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| (54) Title: ANALYTICAL TEST DEVICE AND METHOD OF USE (57) Abstract The present invention provides an analytical test device for conducting assays of biological fluids. Methods for carrying out the assays with the disclosed analytical test devices are also provided. | | |

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ANALYTICAL TEST DEVICE AND METHOD OF USE**FIELD OF THE INVENTION**

This invention relates to a novel analytical test device for analysis of biological fluids such as urine. The invention further relates to methods for detecting analytes in fluids using the test device.

BACKGROUND INFORMATION

The sampling and testing of biological fluids such as urine for the presence of analytes provide important information regarding various health-related matters, including pregnancy and conception.

In recent years, test devices have undergone nearly continuous refinement in an effort to simplify and speed the process of detecting selected ligand in fluids. As a result of this work, current test devices use an immunoassay for determining pregnancy or conception. In these devices a reagent, such as an antibody, specifically reacts with an analyte to form a complex, which can usually be detected by the unaided eye.

Current pregnancy test devices assay for hormones associated with pregnancy, such as, for example, chorionic gonadotrophin (hereinafter "hCG") since the presence of hCG in urine is usually an indicator that a woman is pregnant. Such test devices obtain qualitative results indicating either the presence or absence of hCG. Typically, a pregnancy immunoassay contains an antibody directed against hCG. The reaction complex can then be viewed by the user.

Conception test devices also assay for hormones associated with the ovarian cycle, such as, for example, luteinizing hormone (hereinafter "LH"). LH is present normally in urine but its concentration increases
5 markedly during ovulation, the time at which a woman is most likely to conceive. Thus, the probability that a woman can conceive a child varies directly with LH concentration. Such test devices obtain
10 semi-quantitative results regarding the relative concentration of LH in the urine. Typically, a conception immunoassay contains an antibody directed against LH and a separate detection antibody.

In known devices, the fluid to be tested wicks
15 up through an absorbent membrane that is in fluid flow contact with the reagents that detect an analyte in a fluid. A major problem with this type of device is that as the fluid progresses through the membrane, the front edge of the fluid is uneven. The uneven fluid front will
20 cause the results to be smeared, produce false negatives and make the results difficult to detect. These artifacts are called "leading edge effects." An uneven fluid front can arise for a variety of reasons, for example, because the sample is not uniformly applied to
25 the absorbent membrane.

Therefore, a need exists for a device that does not produce an uneven fluid front and associated leading edge effects. The present invention provides a device
30 that eliminates the uneven fluid front problem and related methods of using such a device.

SUMMARY OF THE INVENTION

The present invention provides an analytical test device for analyzing biological fluids, for example, urine. The device has a top with one or more display ports, and optionally, a sample aperture, an entry port and a vertical bar. The device also has a bottom with an upper plane, a slope and a contacting surface attached to the slope at a descending angle, optionally, a lower plane between the slope and contacting surface and, optionally, an entry port. The device further has a strip made up of an absorbent membrane, a reaction membrane with one or more reagents that form a reaction complex with an analyte in a fluid sample and, optionally, a collecting pad.

The absorbent membrane, the reaction membrane and the collecting pad, when present, are in fluid flow contact with one other. The strip is placed on the bottom such that the reaction membrane is visible through the display port and the absorbent membrane is on the slope and on the lower plane when present. The fluid sample contacts the absorbent membrane by first contacting the contacting surface when the top and bottom are assembled.

In one embodiment, the analytical test device has the contacting surface extending outward from the bottom. Here the absorbent membrane is in fluid flow contact with the contacting surface through the entry port. The top also has a vertical bar that deflects the absorbent membrane to follow the contour of the slope.

The present invention also provides methods for detecting an analyte in a fluid sample, comprising contacting the fluid sample to the contacting surface of the analytical test device and detecting the analyte by
5 observing the reaction complex through the display port. In one method the reaction complex is visible.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Figure 1 is a perspective view of an analytical test device containing a contacting surface extending outward from the bottom.

Figure 2 is an exploded view showing the
15 components of the analytical test device of Figure 1.

