sample. U.S. Patent No. 4,806,312 to Greenquist describes a multizone analytical element having detectable signal concentration zone. U.S. Patent No. 4,627,445 to Garcia *et al.* describes a hand-held portable medical diagnostic system for checking measurement of blood glucose, urea nitrogen, hemoglobin, or blood components, where a disposable needle or lance probe

package carries a chemical reagent strip such as blood reacting chemistry, a visual readout, and a computer system.

U.S. Patent No. 4,197,734 to A. Rosenberg describes an apparatus that is capable of measuring the clotting time of blood. The apparatus includes a support frame, which supports a syringe containing a blood sample, and a turntable that rotates. Blood from the syringe drops onto the turntable where the clotting time is automatically and graphically depicted by a chart that is rotated on the turntable. The apparatus can also be employed to determine variations in the viscosity of blood plasma and other fluids.

U.S. Patent No. 3,486,859 to Greiner *et al.* describes an apparatus that has a double arm holder with blood liquid reactant chambers that are connected to each other via a small capillary conduit. An air pump is provided for applying pressure changes to one of the chambers to effect periodic mixing of the liquids via the capillary conduit. Indicator means are included to detect the progressive restriction of the capillary conduit upon coagulation of the blood.

The methods described above have severe limitations which make them extremely challenging for home use. Some of the methods require special blood preparations and handling, making them suitable only for a central clinic with well-trained staff, while others are expensive, or not accurate.

The Avie™ A1c System test (MEC Dynamics Corp, San Jose, CA) is a point of care system (POC) that quantitatively measures % glycated hemoglobin (HbA1c) in capillary and venous whole blood. , to monitor glycemic control in patients with diabetes mellitus.. Whole blood is directly applied to the sample port of the Avie A1c test cartridge, containing antibodies to HbA1c deposited in a mixing chamber and antigen conjugates on a solid membrane support, and the cartridge is then inserted into the Avie A1c reader. Avie A1c diluent is then added to lyse the red blood cells in the sample, which allows both the HbA1c and total hemoglobin (Hb) analytes to be measured photometrically at their own distinct wavelengths. In its original design [shown in Figures 1 and 2] the Avie A1c test cartridge employed a closeable bridge functionality to fluidically connect the mixing chamber to downstream components, and the diluent well connected indirectly with the mixing chamber via the sample port. In this prior art design the bridge arrangement prevents flow of the diluent/blood mixture from the mixing well to the reagent strip for a predetermined time, after which the bridge is lowered by action of the reader and flow is engaged. Unfortunately , however, final A1c hemoglobin measurements were inconsistent with this design, due in part to excess diluent flow but also to sample collection challenges.

Thus the need for assay systems for detecting analytes that are accurate, convenient, and inexpensive continues. The systems and methods described herein meet these and other needs.

SUMMARY

The present invention provides methods and micro technological systems for performing assays for determining the presence of one or more preselected analytes in a sample. The systems include a disposable test cartridge that can be inserted into a test analysis machine, also referred to herein as a reader. The test cartridge isolates the sample such that it is not in contact with the reader and the sample is not contaminated.

The test cartridge of the present invention comprises a plurality of defined wells on a solid support, preferably linked by capillary channels. One aspect of the present invention relates to a test cartridge comprising a first solid support comprising a diluent well, a reaction well, and a sample port; wherein the diluent well and the reaction well are in direct fluidic communication via a first capillary channel; wherein the sample port and the reaction well are in direct fluidic communication and at different elevations; wherein the sample port comprises: a sample channel having an exterior opening adapted to admit a liquid sample comprising a first analyte, and an interior opening connecting the sample channel to the reaction well via a shelf; wherein the sample channel and the shelf are adapted to hold a first amount of the liquid sample; and wherein the shelf prevents the first amount of the liquid sample from entering the reaction well unless a diluent is present in the reaction well. In preferred embodiments, the sample port and the diluent well are not in direct fluidic communication. In particularly preferred embodiments, the liquid sample comprises whole blood and the first amount of such sample is from about 0.1 μl to about 0.3 μl, more preferably about 0.2 μl.

A further aspect of the present invention relates to methods for determining the concentration of a first analyte in a liquid sample, the method comprising: providing a test cartridge as described herein; placing a first amount of the sample in the sample port; inserting the test cartridge into a reader, whereafter the reader prompts a user to add the diluent to the diluent well; adding the diluent to the diluent well, wherein the diluent flows from the diluent well via the first capillary channel to the reaction well, wherein the diluent in the reaction well draws the sample from the sample port over the edge of the shelf into the reaction well to form a mixture of the diluent and the liquid sample in the reaction well; wherein the reader is adapted to measure the concentration of the first analyte in the liquid sample.

In another aspect, the test cartridge further comprises a mixing well, wherein the reaction well and the mixing well are in fluidic communication via a second capillary channel, and wherein the second capillary channel comprises a closable gate. In preferred embodiments, the closeable gate comprises a collapsible tube, *e.g.* a silicon tube or the like.

In some embodiments the test cartridge further comprises a reagent strip comprising a first capture zone wherein mixing well and the reagent strip are in fluidic communication via a third capillary channel, wherein the first capture zone comprises a first antigen that specifically binds to a complex

comprising the first analyte and the antibody, and a second capture zone comprising a second antigen that binds to the antibody. In some embodiments, the first analyte is HbA1c. In some embodiments, the liquid sample further comprises a reference analyte. In some embodiments, the reader is adapted for quantifying the reference analyte in the reaction well. In some embodiments, the reference analyte is hemoglobin. A further aspect of the present invention relates to methods for determining the percentage of hemoglobin in a sample that is HbA1c, comprising determining the total hemoglobin from the reaction well cell and the total HbA1c from the capture zone; and dividing the total HbA1c by total hemoglobin to obtain the percentage of hemoglobin that is HbA1c.

The surface of the wells and the capillary channels can be coated with reagents that assist the flow of liquids through the wells and capillary channels of the test cartridge. Inside at least one of the wells may be at least one magnetic stir bar which can be attracted magnetically and driven by a magnetic moving device arranged outside the test cartridge in the test analysis machine. The magnetic stir bar is capable of executing motions that mix the reaction components, move reaction components, exchange or systematically deliver reagents to targets in the cartridge, and the like.

The test cartridge can be placed in a portable handheld machine or "reader" having sensors configured to detect and or quantify the presence of at least one analyte in the wells or at other analyte detection sites within the test cartridge, while responding to the physically detectable changes, producing signals which correlate to the presence of and/or amount of the selected analyte in the sample. The detection system can comprise detectable reagents that interact with the selected analyte, whereby a detectable result occurs in relation to the presence of an analyte. A signal from the sensors can be converted to an appropriate output on a visual display window on the external part of the reader.

To test a sample, a sample can be introduced into the test cartridge via the sample port and the test cartridge can then be inserted into the reader. Diluent can be placed in the diluent well of the test cartridge and pass into the reaction well via the first capillary channel. The sample is drawn toward the edge of the shelf in the sample channel after introduction at the sample port, and stays there until flushed from the edge by diluent in the reaction well. A sensor detects the movement and sends a signal to spin the magnet which, in turn, spins the stir bar at the appropriate times in a clockwise motion. The diluent then flushes the sample off of the shelf and continues to mix with the sample within the reaction well. For timed assays, a microprocessor contained within the reader begins a time count while the sensors, which can be electrical or optical, monitor various parts of the test cartridge for specific analyte responses. When detected and/or quantified, the results are reported qualitatively and/or quantitatively with appropriate units in the display window on the reader.

The motion and the sensors can be microprocessor controlled. A heater assembly can be activated in the reader, for temperature-sensitive assays such as coagulation tests and the temperature can be held constant or varied in a predetermined way through the duration of the assay.

These and other aspects of the present invention will become evident upon reference to the following detailed description. In addition, various references are set forth herein which describe in more detail certain procedures or compositions, and are therefore incorporated by reference in their entirety.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 illustrates a view of a prior art test cartridge design showing the closeable bridge functionality.

Figure 2 illustrates a view of a prior art test cartridge design showing the sample port.

Figure 3 illustrates a view of an exemplary test cartridge (10) of the invention. The test cartridge comprises a first solid support (20) having pegs (40) for attachment to a second solid support (not shown). The first substrate comprises a diluent well (50) in fluidic communication with a reaction well (70) via a first capillary channel (60). Also in fluidic communication with the reaction well is sample port (80). The first substrate further comprises a mixing well (110) in fluidic communication with the reaction well via a second capillary channel (100) comprising a silicone tube (90). The mixing well is in fluidic communication with a reagent strip (not shown) held in a reagent strip holder (140) via a third capillary channel (120) having a distal opening (130).

Figure 4 illustrates another view of the test cartridge (10) showing the silicone tube (90) and the second substrate (30) having a hole that communicates with the diluent well (50).

Figure 5 illustrates another view of the test cartridge showing the first capillary channel (60), the reaction well (70). The sample port comprises an exterior opening (150) adapted to admit a liquid sample, a sample channel (160) adapted to hold a first volume of the liquid sample and an interior opening (170) connecting the sample channel to the reaction well. Also depicted is the silicone tube (90) having a lumen (180) in fluidic communication within the second capillary channel (100).