Figure 3 is an enlarged sectional view along line 3-3 of the analytical test device of Figure 1.

20 Figure 4 is a perspective view of an embodiment of the analytical test device with a contacting surface extending outward from the bottom.

Figure 5 is an exploded view showing the
25 components of the analytical test device of Figure 4.

Figure 6 is an enlarged sectional view along line 6-6 of the analytical test device of Figure 4.

30 Figure 7 is a perspective view of another embodiment of the analytical test device with a contacting surface extending outward from the bottom and a cap.

Figure 8 is an exploded view showing the components of the analytical test device of Figure 7.

Figure 9 is an enlarged sectional view along
5 line 9-9 of the analytical test device of Figure 7.

Figure 10 is a perspective view of another embodiment of the analytical test device with a sample aperture on the top.

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Figure 11 is an exploded view showing the components of the analytical test device of Figure 10.

Figure 12 is an enlarged sectional view along
15 line 12-12 of the analytical test device of Figure 10.

Figure 13 is a perspective view of an embodiment of the analytical test device with a sample aperture on the top.

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Figure 14 is an exploded view showing the components of the analytical test device of Figure 13.

Figure 15 is an enlarged sectional view along
25 line 15-15 of the analytical test device of Figure 13.

Figure 16 is a perspective view of the absorbent and reaction membranes of the analytical test device employing a sandwich assay system showing a
30 positive assay result.

Figure 17 is a perspective view of the absorbent and reaction membranes of the analytical test device employing a sandwich assay system showing a
35 negative assay result.

Figure 18 is a perspective view of the absorbent and reaction membranes of the analytical test device employing a sandwich assay system showing an inconclusive assay result.

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Figure 19 is a perspective view of the absorbent and reaction membranes of an embodiment of the analytical test device employing a competitive assay system showing a positive assay result.

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Figure 20 is a perspective view of the absorbent and reaction membranes of an embodiment of the analytical test device employing a competitive assay system showing a negative assay result.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to an analytical test device that eliminates the uneven fluid front and leading edge effects associated with prior devices. The device ensures that fluid sample comes into fluid flow contact with the membrane that contains the reagents to detect the analyte, evenly and at the same height across the membrane, thereby producing an even fluid front.

25

By laying the device flat, the user can simply apply fluid sample to the contacting surface, allowing the fluid sample to run down into the device where the fluid sample gradually builds up until it comes into fluid flow contact with the membrane. Thus, an even fluid front will be formed on the membrane because the fluid sample level rises gradually and contacts the membrane evenly and at the same height across the membrane. Optionally, the user can tilt the device so

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that when fluid sample is applied to the contacting surface, the fluid sample remains on the contacting surface. After the user decides that a sufficient amount of fluid sample is collected, the device is laid flat so
5 that the fluid sample runs down into the device and comes into fluid flow contact with the membrane evenly and at the same height across the membrane, thereby producing an even fluid front.

10 A further advantage of the analytical test device is that the user can control when the assay starts. Thus, if desired, the user can add more than one fluid sample to the contacting surface before laying the device flat. This feature is especially important where
15 the user only wishes to know whether an analyte is present in more than one fluid sample. In prior devices, once the fluid sample is added, the assay proceeds and the reagents are depleted before a second fluid sample can be added. Thus, the user of those devices is not
20 given the time to test additional samples.

The analytical test device of the present invention includes a top and a bottom. The top has a display port that allows the user to see the results.
25 Preferably, the top will have a first display port and a second display port that are in close proximity for easy comparison of results. Optionally, the top will also have a sample aperture. The bottom has an entry port for the fluid sample. Inside the device is a strip formed by
30 an absorbent membrane, a reaction membrane and, optionally, a collecting pad. The absorbent membrane and the reaction membrane contain the reagents to detect an analyte in a fluid sample. When the top is placed on the bottom in the assembled device, the absorbent membrane
35 will be positioned on the bottom toward the entry port

and the reaction membrane will be positioned on the bottom underneath the display port.