Figure 6 illustrates another view of the test cartridge showing the first capillary channel (60), the reaction well (70), and the silicone tube (90). Also depicted is the sample channel (160) and the shelf (190) at the interior opening of the sample port, wherein the shelf prevents the first amount of the liquid sample from entering the reaction well unless a diluent is present in the reaction well.

Figure 7 illustrates another view of the test cartridge showing one of the pegs (40) used to connect the first and second solid supports, the mixing well (110), the second capillary channel (120) having a distal opening (130), and the reagent strip holder (140).

Figure 8 illustrates another view of the test cartridge showing the reaction well (70), the sample channel (160), and the shelf (190). The reaction well comprises a well floor, the shelf comprises a shelf floor, and the sample channel comprises a sample channel floor, wherein the well floor is at a first elevation, and the shelf floor and the sample channel floor are at a second elevation; the floors are parallel; and the difference between the first elevation and the second elevation is about 0.010". Also shown is the height of the sample channel, 0.005".

DETAILED DESCRIPTION

Definitions

Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

As used herein, the term "subject" encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the Mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish and the like. The term does not denote a particular age or gender.

The term "antibody" as used herein, includes, but is not limited to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). "Antibody" also includes, but is not limited to, a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize the antigen-specific binding region (idiotype) of antibodies produced by the host in response to exposure to trichomonas antigen(s). Examples include polyclonal, monoclonal, chimeric, humanized, and single chain antibodies, and the like. Fragments of immunoglobulins, include Fab fragments and fragments produced by an expression library, including phage display. See, *e.g.*, Paul, Fundamental Immunology, 3rd Ed., 1993, Raven Press, New York, for antibody structure and terminology.

The terms "specifically binds to" or "specifically immunoreactive with" refers to a binding reaction which is determinative of the presence of the target analyte in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated assay conditions, the specified binding

moieties bind preferentially to a particular target analyte and do not bind in a significant amount to other components present in a test sample. Specific binding to a target analyte under such conditions may require a binding moiety that is selected for its specificity for a particular target analyte. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular antigen. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with an analyte. See Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will provide a signal to noise ratio at least twice background and more typically more than 10 to 100 times background.

As used herein, the terms "label" and "detectable label" refer to a microparticle or a molecule capable of detection, including, but not limited to, colored microparticles, radioactive isotopes, fluorescers, chemiluminescers, chromophores, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, ligands (*e.g.*, biotin, avidin, strepavidin or haptens) and the like.

As used herein, a "solid support" refers to a solid surface such as a plastic plate, magnetic bead, latex bead, microtiter plate well, glass plate, nylon, agarose, acrylamide, and the like.

"Specific" in reference to the binding of two molecules or a molecule and a complex of molecules refers to the specific recognition of one for the other and the formation of a stable complex as compared to substantially less recognition of other molecules and the lack of formation of stable complexes with such other molecules. Exemplary of specific binding are antibody-antigen interactions, enzyme-substrate interactions, polynucleotide hybridizations and/or formation of duplexes, cellular receptor-ligand interactions, and so forth.

OVERVIEW

The invention pertains to a disposable strip (also referred to herein as "test cartridge"), that can be used for performing qualitative and quantitative immuno- and chemical assays. On the strip are at least three wells, where the wells can be in fluidic communication with each other via capillary channels. A sample, preferably a liquid sample, for analysis is introduced into a reaction well via a sample port. Diluent is added to a diluent well and moves into the reaction well via a capillary channel where it mixes with the liquid sample. A reference analyte in the sample may be measured in the reaction well. The mixture moves from the reaction well to a mixing well via a second capillary channel comprising a gate, and from the mixing well to a reagent strip where other analytes in the sample can be measured. The disposable strip can be placed in an analyzer (also referred to herein as a "reader") that detects the

individual components of the sample and the total analyte in the sample. The analyzer includes a display system that can display the results of the analysis as well as provide instructions during the operation of the assay.

In one application, the percent total Hb that is HbA1c in human red blood cell can be determined. Blood from a subject can be deposited in the sample port on the test cartridge, which is then placed in the reader. In the diluent well can be placed a reagent that flows into the reaction well via a capillary channel, where it draws in the sample from the sample port. In the reaction well can be placed a known amount of an antibody specific for HbA1c, and a magnetic stirrer. When blood moves into the reaction well, the magnet stirs the liquids in the well thereby mixing them well. The reagent lyses the cells thereby releasing the hemoglobin from the red blood cells. The concentration of Hb in the reference cell can be measured using infrared or ultraviolet measurements. The antibody binds to HbA1c. An open gate connects the reaction well to the downstream components of the test cartridge. After a specified period of time, the reader can close the gate and prevent further flow out of the reaction well. The liquid in the reaction well then flows through a gate, further capillary channels, and a mixing well towards one or more capture zones. The capture zones have immobilized on them antigens that bind to the bound antibody complex only, and on a separate part of the zone other antigens that bind to all antibodies. The antibody-HbA1c complex can be captured by the antigens in the first part of the capture zone, and all the antibodies can be captured by the antigens in the latter part of the capture zone. A detection system can be used to detect the antibodies bound in the first and the second part of the capture zone. The ratio and/or the sum of the two zones can be used to quantify the amount of HbA1c present in the sample. The ratio of the first zone to the total hemoglobin from the reference cell can provide the percentage of HbA1c in the blood sample. The results can be displayed on the reader.

MICRO MECHANICAL SYSTEM

The invention provides a test cartridge, a portable handheld machine (*i.e.*, a reader), and a combination comprising a test cartridge and a reader. The test cartridge can be placed on the machine for performing assays, for detecting analytes, and for displaying information such as instructions and results. One aspect of the test cartridge is illustrated in Fig. 1. The test cartridge can be made by joining together two or more solid supports with grooves present in at least one of the supports. The solid support can be rectangular, circular, oval, or any shape. The support can be made from a suitable material that is selected on its properties, such as good thermal conductivity, clarity for optical transmission, mechanical properties for easy welding, surface properties that allow for uniform coating and stability of reagent, and neutrality to the liquid medium to prevent interference with the assay. For this purpose, suitable plastics include those with high free surface energies and low water sorption, including PETG, polyester

(Mylar®), polycarbonate (Lexan®), polyvinyl chloride, polystyrene, SAN, acrylonitrile-butadiene-styrene (ABS), particularly ABS supplied by Borg Warner under the trade name Cycolac, among others. When the solid support is a hydrophobic plastic, it can be treated by art-known methods to render the surfaces hydrophilic, such as by plasma etching and by corona treatment. Alternatively and equivalently, a commercially-available molded solid support can be used in the practice of the invention.

For purposes of illustration, this embodiment of the invention is described by reference to a test cartridge formed by joining two solid supports. At least one of the solid supports has grooves or cavities that serve as the wells (50, 70, 110), and capillary channels (60, 100, 120). The grooves can be any geometric shape, and are preferably circular. The grooves have dimensions that are sufficient volume to hold the samples and to allow for the reaction and mixing to occur. Thus, the circular grooves can have a diameter of between about 0.01 mm to about 100 mm, depending on the length and width of the support material, and can have a height of about 0.001 mm to about 4 mm, depending on the thickness of the support material. The diameter and height of the grooves can be easily determined by the one of skill in the art. In one aspect of the invention, one of the support pieces has holes drilled through to the grooves where the holes serve as the vent holes. Further, the holes can allow access to the well where the diluent will be placed, such as the diluent well (50), 1. Prior to the joining of the two pieces, a mixing bar can be inserted in the desired reaction well (70).

In some embodiments the solid supports are plastic. In the molding process, energy-directing ridges may be present at least on the outline adjacent to the periphery of the grooves of at least one of the two plastic pieces. When welded ultrasonically, the two plastic pieces are glued together along the energy ridges forming an air-tight seal around the wells and channels, with the only access to the exterior from the wells being the vent holes and the access to the diluent well. The surface of the reaction well can optionally be slightly textured for use with moveable members such as magnetic stir bars. The texturing can accommodate a disjoining pressure, Π. Π, is the pressure, in the case of two plates immersed in a medium, in excess of the external pressure, that must be applied to the medium between the plates to maintain a given separation. In this case, Π is numerically just the force of attraction or repulsion between the moveable member and the surfaces of the reaction well per unit area. The wider the moveable member, the greater would be the pressure between the surfaces and texturing would eliminate any undesired clamping of the moveable member onto the walls of the reaction well. A more general definition for disjoining pressure is

$$\Pi = -1/Ă(\delta G/\delta x)\ Ă, T, V$$

where

Ă = Area

T = Temperature

V = Volume

G = Gibb's free energy

The moveable member can be made by use of stainless steel or a combination of stainless steel with any other desired material so that it is capable of being attracted and driven by an external magnetic moving device. The material can be any form of magnetizable alloy with a stainless covering to prevent corrosion or specially coated for bonding of specific molecules. The thickness of the movable member is based on the height of the reaction well. It has to be small enough to fit in the reaction well and move freely. For a reaction well cavity of a height of 0.010 inches, the thickness of the moveable member can be between about 0.007 to about 0.008 inches

An assembled test cartridge is illustrated in Figure 2, where 10 is the first solid support having grooves or cavities that serve as the wells, and capillary channels, 30 the second solid support allowing access to the diluent well (50), and 90 is a closable bridging functionality between the reaction well and the downstream components.