The bottom is divided into an upper plane and a slope. As used herein, the term "slope" refers to a surface or plane of the bottom of the device that is at such an angle in relation to the other plane or planes of the bottom that it slows the rate of fluid sample wicking through the absorbent membrane. Thus, the slope prevents an excess amount of fluid sample migrating through the absorbent membrane, which would otherwise compromise the assay.

Also, the bottom has a contacting surface attached to the slope at a descending angle. If the top has the optional sample aperture, the contacting surface is inside the assembled top and bottom below the sample aperture. However, if the top does not have the sample aperture, the contacting surface extends outward from the bottom of the device and is indirectly connected to the slope by a lower plane of the bottom. When the lower plane is present, the absorbent membrane will extend along the lower plane and along the slope. Preferably, the contacting surface extending outward from the bottom will be inside a protruding sample well.

Also, the device preferably has a vertical bar extending downward from the top. As used herein, the term "vertical bar" refers to a part of the top of the device extending downward from the top at a point just in front of the slope that holds or deflects the absorbent membrane to follow the contour of the slope. Optionally, the vertical bar can extend downward far enough to only allow fluid sample to proceed further into the device by wicking through the absorbent membrane.

An amount of fluid sample, such as urine, is applied to the contacting surface through the sample aperture or through the protruding sample well. As described above, if desired, more than one fluid sample
5 can be applied or contacted with the contacting surface by tilting the device before adding fluid sample. Tilting the device, therefore, can control the start of the assay.

10 The fluid sample then proceeds by capillary action (i.e., wicking) through the absorbent membrane. Bound evenly and at the same height extending from side to side of the absorbent membrane at a downstream point spatially separated from where the fluid sample first
15 contacts the absorbent membrane and downstream from the vertical bar, when present, is a diffusively bound labeled reagent. The spatial separation ensures that the fluid sample wicks up through the absorbent membrane to contact the diffusively bound labeled reagent. Thus, the
20 diffusively bound labeled reagent stays within the absorbent membrane and does not migrate into the fluid sample, which would otherwise destroy performance of the assay.

25 If analyte is present in the fluid sample it will complex with the diffusively bound labeled reagent. The fluid sample containing either diffusively bound labeled reagent alone or a diffusively bound labeled reagent/analyte complex (i.e., "first complex") will move
30 by wicking up to a "test region" in the reaction membrane. Bound evenly and at the same height across the reaction membrane in the test region is a non-diffusively bound reagent capable of complexing the first complex. The non-diffusively bound reagent may be non-diffusively
35 bound to the reaction membrane by means known in the art,

including covalent bonding or attachment to an insoluble protein-coated surface (see, for example, U.S. Patent No. 4,200,690, which is incorporated herein by reference). Preferably, the non-diffusively bound reagent will be
5 bound in a bar shape extending from side to side of the reaction membrane in a manner that is similar to the attachment of the diffusively bound labeled reagent to the absorbent membrane.

10 If a first complex is present in the fluid sample, the analyte portion of the first complex will bind to the non-diffusively bound reagent creating a detectable signal, preferably a visible straight line or bar extending from side to side of the reaction membrane
15 at the first display port. As a control, the fluid sample will continue its migration along the reaction membrane past the first display port to a "control region" portion of the reaction membrane.

20 The control region is so called because bound evenly and at the same height extending from side to side of the reaction membrane is a non-diffusively bound control reagent. The diffusively bound labeled reagent, complexed with analyte or not, will bind to the
25 non-diffusively bound control reagent present in the control region. This binding will create a detectable signal, preferably a straight line or bar extending from side to side of the reaction membrane. For ease of comparison, the shape and orientation of the
30 non-diffusively bound control reagent should be similar to the shape and orientation of the non-diffusively bound reagent. In addition, the non-diffusively bound control reagent may be non-diffusively bound in the manner similar to that of the non-diffusively bound reagent,
35 described above. Thus, when the top is aligned with

bottom in the assembled device the diffusively bound
labeled reagent will be bound on the absorbent membrane
near the contacting surface, the non-diffusively bound
reagent will be bound beneath first display port and the
5 non-diffusively bound control reagent will be bound
beneath second display port.

The results can then be interpreted. If a
signal appears at the first display port and a signal
10 appears at the second display port, the test is positive
for the presence of analyte. If there is no detectable
signal at the first display port compared to the signal
at the second display port, the test is negative for the
presence of analyte. If there is no signal at the second
15 display port the user is alerted that the test results
are compromised and that the test should be repeated.

Alternatively, the analytical test device can
employ a competitive assay system. A diffusively bound
20 labeled reagent capable of competing with the analyte for
a non-diffusively bound reagent is applied evenly and at
the same height extending from side to side of the
absorbent membrane. It is positioned at a downstream
point spatially separated from the point where the
25 absorbent membrane comes into contact with the fluid
sample and downstream from the vertical bar, when
present. The diffusively bound labeled reagent here does
not complex with the analyte. A non-diffusively bound
reagent that can bind either the analyte or the
30 diffusively bound reagent is applied evenly and at the
same height extending from side to side of the reaction
membrane at a point beneath the display port. In this
embodiment of the analytical test device, the top need
only have one display port, as no comparison is required.
35 However, if desired, a control region may be incorporated

to demonstrate the integrity of the reagents and device. In this embodiment, the presence of a signal, such as a solid straight line or bar across the reaction membrane in the display port is a validation of the assay and
5 indicates that no analyte is present in the fluid sample. No signal in the display port indicates the presence of analyte in the fluid sample.

The analytical test device allows the assay to
10 be performed in a single step. The user only has to apply the fluid sample to the contacting surface, and then observe the results.

The analytical test device is assembled by
15 placing the absorbent membrane and the reaction membrane on the bottom and then the top is then placed on the bottom to ensure a tight fit. One skilled in the art would understand that any suitable means to ensure a tight fit between the top and bottom can be used. For
20 example, the top can be fitted on the bottom with snaps or glue.

Also, to ensure fluid retention, the analytical test device of the present invention is preferably made
25 of a nonporous plastic, such as the commercially available plastic "ABS" supplied by the Monsanto Company of St. Louis, MO. In an embodiment of the device, once a cap is placed over the contacting surface extending outward from the bottom, the device becomes substantially
30 fluid-tight and evaporation of the fluid sample is minimized. This configuration also has the advantage of avoiding spillage and the need for any further contact between the sample and user.

Several features of the analytical test device assist in avoiding compromised results. First, the simultaneous migration of an excessive amount of fluid sample is limited by requiring the fluid sample to ascend through the absorbent membrane by capillary action, rather than applying the fluid sample to the absorbent membrane directly. Second, the spatial separation of the diffusively bound labeled reagent on the absorbent membrane from the point at which the fluid sample first contacts the absorbent membrane, ensures that the assay is not compromised by reagent leaking out into the fluid sample. Third, the impact of leading edge effects caused by depletion of bound reagents or labels, are minimized by physically separating the test and control regions, by applying the fluid sample evenly and at the same height across the absorbent membrane and by using the presence of a reaction, rather than the appearance of a given shape, (such as a "+" or a "-"), to signify a positive or negative result. In addition, the entire process is simplified by having the label bound to the absorbent membrane placed within the device, thus avoiding any need for the user to add a reagent or mix it with fluid sample.

As described above, the present invention is also ideally suited to the application of more than one fluid sample to a single analytical test device. In prior devices, such as the one described in May et al., U.S. Patent No. 5,602,040, issued February 11, 1997, the device (hereinafter the '040 device) is typically held in a stream of urine. The protruding sample aperture in the '040 device is in contact with the dry porous carrier inside the hollow casing of the device. Thus, the test proceeds immediately upon application of the sample.

Therefore, such a device is suited for the testing of only one sample at a time.

Moreover, since the user of the '040 device typically holds the protruding bibulous member in the urine stream, the sample would likely be unevenly applied to the membrane. Thus, the '040 device suffers from leading edge effects created by an uneven solvent front along the membrane. Accordingly, the reagents and bound labels could be depleted and the results could be misleading. In contrast, the user of the present analytical test device is assured that the solvent front formed by the fluid sample will be uniform since the fluid sample contacts the absorbent membrane evenly and at the same height across the absorbent membrane. Therefore, the present analytical test device provides more reliable results.