An example of a detection system for automated detection of analytes for use with the present test cartridge and associated methods comprises an excitation source, a monochromator (or any device capable of spectrally resolving light components, or a set of narrow band filters) and a detector array. The excitation source can comprise infrared, blue, or UV wavelengths, and the excitation wavelength can be shorter than the emission wavelength(s) to be detected. The detection system may be: a broadband UV light source, such as a deuterium lamp with a filter in front; the output of a white light source such as a xenon lamp or a deuterium lamp after passing through a monochromator to extract out the desired wavelengths; any of a number of continuous wave (cw) gas lasers, including but not limited to any of the argon-ion laser lines (457, 488, 514, *etc.* nm) or a HeCd laser; solid-state diode lasers in the blue such as GaN and GaAs (doubled) based lasers or the doubled or tripled output of YAG or YLF based lasers; or any of the pulsed lasers with output in the blue.

The emitted light from the sample or the reactants in the reaction well can be detected with a device that provides spectral information for the substrate, *e.g.*, a grating spectrometer, prism spectrometer, imaging spectrometer, or the like, or use of interference (bandpass) filters. Using a two-dimensional area imager such as a CCD camera, many objects may be imaged simultaneously. Spectral information can be generated by collecting more than one image via different bandpass, longpass, or shortpass filters (interference filters, or electronically tunable filters are appropriate). More than one imager may be used to gather data simultaneously through dedicated filters, or the filter may be changed

in front of a single imager. Imaging based systems, like the Biometric Imaging system, scan a surface to find fluorescent signals.

Other embodiments appropriate for the systems described herein include the use of a reagent-coated membrane (also referred to herein as a "reagent strip") as part of the test cartridge positioned in a way that allows continuity and directed sample flow within the entire test cartridge system. The sensory systems would be positioned to be capable of monitoring the membrane portions of the strip for the analyte or responses being tested for.

Test Cartridges

One aspect of the present invention relates to a test cartridge comprising a first solid support comprising a diluent well, a reaction well, and a sample port; wherein the diluent well and the reaction well are in direct fluidic communication via a first capillary channel; wherein the sample port and the reaction well are in direct fluidic communication and the sample port and the diluent well are not; wherein the sample port comprises: a sample channel having an exterior opening adapted to admit a liquid sample comprising a first analyte, and an interior opening connecting the sample channel to the reaction well via a shelf; wherein the sample channel and the shelf are adapted to hold a first amount of the liquid sample; and wherein the shelf prevents the first amount of the liquid sample from entering the reaction well unless a diluent is present in the reaction well.

In some embodiments the reaction well comprises a well floor. In some embodiments, the shelf comprises a shelf floor. In some embodiments, the sample channel comprises a sample channel floor. In some embodiments, the well floor is at a first elevation. In some embodiments, the shelf floor and the sample channel floor are at a second elevation. In some embodiments, the floors are parallel. In some embodiments, the difference between the first elevation and the second elevation is from about 0.015" to about 0.005". In some embodiments, the difference between the first elevation and the second elevation is about 0.010". In some embodiments, the shelf floor has a surface area of about 0.0010 in^2 to 0.0015 in^2, more preferably about 0.0012 in^2 to 0.0013 in^2, most preferably about 0.00125 in^2.

In some embodiments, the test cartridge further comprises a second solid support comprising a hole wherein the first solid support and the second solid support are joined and the hole communicates with the diluent well. In some embodiments, the first solid support and the second solid support are independently selected from plastic, glass, nylon, metal, and combinations thereof. In some embodiments, the first solid support is plastic. In some embodiments, the first solid support is glass. In some embodiments, the first solid support is nylon. In some embodiments, the first solid support is metal. In some embodiments, the second solid support is plastic. In some embodiments, the second solid support is

glass. In some embodiments, the second solid support is nylon. In some embodiments, the second solid support is metal. In some embodiments, the first solid support and the second solid support are plastic.

Assay results vary considerably if the patient has to squeeze his or her finger to get enough blood into the device to perform the assay, because the blood is then coming from tissue which disrupts the results. One aspect of the present invention relates to a test cartridge having a sample port specifically adapted for improved performance and reliability/consistency of results. In some embodiments, the first amount of the liquid sample is from about 0.02 μL to about 2.0 μL. In some embodiments, the first amount of the liquid sample is less than about 1.0 μL. In some embodiments, the first amount of the liquid sample is from about 0.5 μL to about 0.2 μL. In some embodiments, the first amount of the liquid sample is about 0.3 μL. In some embodiments, the first amount of the liquid sample is about 0.4 μL. In some embodiments, the first amount of the liquid sample is about 0.5 μL. In some embodiments, the first amount of the liquid sample is about 0.08 μL. In some embodiments, the first amount of the liquid sample is about 0.29 μL. In some embodiments, the first amount of the liquid sample is about 0.35 μL. In some embodiments, the first amount of the liquid sample is about 0.47 μL. In some embodiments, the first amount of the liquid sample is from about 0.04 μL to about 1.0 μL. In some embodiments, the first amount of the liquid sample is from about 0.08 μL to about 0.5 μL. Preferably, the first amount of the liquid sample is about 0.1 μL to about 0.3 μL. In particularly preferred embodiments, the first amount of the liquid sample is about 0.2 μL. The sample channel (160) and the shelf (190) as shown in Figure 7 together hold the appropriate amount of blood within the sample channel for mixing with the diluent when added. In some embodiments, the test cartridge further comprises a moveable member in the reaction well. The moveable member may be a magnetic stir bar which rotates clockwise around the reaction well to draw the blood into the well.

Accurate analyte measurement requires consistent analyte dilution at the site of detection. A gate within the second capillary channel connecting the reaction well to the analyte detection site should be closable after a predetermined time to ensure that the analyte is not contaminated with excess diluent. One aspect of the present invention relates to a test cartridge specifically adapted for improved performance and reliability/consistency of results by having a mixing well that draws the reagent/blood mixture down from the reaction well, wherein addition of further reagent and/or blood is stopped after a certain time frame. One aspect of the present invention relates to a test cartridge comprising a second capillary channel to fluidically connect the reaction well to the mixing well, wherein the second capillary channel comprises a closeable gate. In preferred embodiments, the gate comprises a collapsible tube the lumen of which is in fluidic communication with the second capillary channel. In particularly preferred embodiments, the collapsible tube comprises a silicone tube.

In some embodiments the second solid support comprises a vent hole in communication with the reaction well. In some embodiments the second solid support comprises a vent hole in communication with the mixing well. In some embodiments the second solid support comprises a first vent hole in communication with the mixing well, and a second vent hole in communication with the mixing well.

One aspect of the present invention relates to kits comprising a plurality of test cartridges and suitable packaging. In some embodiments the test cartridges are packaged in groups of 10-15 cartridges/vial. The kits may further comprise diluent, which may be separately packaged in a second vial. In some embodiments the diluent comprises 2% detergent buffered solutions with 0.6% sodium chloride. In some embodiments each vial of cartridges comes with its own vial of diluent. The kits may further comprise a portable handheld device, also referred to herein as a reader, for use with the test cartridges to quantify one or more analytes present in a sample applied to the test cartridge. For measuring HbA1c, the reader may be calibrated using the National Glycohemoglobin Standardization Program (NGSP) certified laboratory value assigned blood samples. Lot specific information for test cartridges may be transferred to the reader by radio frequency identification (RFID).

Methods

A further aspect of the present invention relates to methods for determining the concentration of a first analyte in a liquid sample, the method comprising: providing a test cartridge as described herein; placing a first amount of the sample in the sample port; inserting the test cartridge into a reader, whereafter the reader prompts a user to add the diluent to the diluent well; adding the diluent to the diluent well, wherein the diluent flows from the diluent well via the first capillary channel directly to the reaction well, wherein the diluent in the reaction well draws the sample from the sample port over the edge of the shelf into the reaction well to form a mixture of the diluent and the liquid sample in the reaction well; wherein the reader is adapted to measure the concentration of the first analyte in the liquid sample.

In some embodiments, the test cartridge further comprises a stir bar in the reaction well, wherein the reader comprises a magnetic moving device which stirs the mixture of the diluent and the liquid sample in the reaction well by magnetically rotating the stir bar.

In some embodiments, the test cartridge further comprises an antibody deposited in the reaction well, wherein the antibody is specific for the first analyte, and wherein the first analyte combines with the antibody in the reaction well. In some embodiments, the antibody is conjugated to a detectable label. In some embodiments, the antibody is conjugated to a colored microparticle.

In some embodiments, the liquid sample is selected from venous whole blood, capillary whole blood, and combinations thereof. In some embodiments, the first analyte is HbA1c. In some

embodiments, step (b) comprises lancing the skin of a subject causing a blood droplet to form and touching the exterior opening of the sample port to the blood droplet to fill the sample channel with the first amount of the sample.

In some embodiments, the diluent comprises a lysing agent. In some embodiments, the lysing agent is a nonionic surfactant. In some embodiments, the diluent further comprises sodium chloride.

In some embodiments, the liquid sample further comprises a reference analyte, and wherein the reader quantifies the reference analyte in the reaction well. The method according to claim 31, wherein the reference analyte is hemoglobin. In some embodiments, the hemoglobin is converted to methemoglobin prior to quantification. In some embodiments, the reader quantifies the methemoglobin photometrically. In some embodiments, the reader quantifies the methemoglobin photometrically at 420 nm.