As used herein, the terms "ligand" and "control reagents" are used interchangeably, and refer to a molecule to which another molecule will bind. A ligand or control reagent can be human, animal or plant in origin. For purposes of this invention, these may include LH, hCG or other naturally occurring biological ligands in serum, plasma, urine or other fluids and will preferably include analytes. Specifically, it will be appreciated by those skilled in the art that the ligand or control reagent or analyte may be a protein, peptide, amino acid, nucleic acid, sugar, hormone, steroid, vitamin, toxin in the sample fluid, pathogenic microorganism and metabolites from analogs of such analytes, or antibodies to these substances. They may also include hormones, haptens, immunoglobulin, polynucleotides, drugs and infectious disease agents

(bacterial or viral) such as *Streptococcus*, *Neisseria*, *Chlamydia*, *Gonorrhea* and HIV.

As used herein, the term "reagent" refers to
5 receptor molecules which bind to a ligand. Reagents may,
in this context, include any naturally occurring or
synthetic biological receptor and will preferably include
antibodies. The antibodies may be polyclonal or
monoclonal. For simplicity, the terms antibody and
10 analyte will be used interchangeably with reagent and
ligand, respectively, throughout this disclosure. It
will be appreciated by those skilled in the art, however,
that the invention is not limited to use with analytes
and antibodies.

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Ligand-reagent pairs useful in the present
invention include specific binding pairs such as antigens
and antibodies, or fragments of antibodies, both
polyclonal and monoclonal, lectins and carbohydrates,
20 hormones and hormone receptors, enzymes and enzyme
substrates, biotin and avidin, vitamins and vitamin
binding proteins, complementary polynucleotide sequences,
drugs and receptors, enzymes and inhibitors, apoproteins
and cofactors, growth factors and receptors, and the
25 like. Biotin and avidin derivatives may also be used,
including biotin analogs/avidin, biotin/streptavidin, and
biotin analogs/streptavidin. Members of the complex may
be "engineered," that is, made by synthetic means. Such
techniques are well known in the art, and include
30 techniques for chimeric and humanized antibodies and
fragments thereof, synthetic peptides, and synthetic RNA
and DNA oligonucleotides.

Any known reagent can be used in any known
35 format such as, for example, sandwich and competitive

binding formats, to specifically detect an analyte in a fluid sample. Examples of such reagents are those disclosed in: H. J. Friesen, United States Patent No. 4,861,711, issued August 29, 1989; J. Bunting, United States Patent No. 4,271,140, issued June 2, 1981; May et al., United States Patent No. 5,622,871, issued April 22, 1997; May et al., United States Patent No. 5,656,503, issued August 12, 1997; May et al., United States Patent No. 5,602,040, issued February 11, 1997; and R. Rosenstein, United States Patent No. 5,591,645, issued January 7, 1997 (each of which is incorporated by reference herein). Such reagents can form a detectable complex with such ligands as listed above.

Preferred reagents include antibodies to a hormone or infectious disease agent. Preferred antibodies include anti-hCG antibodies and anti-human LH antibodies, especially of the IgG class, and even more so murine monoclonal antibodies and especially those that have been affinity purified. One skilled in the art would recognize, however, that polyclonal antibodies could also be employed in the present invention.

By the term "non-diffusively bound" is meant covalent or non-covalent attachment to the membrane such that the advancing fluid sample does not cause movement of the non-diffusively bound reagent or non-diffusively bound control reagent from the place it is applied on the reaction membrane. Conversely, by the term "diffusively bound" is meant placement such that the advancing fluid sample does cause movement of the diffusively bound labeled reagent from the place it is applied on the absorbent membrane.

As used herein, the term "fluid sample" refers to a material suspected of containing an analyte. The fluid sample can be used directly as obtained, for example, from any biological source. The fluid sample
5 can also be obtained from an organism and the relevant portion extracted or dissolved into a solution. For example, the fluid sample can be a physiological fluid, such as, for example, saliva, ocular lens fluid, cerebral spinal fluid, sweat, blood, pus, mucus, serum, urine,
10 milk, ascites fluid, synovial fluid, peritoneal fluid, amniotic fluid, and the like. In addition, the fluid sample fluid can be extracted from throat swabs, feces, or from other biological samples.

15 As used herein, the term "label" refers to a molecule that directly or indirectly mediates the production of a signal (such as a color change) which is used in assay procedures to indicate the presence or absence of analyte in a fluid sample. Labels may include
20 enzymes, fluorescent molecules and will preferably include metal sols. Labels include colored latex spheres and colloidal metals. Labels include those disclosed by D. Yost et al., United States Patent No. 4,954,452, issued September 4, 1990; J. Leuvering, United States
25 Patent No. 4,313,734, issued February 2, 1982; P. Tarcha et al., United States Patent No. 5,252,459, issued October 12, 1993; T. Gribnau et al., United States Patent No. 4,373,932, issued February 15, 1983; and R. Campbell, United States Patent No. 4,703,013, issued October 27,
30 1987 (each of which is incorporated by reference herein).

Alternatively, the label can be colored latex particles (see Campbell, U.S. Patent No. 4,703,017, issued October 27, 1987, which is incorporated by
35 reference herein) or can be an enzyme that has reacted

with a colorless substrate to give a colored product and is encapsulated, for example, in a liposome (see E. Soini, United States Patent No. 5,518,883, issued May 21, 1996, which is incorporated herein by reference). The label may also be an inducible property of the particles, such as colorable latex particles (see Gribnau et al., U.S. Patent No. 4,373,932, issued February 15, 1983, and de Jaeger et al., U.S. Patent No. 4,837,168, issued June 6, 1989, both of which are incorporated by reference herein).

Alternatively, the label can be fluorescent molecules, such as the rhodamine, fluorescein, or umbelliferone series, employed by themselves or with a quencher molecule (see, for example, Ullman et al., U.S. Patent No. 3,996,345, issued December 7, 1976 and Tom et al., U.S. Patent No. 4,366,241, issued December 28, 1982, both of which are incorporated herein by reference). Chemiluminescent molecules, such as luminol, luciferin, lucigenin, or oxalyl chloride can be used as a signal means (see, for example, Maier, U.S. Patent No. 4,104,029, issued August 1, 1978, which is incorporated herein by reference). Finally, enzymatic systems that react with a colorless substrate to give a colored product, such as horseradish peroxidase, alkaline phosphatase, indoxyl phosphate and aminoethylcarbazole may be used as labels.

Analytical test devices using labeled reagents are also provided in K. May, United States Patent No. 5,656,503, issued August 12, 1997. However, in those devices, the mobility of the labeled reagent is facilitated by using sugar in an amount effective to reduce interaction. Sugar is not used in the present invention to assist mobility of the labeled reagent.

Diffusively bound complexed antibodies can be impregnated into the immunoassay within the absorbent membrane. The diffusively bound complexed antibodies are located upstream from a zone on the reaction membrane of the immunoassay that contains immobilized antibodies. The diffusively bound label complexed antibodies bind to the analyte and are carried to the zone containing the immobilized antibodies where a sandwich antibody-hormone complex is formed and detected. However, if desired, both the diffusively bound and non-diffusively bound antibodies may be disposed on a single membrane.

In one embodiment, the diffusively bound labeled reagent will be labeled according to means known in the art. For purposes of producing a clearly visible reaction, labels of metal sols are preferred, with labels of colloidal gold or selenium being most preferred. An example of a suitable product is colloidal gold available from Janssen Life Sciences Products. These colloidal metals will produce colored reactions without addition of further reagents. However, fluorescent compounds (such as fluorescein and phycobiliprotein) and enzymes (such as those identified in U.S. Patent No. 4,275,149, which is incorporated by reference herein), may also be used. To maximize contact of analyte with labeled reagent, the latter should be bound to the membrane across its face; i.e., from one side to the other (see, bar 354 shown in phantom on Figures 16, 17 and 18).