In some embodiments, the first solid support further comprises a mixing well, wherein the reaction well and the mixing well are in fluidic communication via a second capillary channel, wherein the mixture of the diluent and the liquid sample flows from the reaction well via a second capillary channel into the mixing well. In preferred embodiments, the second capillary channel comprises a gate, wherein the mixture of the diluent and the liquid sample flows from the reaction well via the closable bridging functionality into the second capillary channel. In some embodiments, the gate comprises a collapsible tube, wherein the reaction well and the mixing well are in fluidic communication via the second capillary channel further comprising the lumen of the collapsible tube, wherein the mixture of the diluent and the liquid sample flows from the reaction well via the lumen of the silicone tube and the second capillary channel. In some embodiments, the reader closes the gate after a first time period, wherein closing the gate after the first time period stops the mixture of the diluent and the liquid sample flowing from the reaction well via the gate to the second capillary channel after a first volume of the mixture of the diluent and the liquid sample has entered the mixing well via the second capillary channel.

In some embodiments, the test cartridge further comprises a reagent strip comprising a first capture zone wherein the mixing well and the reagent strip are in fluidic communication via a third capillary channel, wherein the mixture of the diluent and the liquid sample flows from the mixing well via the third capillary channel onto the reagent strip. In some embodiments, the first capture zone comprises a first antigen that specifically binds to a complex comprising the first analyte and the antibody, wherein the reader quantifies the complex comprising the first analyte and the antibody bound to the first antigen. In some embodiments, the reagent strip further comprises a second capture zone. In some embodiments, the second capture zone comprises a second antigen that binds to the antibody, wherein the reader quantifies the complex comprising the antibody bound to the second antigen.

OPERATION

A general mode of operation of the device shown involves the application of a sample, preferably a liquid sample, directly to the sample port of the test cartridge described shown in Figures 1-5. In some embodiments the liquid sample is venous whole blood, capillary whole blood, or combinations thereof. Single use, auto-disabling lancing devices may be used to obtain a blood sample.

The test cartridge is inserted into a receptacle on the reader that allows the test cartridge in one orientation only. The reader may have a molded plastic housing which contains the electronics, optics, motors for locating mechanisms, LCD display and the power button. A printer may be provided for the reader. For an assay with a specific temperature requirement, a heater assembly in the reader heats the test cartridge to the desired temperature controlled by a microprocessor in the reader. An LCD prompts simple steps, after the test cartridge is inserted and the reader is turned on, which an operator can follow including the addition of the diluent to the diluent well. The reader can optionally have sensors to determine the presence of adequate amounts of sample and diluent in the wells, a mechanism to initiate and stop the timing of the assay, and a mechanism to close the gate mechanism. The sensors detect the signals from the completion of the reaction, such as measuring the transmission of an optical signal emitted and directed through the walls of the reaction well.

The applied sample is accurately distributed into the reaction and mixing wells via the capillary channels. The defined modes of movement of the moveable member ensure proper mixing of the diluent and sample and also contribute to proper flow between wells. For assays that require quantification of an analyte, the sensory system monitors the changes either in the reaction well, the reagent strip or the moveable members, until the desired end point is achieved. For assays requiring just the determination of the presence of an analyte, the sensory system monitors the specific parts of the test cartridge for the appropriate duration of time. The microprocessor computes the results quantitatively or qualitatively, which are displayed on the LCD. The test cartridge can then be removed at the end of the assay and disposed.

Thus, the operator inserts the test cartridge into test cartridge receptacle in the reader. The operator then pushes a start button, which could automatically be activated by the test cartridge itself, waits for a prompt to add diluent, and then obtains the results from the display, typically within a few minutes or seconds, depending on the assay type.

DETECTION OF GLYCATED HEMOGLOBIN (HbA1c)

Glycated hemoglobin refers to a series of minor hemoglobin components that are formed through the attachment of glucose to the hemoglobin molecule. The human red blood cell is freely permeable to glucose. Within each red blood cell, glycated hemoglobin is formed at a rate that is directly proportional

to the ambient glucose concentration. Approximately 97% of the total hemoglobin in circulating red blood cells is hemoglobin A. Hemoglobin A consists of four polypeptide chains, two a-chains and two b-chains. Glycation of the Hemoglobin A occurs through the covalent coupling of glucose with the N-terminal valine amino acid of each b-peptide chain. An unstable Schiff base (aldimine) is initially formed which then undergoes an irreversible Amadori rearrangement to form a stable ketoamine, Hemoglobin A1c (HbA1c).

The life-span of hemoglobin A containing red blood cells averages 120 days. The percentage of Hemoglobin A that is glycated to HbA1c is directly proportional to the time that red blood cells are exposed to glucose and to the average glucose concentration encountered. Measurement of the HbA1c fraction gives an integrated picture of the average blood glucose concentration during the half-life of the red cells, that is, over the last 60 days. The level of HbA1c is usually expressed as a percentage of total hemoglobin.

In normal subjects, HbA1c is typically in the range 3-6% of total hemoglobin. In patients with elevated glucose levels *e.g.* in the case of Type I and Type 2 diabetes, the level may rise to twice the upper limit of normal or more.

Long-term control of glucose levels in diabetics is very important. Too much glucose in the blood over many years can damage the eyes, kidneys and nerves. It also increases the risk for heart and blood vessel disease. The measurement of HbA1c as a percentage of total hemoglobin provides a valuable means of assessing the long-term control of glucose levels and also constitutes an important risk indicator for identifying Type 1 and Type 2 diabetics.

A sample of blood from a subject is deposited in the sample port of the test cartridge (Figure 3). The blood moves to the reaction well via the sample channel. The reaction well can serve as the venue where total Hb is measured. HbA1c in the blood sample is measured on the reagent strip. The ratio of HbA1c to the total Hb provides the percentage of total hemoglobin that is HbA1c.

In some embodiments, the reaction well contains a lysing agent. The lysing agent lyses the whole blood samples thereby releasing the hemoglobin. The lysing agents are typically surfactants, and preferably nonionic surfactants, such as for example TRITON™ X-100 (4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol). In some embodiments, the reaction well additionally contains an antibody that can detect HbA1c. The antibody can be a monoclonal or polyclonal antibody, or antibody fragment containing the antigen binding site, or complementarity determining region (CDR), such as an $F(ab')_2$ or Fab fragment. The antibody may be labeled with a detectable label. In some embodiments, the detectable label may be a colored microparticle. In some embodiments, the detectable label may be a radioactive, fluorescent or chemiluminescent substance, or an enzyme. Alternatively, a

labeled second antibody which recognizes the species specific Fc fragment of the first antibody may also be used.

In some embodiments, the detectable label is a microparticle. The word "microparticle" may refer to individual plastic spheres in the size range of 0.03 μM to 2 μM in diameter. Microparticles may be colored or fluorescent. A suspension of microparticles has the milky appearance of latex rubber. For this reason, microparticle suspensions have historically been referred to as latex. Other terms such as microsphere, nanosphere, bead, are used to describe the same product. Examples of suitable microparticles include colored microparticles, such as blue latex microparticles.

In some embodiments, the detectable label is a fluorescent molecule. Examples of suitable fluorescent labels include fluorescein (FITC), 5,6-carboxymethyl fluorescein, Texas red, nitrobenz-2-oxa-1,3-diazol-4-yl (NBD), coumarin, dansyl chloride, rhodamine, 4'-6-diamidino-2-phenylinodole (DAPI), and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. Preferred fluorescent labels are fluorescein (5-carboxyfluorescein-N-hydroxysuccinimide ester), rhodamine (5,6-tetramethyl rhodamine), substituted rhodamine compounds, and the cyanine dyes Cy3, Cy3.5, Cy5, Cy5.5 and Cy7. The absorption and emission maxima, respectively, for these fluorophores are: FITC (490 nm; 520 nm), Cy3 (554 nm; 568 nm), Cy3.5 (581 nm; 588 nm), Cy5 (652 nm: 672 nm), Cy5.5 (682 nm; 703 nm) and Cy7 (755 nm; 778 nm), thus allowing their simultaneous detection. The fluorescent labels can be obtained from a variety of commercial sources, including Molecular Probes, Eugene, OR and Research Organics, Cleveland, Ohio. As another alternative, in place of an added label, the bound hemoglobin itself, due to its peroxidase-like properties, can generate a detectable signal. This is accomplished by adding hydrogen peroxide, with or without addition of another substrate (*e.g.* isoluminol).

The cells in the reaction well are lysed by a reagent in the diluent. The total amount of hemoglobin can be obtained by spectroscopic methods, such as measuring in the UV region or the infrared region. The spectroscopic apparatus is known in the art and is incorporated within the reader. In particular, the measurements can be made at 880 nm and at 580 nm. Alternatively the diluent may comprise a reagent that converts the hemoglobin from the lysed cells into methemoglobin, which can be measured at 420 nm. In the reaction well, the antibodies bind to HbA1c. In order to ensure complete reaction, the liquids in the reaction wells can be magnetically stirred, and optionally heated to a higher temperature.