As used herein, the term "metal label" includes labels of metal sols; i.e., metal or metal compounds such as metal oxides, metal hydroxides, metal salts or polymer nuclei coated with a metal or metal compound. These metal labels may include dry forms of any of the above-named metals or metal compounds, and will

preferably include colloidal gold in dry form. For example, the metal label can be composed of a metal sol, a selenium sol or a carbon sol (see, for example, Leuversing et al., U.S. Patent No. 4,313,734, issued February 2, 1982; Moremans et al., U.S. Patent No. 4,775,636, issued October 4, 1988; Yost et al., U.S. Patent No. 4,954,452, issued September 4, 1990; and Kang et al., U.S. Patent No. 5,559,041, issued September 24, 1996, each of which is incorporated by reference herein).

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Depending on the context in which it is used, "reaction complex" or "complex" shall mean a complex of analyte and diffusively bound labeled reagent complex first produced in the assay ("first complex"), a complex of first complex and the non-diffusively bound reagent produced second in the assay ("second complex"), or a complex of the second complex and the non-diffusively bound control reagent produced third in the assay ("third complex"). The complex can be made up of a ligand-reagent pair that has spatial and/or polar features which permit it to bind specifically to each other. By "first member" of the complex is meant the member bound, covalently or non-covalently, at least to a sub-population of the particles. The "second member" of a complex refers to the corresponding binding member of the pair non-diffusively bound in the control region.

As used herein, the term "sample aperture" refers to an opening on the top to which the fluid sample can be added by any means, such as for example, dropping or by pipette. Preferably, the sample aperture will be located on the top at a point over the contacting surface.

30

The term "entry port" refers to an aperture on the embodiment of the device containing the contacting surface extending outward from the bottom. The entry port allows fluid sample to enter the assembled top and
5 bottom from the contacting surface.

The term "display port" refers to any means whereby visual access to the reaction membrane can be gained. In one embodiment, a display port will be an
10 aperture in the top positioned over the reaction membrane. In another embodiment there will be two display ports. A display port also encompasses all or any part of the top made of a clear or transparent material so that the reaction membrane can be seen.
15 Thus, if the entire top is made of clear or transparent material, then the entire top is the display port. In addition there may be a clear or transparent membrane placed between the reaction membrane and the aperture of the display port in order to prevent evaporation of fluid
20 sample, to prevent fluid sample or other fluid from entering the display port, or to prevent contamination of the reaction membrane by touching, all of which can affect the performance of the assay.

25 The term "contacting surface" refers to a surface of the analytical test device that first contacts the fluid sample when the fluid sample is added. It is contemplated that the contacting surface could encompass, for example, a plane, a curved or bent plane, a groove or
30 a channel. The contacting surface will be disposed at a descending angle to the slope. In the embodiment of the analytical test device containing the contacting surface extending outward from the bottom, the contacting surface will be attached at a descending angle to the slope and
35 indirectly to the slope through the optional lower plane

of the bottom. Here, the lower plane and upper plane are preferably parallel to one another and the contacting surface is not parallel to either the lower plane or the upper plane. In the embodiment of the analytical test device with the sample aperture on the top, the contacting surface can be attached to the slope directly and will be directly beneath the sample aperture. In this embodiment, the contacting surface, preferably, will not be parallel to the upper plane.

10

As used herein, the term "descending angle" refers to any angle, other than 180° , between the contacting surface and the upper plane. Thus, it is contemplated that the contacting surface will not be parallel to either the lower plane or upper plane of the bottom of the analytical test device. Also preferably, fluid sample contacted with the contacting surface should run into the absorbent membrane with the aid of gravity. However, tilting the device can control movement of the fluid sample.