The sample/diluent mixture moves, via the gate mechanism and the second capillary channel into the mixing well. After a specified period of time, the reader closes the gate and prevents further flow out of the reaction well to the mixing well to avoid over-dilution of the sample. The diluent/sample mixture having the proper concentration flows from the mixing well through the third capillary channels towards the capture zones on the reagent strip. The capture zones can comprise antigens or other compounds that

can specifically bind to the antibody-HbA1c complex, any antibody, and the like or combinations thereof. Thus, in one aspect, the first capture zone contains antigens that specifically bind to the antibody-HbA1c complex, while the second capture zone contains antigens that bind to the antibody and the antibody-HbA1c complex. An absorbent pad may be provided that absorbs all the liquid and can help in drawing the liquid from the wells to the reagent strip.

The amount of material in each capture zone can be determined by using the detection systems described above. Calibrators or standards that are run with the assay provide calibration (or standard) curves from which the % HbA1c in the sample is determined using the measured signal. The sum of all the capture zones preferably equals the amount of antibody that was placed in the reaction well, and can provide an internal control to determining the percentage of reaction that has occurred. The concentration of the antibody-HbA1c complex can be determined from the reading of the first capture zone. The % HbA1c in the blood sample can be determined by dividing the concentration of the antibody-HbA1c complex with the total concentration of hemoglobin.

In some embodiments, the test cartridge further comprises an antibody deposited in the reaction well, wherein the antibody is specific for the first analyte. In some embodiments, the antibody is conjugated to a detectable label. In some embodiments, the antibody is conjugated to a colored microparticle.

In some embodiments, the liquid sample is selected from venous whole blood, capillary whole blood, and combinations thereof. In some embodiments, the first analyte is HbA1c. In some embodiments, liquid sample further comprises a reference analyte. In some embodiments, the reaction well is adapted for quantifying the reference analyte. In some embodiments, the reference analyte is hemoglobin. In some embodiments, the diluent is adapted for converting the hemoglobin to methemoglobin prior to quantification. In some embodiments, the reaction well is adapted for quantifying methemoglobin.

In some embodiments, the test cartridge further comprises a reagent strip comprising a first capture zone wherein mixing well and the reagent strip are in fluidic communication via a third capillary channel. In some embodiments, the first capture zone comprises a first antigen that specifically binds to a complex comprising the first analyte and the antibody. In some embodiments, the reagent strip further comprises a second capture zone. In some embodiments, the second capture zone comprises a second antigen that binds to the antibody.

While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A test cartridge comprising a first solid support comprising a diluent well, a reaction well, and a sample port;

wherein the diluent well and the reaction well are in fluidic communication via a first capillary channel;

wherein the sample port and the reaction well are in fluidic communication;

wherein the sample port comprises: a sample channel having an exterior opening adapted to admit a liquid sample comprising a first analyte, and an interior opening connecting the sample channel to the reaction well via a shelf; wherein the sample channel and the shelf are adapted to hold a first amount of the liquid sample; and wherein the shelf prevents the first amount of the liquid sample from entering the reaction well unless a diluent is present in the reaction well.

2. The test cartridge according to claim 1, wherein the first amount of the liquid sample is less than 1 μL.

3. The test cartridge according to claim 1, wherein the first amount of the liquid sample is from about 0.5 μL to about 0.2 μL.

4. The test cartridge according to claim 1, wherein the reaction well comprises a well floor, wherein the shelf comprises a shelf floor, wherein the sample channel comprises a sample channel floor, wherein the well floor is at a first elevation, wherein the shelf floor and the sample channel floor are at a second elevation, wherein the floors are parallel, and wherein the difference between the first elevation and the second elevation is from about 0.015" to about 0.005".

5. The test cartridge according to claim 4, wherein the difference between the first elevation and the second elevation is about 0.010".

6. The test cartridge according to claim 4, wherein the shelf floor has a surface area of about 0.0012 in^2 to 0.0013 in^2

7. The test cartridge according to claim 4, wherein the shelf floor has a surface area of about 0.00125 in^2.

8. The test cartridge according to any one of claims 1 to 7, further comprising a second solid support comprising a hole wherein the first solid support and the second solid support are joined and the hole communicates with the diluent well.

9. The test cartridge according to any one of claims 1 to 8, wherein the first solid support and the second solid support are independently selected from plastic, glass, nylon, metal, and combinations thereof.

10. The test cartridge according to any one of claims 1 to 8, wherein the first solid support and the second solid support are plastic.

11. The test cartridge according to any one of claims 1 to 10, further comprising a stir bar in the reaction well.

12. The test cartridge according to any one of claims 1 to 11, further comprising an antibody deposited in the reaction well, wherein the antibody is specific for the first analyte.

13. The test cartridge according to claim 12, wherein the antibody is conjugated to a detectable label.

14. The test cartridge according to claim 13, wherein the detectable label is a colored microparticle.

15. The test cartridge according to any one of claims 1 to 14, wherein the liquid sample is selected from venous whole blood, capillary whole blood, and combinations thereof.

16. The test cartridge according to claim 15, wherein the first analyte is HbA1c.

17. The test cartridge according to claim 16, wherein the liquid sample further comprises a reference analyte.

18. The test cartridge according to claim 17, wherein the reaction well is adapted for quantifying the reference analyte.

19. The test cartridge according to claim 18, wherein the reference analyte is hemoglobin.

20. The test cartridge according to any one of claims 1 to 19, wherein the first solid support further comprises a mixing well, wherein the reaction well and the mixing well are in fluidic communication via a second capillary channel.

21. The test cartridge according to claim 20, wherein the second capillary channel comprises a gate.

22. The test cartridge according to claim 21, wherein the gate comprises a collapsible silicone tube, wherein the reaction well and the mixing well are in fluidic communication via the second capillary channel and the lumen of the silicone tube.

23. The test cartridge according to any one of claims 1 to 22, further comprising a reagent strip comprising a first capture zone wherein mixing well and the reagent strip are in fluidic communication via a third capillary channel.

24. The test cartridge according to claim 23, wherein the first capture zone comprises a first antigen that specifically binds to a complex comprising the first analyte and the antibody.

25. The test cartridge according to claim 23 or 24, wherein the reagent strip further comprises a second capture zone.

26. The test cartridge according to claim 25, wherein the second capture zone comprises a second antigen that binds to the antibody.

27. A method for determining the concentration of a first analyte in a liquid sample, the method comprising:
    (a) providing a test cartridge according to any one of claims 1 to 10;
    (b) placing a first amount of the sample in the sample port;
    (c) inserting the test cartridge into a reader, whereafter the reader prompts a user to add the diluent to the diluent well;
    (d) adding the diluent to the diluent well, wherein the diluent flows from the diluent well via the first capillary channel directly to the reaction well, wherein the movement of the diluent in the reaction well draws the sample from the sample port over the edge of the

shelf into the reaction well to form a mixture of the diluent and the liquid sample in the reaction well;

wherein the reader is adapted to measure the concentration of the first analyte in the liquid sample.

28. The method according to claim 27, wherein the test cartridge further comprises a stir bar in the reaction well, wherein the reader comprises a magnetic moving device which stirs the mixture of the diluent and the liquid sample in the reaction well by magnetically rotating the stir bar.

29. The method according to claim 27 or 28, wherein the test cartridge further comprises an antibody deposited in the reaction well, wherein the antibody is specific for the first analyte, and wherein the first analyte combines with the antibody in the reaction well.

30. The method according to claim 29, wherein the antibody is conjugated to a detectable label.

31. The method according to claim 29, wherein the detectable label is a colored microparticle.

32. The method according to any one of claims 27 to 31, wherein the liquid sample is selected from venous whole blood, capillary whole blood, and combinations thereof.

33. The method according to claim 32, wherein the first analyte is HbA1c.

34. The method according to claim 32 or 33, wherein step (b) comprises lancing the skin of a subject causing a blood droplet to form and touching the exterior opening of the sample port to the blood droplet to fill the sample channel with the first amount of the sample.

35. The method according to any one of claims 27 to 34, wherein the diluent comprises a lysing agent.

36. The method according to claim 35, wherein the lysing agent is a nonionic surfactant.

37. The method according to claim 35 or 36, wherein the diluent further comprises sodium chloride.

38. The method according to claim 37, wherein the liquid sample further comprises a reference analyte, and wherein the reader quantifies the reference analyte in the reaction well.

39. The method according to claim 38, wherein the reference analyte is hemoglobin.

40. The method according to claim 39, wherein the hemoglobin is converted to methemoglobin prior to quantification.

41. The method according to claim 40, wherein the reader quantifies the methemoglobin photometrically.

42. The method according to claim 41, wherein the reader quantifies the methemoglobin photometrically at 420 nm.

43. The method according to any one of claims 27 to 42, wherein the first solid support further comprises a mixing well, wherein the reaction well and the mixing well are in fluidic communication via a second capillary channel, wherein the mixture of the diluent and the liquid sample flows from the reaction well via a second capillary channel into the mixing well.

44. The method according to claim 43, wherein the second capillary channel comprises a gate, wherein the mixture of the diluent and the liquid sample flows from the reaction well to the mixing well via the gate in the second capillary channel.

45. The method according to claim 44, wherein the gate comprises a silicone tube.

46. The method according to claim 44 or 45, wherein the reader closes the gate after a first time period, wherein closing the gate after the first time period stops the mixture of the diluent and the liquid sample flowing from the reaction well to the mixing well after a first volume of the mixture of the diluent and the liquid sample has entered the mixing well via the second capillary channel.

47. The method according to any one of claims 27 to 46, wherein the test cartridge further comprises a reagent strip comprising a first capture zone wherein the mixing well and the reagent strip are in fluidic communication via a third capillary channel, wherein the mixture of the diluent and the liquid sample flows from the mixing well via the third capillary channel onto the reagent strip.

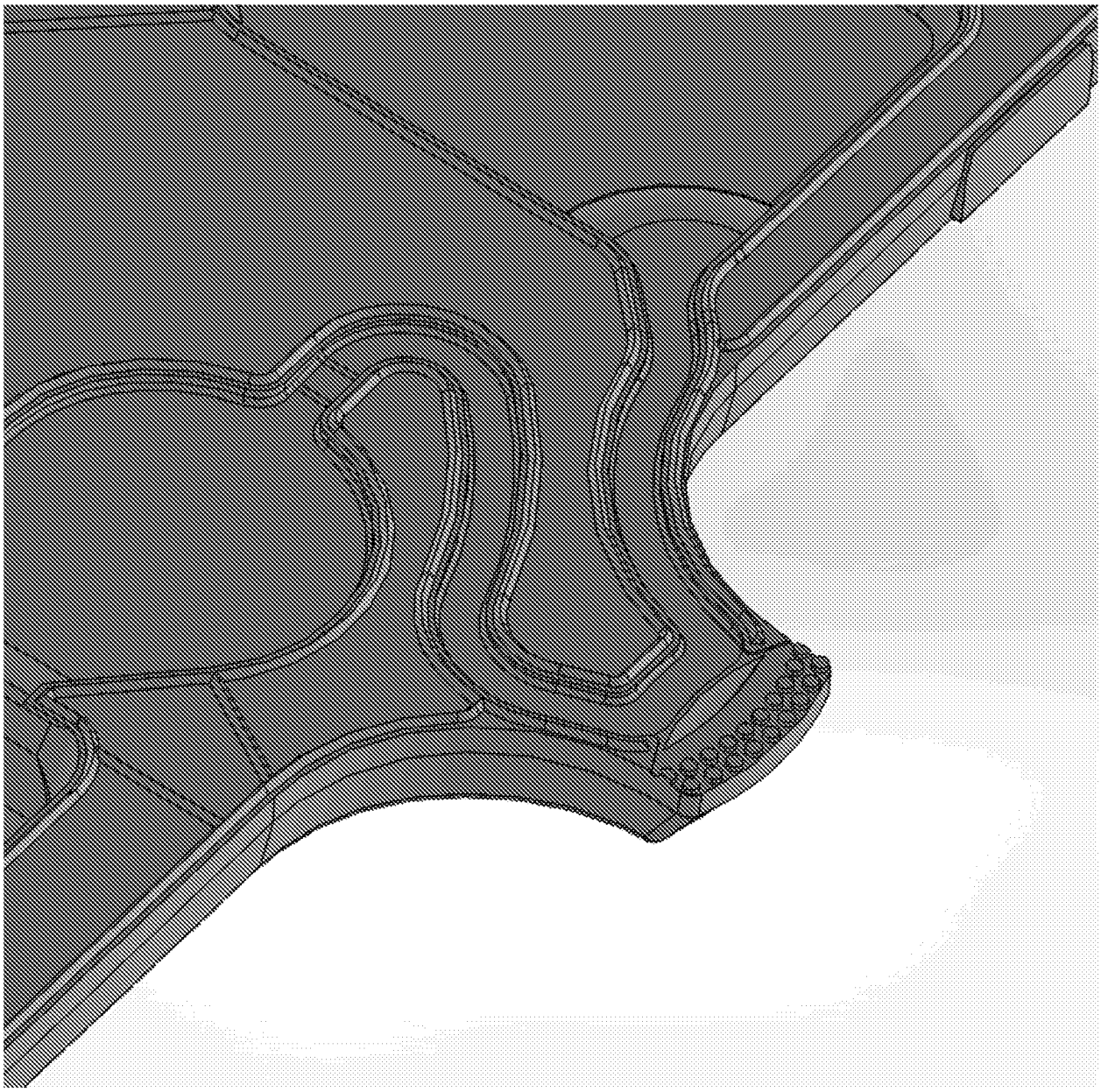
48. The method according to claim 47, wherein the first capture zone comprises a first antigen that specifically binds to a complex comprising the first analyte and the antibody, wherein the reader quantifies the complex comprising the first analyte and the antibody bound to the first antigen.

49. The method according to claim 47 or 48, wherein the reagent strip further comprises a second capture zone.

50. The method according to claim 49, wherein the second capture zone comprises a second antigen that binds to the antibody, wherein the reader quantifies the complex comprising the antibody bound to the second antigen.

Figure 1

Figure 2



3/8

Figure 3

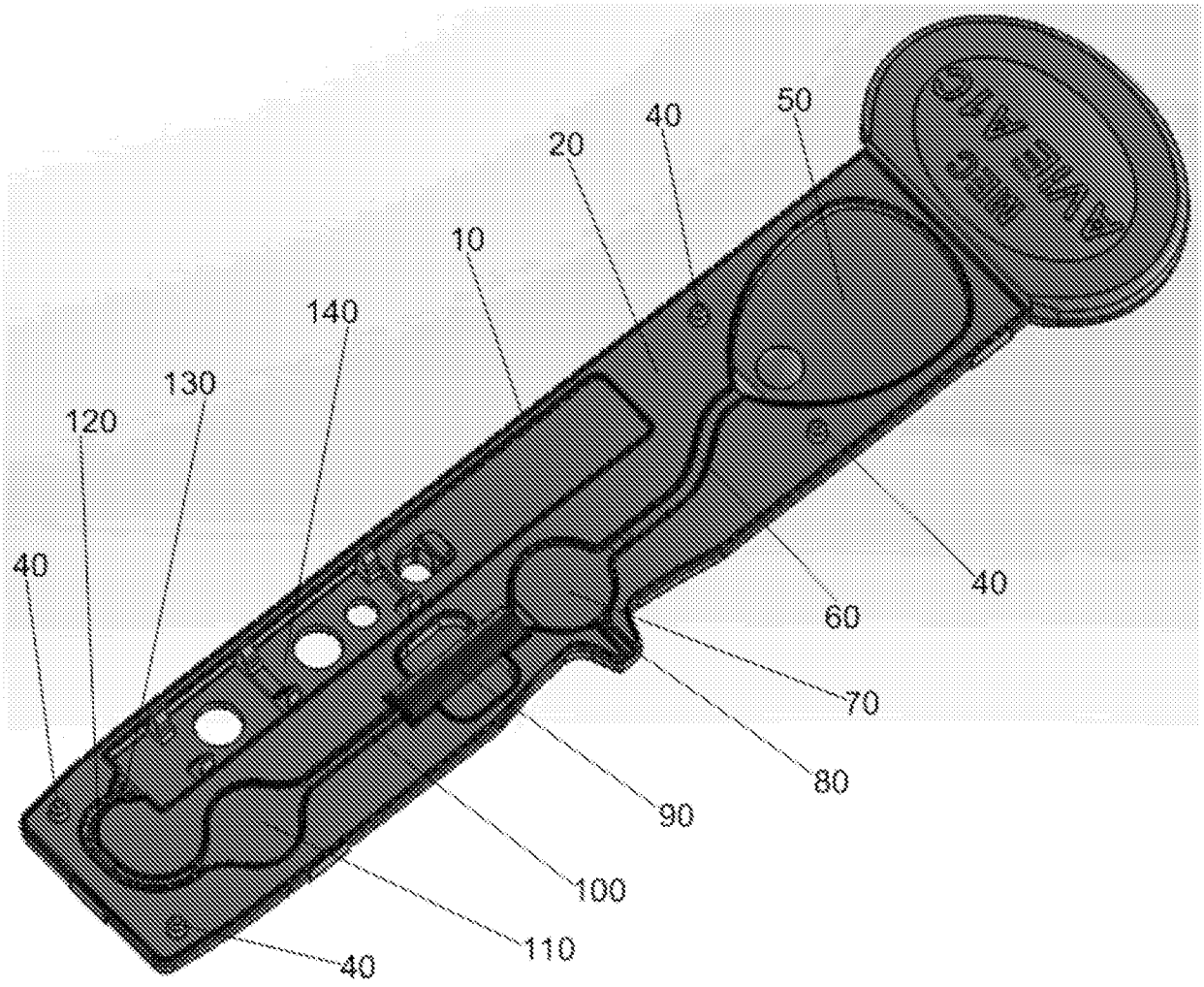


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Figure 4

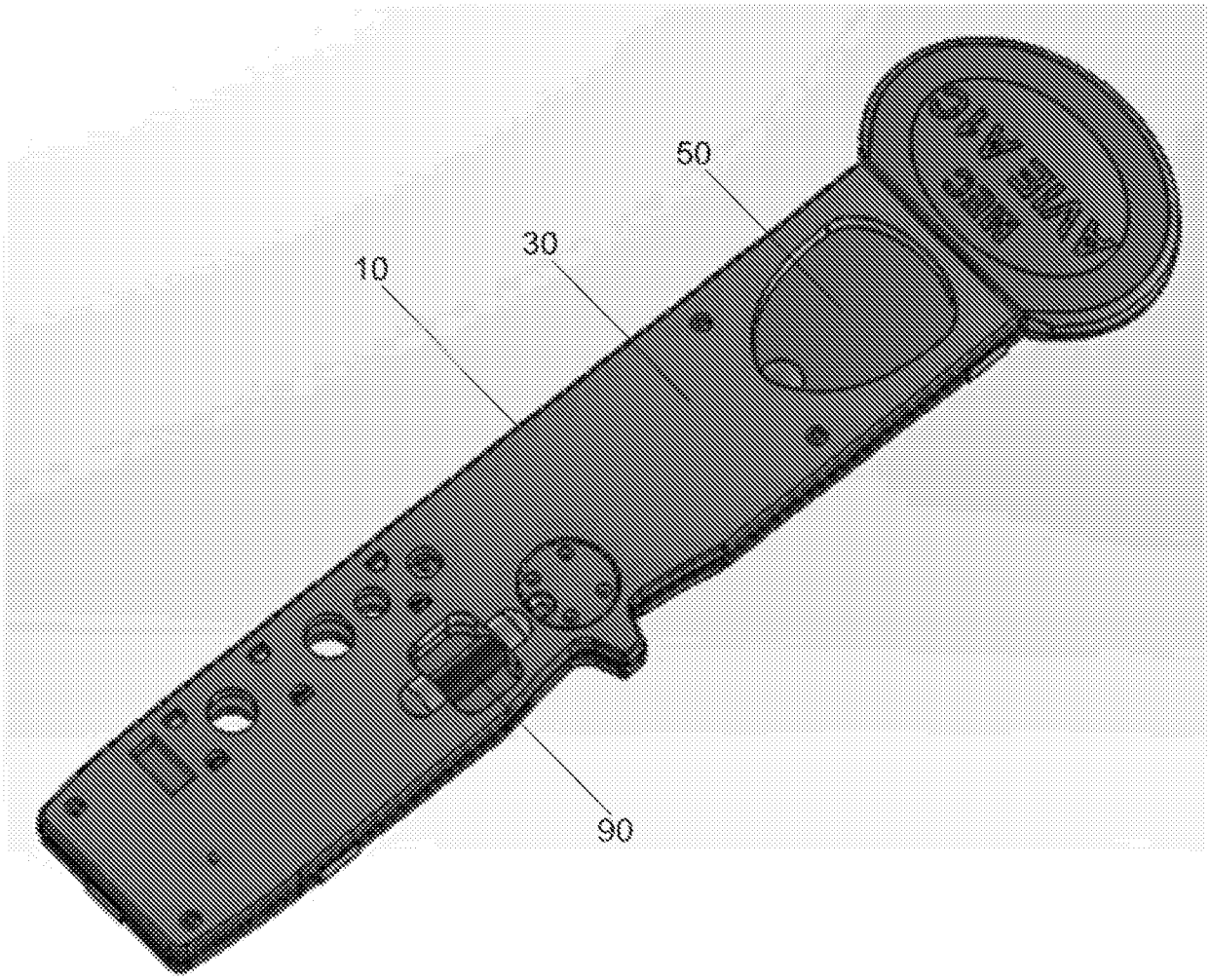


Figure 5

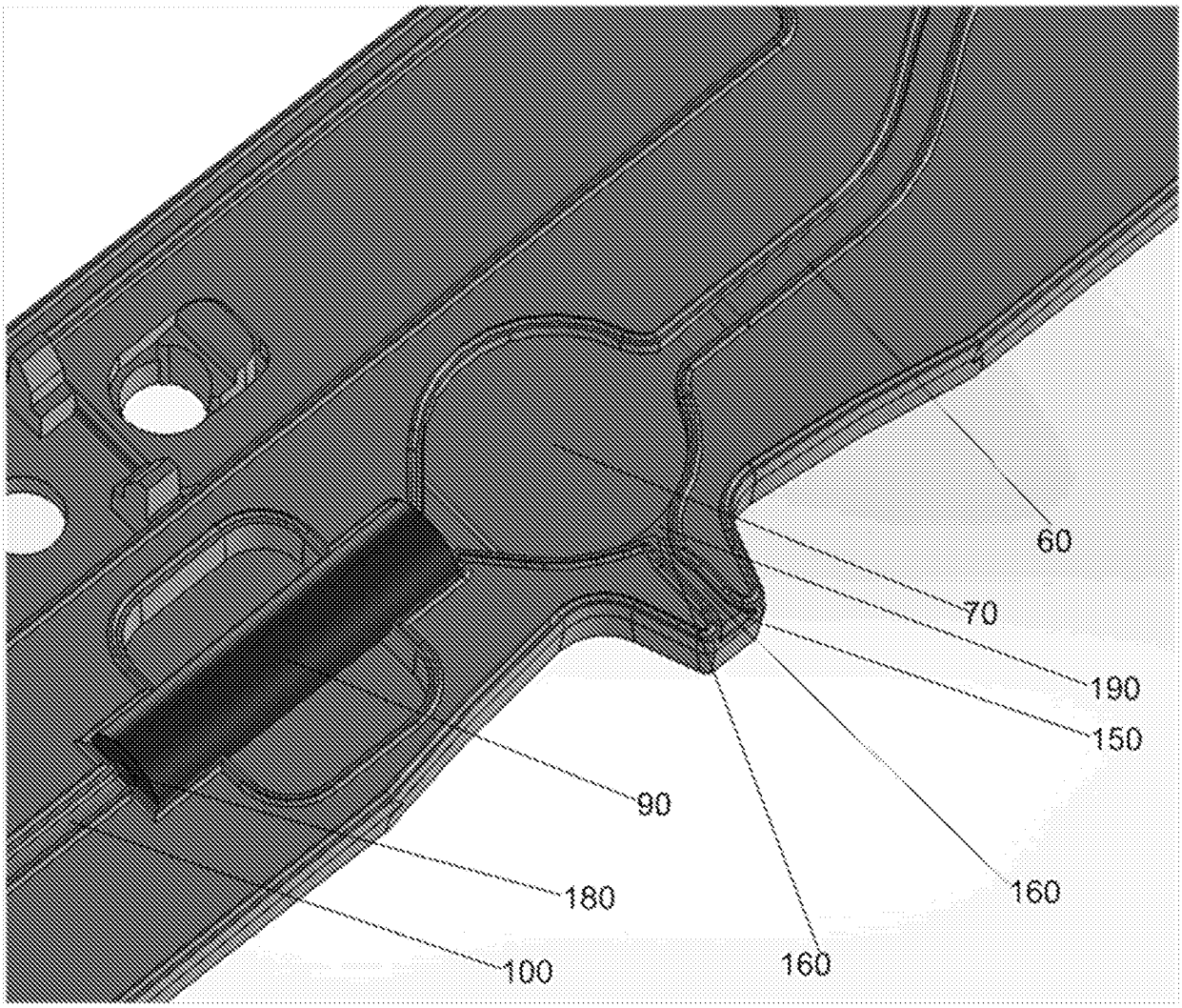


Figure 6

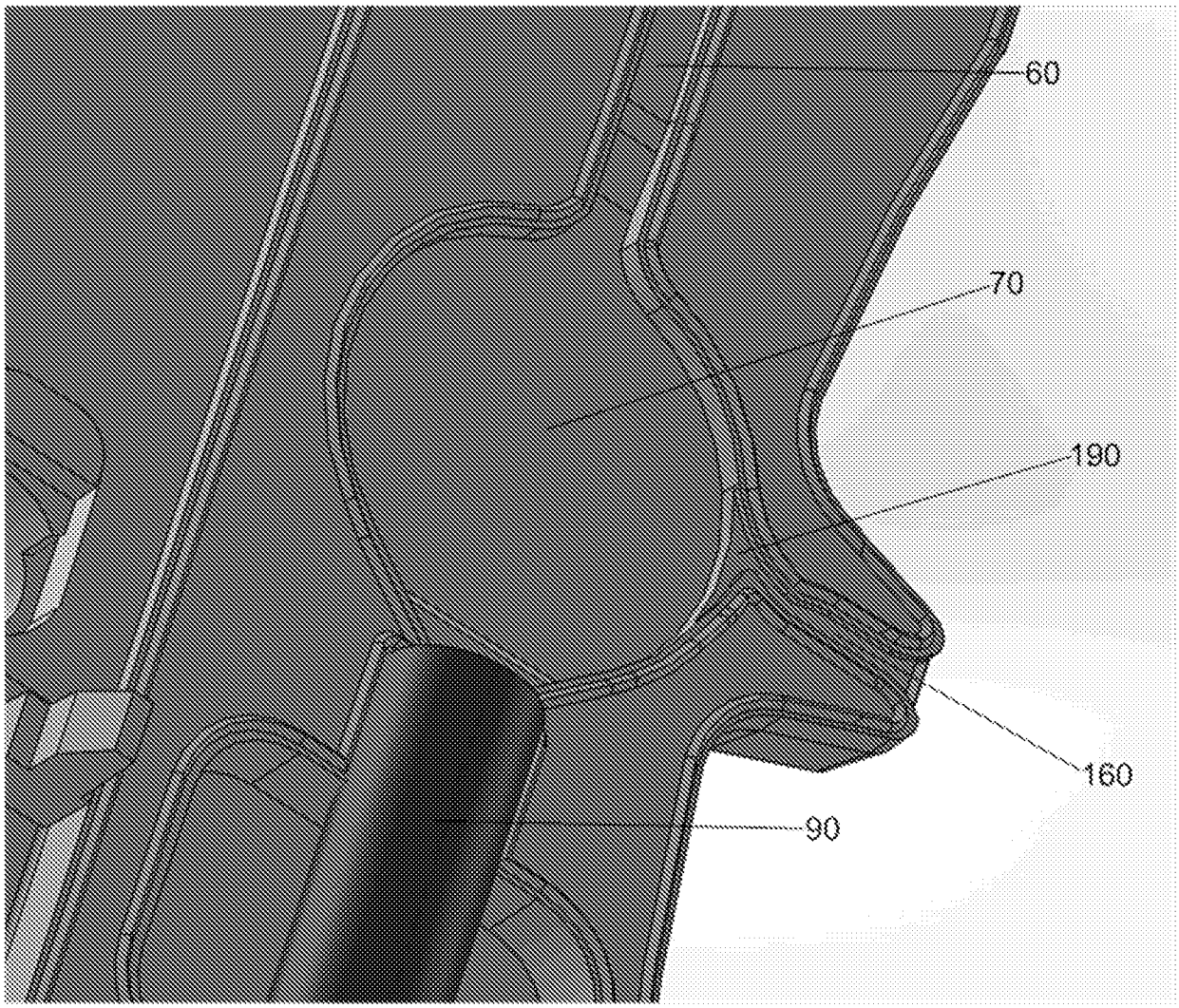


Figure 7

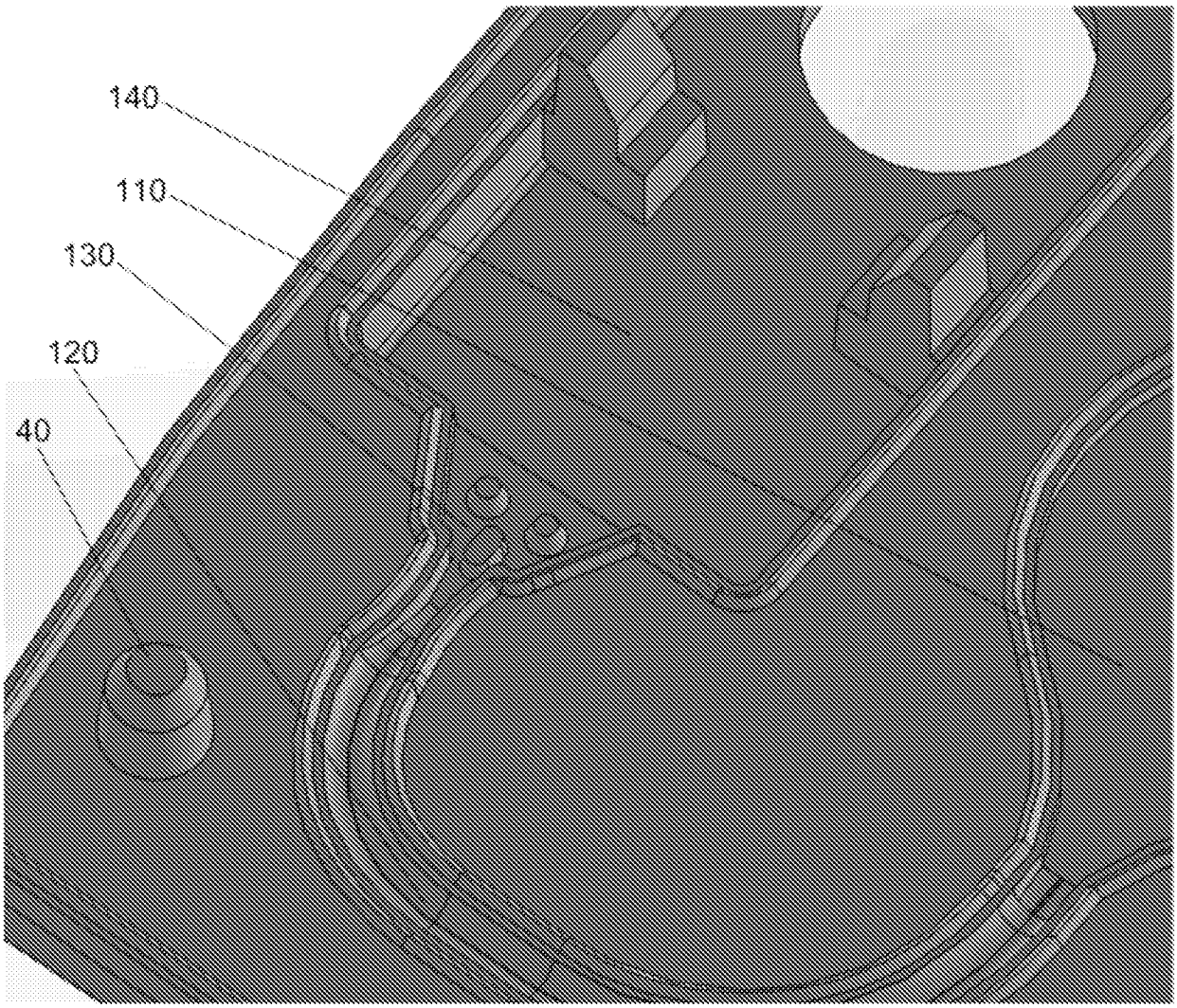
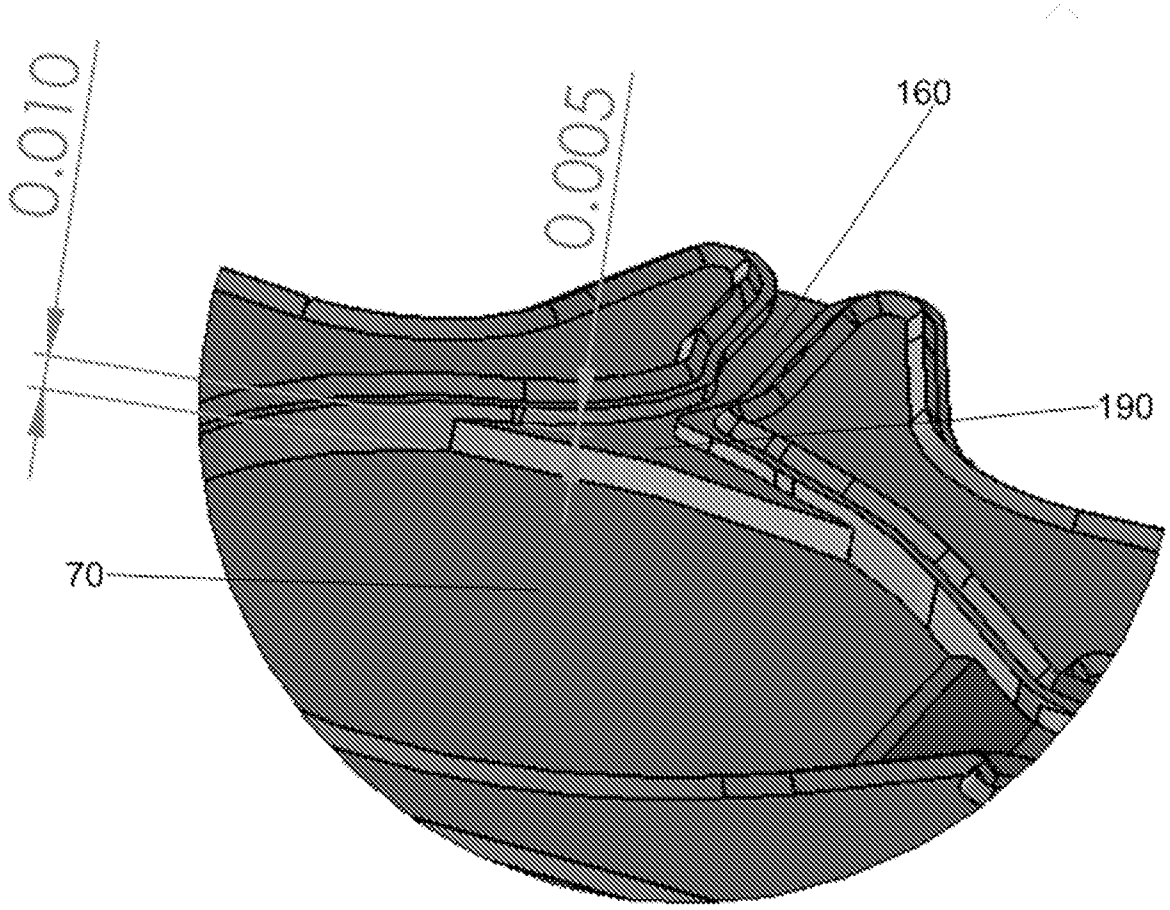


Figure 8



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/069554

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00 G01N33/487
ADD.

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 G01N

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)
EPO-Internal, WPI Data, INSPEC, COMPENDEX, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/009431 A1 (CHO YOONKYOUNG [KR] ET AL) 14 January 2010 (2010-01-14) paragraph [0092]; figure 8B	1-10, 12-20, 23-27, 29-50
Y	US 7 485 118 B2 (BLANKENSTEIN GERT [DE] ET AL) 3 February 2009 (2009-02-03) column 3, line 9 - line 20	1-10, 12-20, 23-27, 29-50
A	US 2014/087359 A1 (NJOROGE SAMUEL [US] ET AL) 27 March 2014 (2014-03-27) paragraph [0186]	11,28

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[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 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

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Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2017	10/04/2017

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