20

The terms "absorbent membrane" and "reaction membrane" refer to any bibulous, porous or fibrous material capable of rapidly absorbing an aqueous fluid and conducting the fluid via capillary attraction. Suitable materials are described, for example, in H. J. Friesen, United States Patent No. 4,861,711, issued August 29, 1989; J. Bunting, United States Patent No. 4,271,140, issued June 2, 1981; May et al., United States Patent No. 5,622,871, issued April 22, 1997; May et al., United States Patent No. 5,656,503, issued August 12, 1997; May et al., United States Patent No. 5,602,040, issued February 11, 1997; and R. Rosenstein, United States Patent No. 5,591,645, issued January 7, 1997 (each of which is incorporated by reference herein). The

35

preferred material for the absorbent membrane is a fiberglass product such as that marketed as "MANNIWEB" or "MANNIGLAS" by Lydall, Inc., Manchester, CT. Other suitable materials include polyethylene or nitrocellulose pads and strips. The means for binding reagents to these materials are well-known in the art. Preferred materials include nitrocellulose, nylon, paper and silica gel. An advantage of a nitrocellulose membrane is that an immobilized antibody described above can be attached without prior chemical treatment. However, antibodies can be immobilized on other materials such as filter paper using well known chemical coupling methods such as, for example, CNBr, carbonyldimidazole or tresyl chloride. The reaction membrane will preferably be a chromatographic strip coated with gelatin to enhance the life of the strip and clarity of any visible reactions produced in the test.

The present invention also provides methods of using the analytical test device for detecting an analyte in a fluid sample, comprising contacting the fluid sample to the contacting surface and observing the reaction complex through the display port. Also preferably, the analytical test device will have a first display port and a second display port, and preferably, the reaction complex will be visible.

Figure 1 shows a perspective view of one embodiment of an analytical test device for performing assays in accord with the invention. Although any appropriate shape may be employed, Figure 1 depicts housing **100** of rectangular shape. Housing **100** has top **102**, in which one opening appears, and bottom **104**. The opening through top **102** is a display port **108** through which the test result can be viewed and compared.

On an end of bottom **104** is protruding sample well **110** and contacting surface **111**. Although Figure 1 shows the protruding sample well **110** and the contacting surface **111** pointed slightly upward at an oblique angle to the end of the housing, it is understood that the protruding sample well **110** or the contacting surface **111** can be placed at any other angle in relation to upper plane **122**, other than 180°.

Figure 2 shows an exploded view of the analytical test device of Figure 1. In this view, it can be seen that bottom **104** has sidewalls **143**, snaps **136**, upper plane **122** and slope **132**. Entry port **140** is located between slope **132** and contacting surface **111**.

As seen in Figure 2, contained within housing **100** will be absorbent membrane **144** and reaction membrane **142** containing the reagents to detect an analyte in a fluid sample.

Absorbent membrane **144** will be disposed along slope **132** to upper plane **122**. Reaction membrane **142** will be disposed along upper plane **122**. Absorbent membrane **144** and reaction membrane **142** will be in contact with; but need not be affixed, to each other.

Figure 3 is an enlarged sectional view of the analytical test device shown in Figure 1. In this view the orientation of contacting surface **111** can be seen.

Figure 4 shows a perspective view of an embodiment of the analytical test device shown in Figure 1. To that end, housing **200** is composed of top **202**,

bottom 204 and has display port 208 positioned on top 202. On an end of bottom 204 is protruding sample well 210 and contacting surface 211. In addition, snaps 236 are shown.

5

Figure 5 is an exploded view of the analytical test device shown in Figure 4 and shows the components, bottom 204, contacting surface 211, sidewalls 243, lower plane 234, slope 232 and upper plane 222. It can be seen that contained within housing 200 is absorbent membrane 244 and reaction membrane 242. Absorbent membrane 244 will be placed along lower plane 234 and along slope 232. Absorbent membrane 244 and reaction membrane 242 will be in contact with, but need not be affixed to each other.

15

Figure 6 is an enlarged sectional view of the analytical test device shown in Figure 4. In this view, the orientation of protruding sample well 210 and contacting surface 211 can be seen. In addition, vertical bar 245 is present to hold absorbent membrane 244 down to follow the contour of slope 232.

Figure 7 shows a perspective view of another embodiment of an analytical test device for performing assays in accord with the invention. Top 302 has two openings forming first display port 306 and second display port 308, which are preferably in close proximity for ease of comparison of results. In addition, housing 300 has handle 318 for the convenience of the user. Also, cap 314 can be placed over protruding sample well 310. Cap 314 can be formed from a single piece or, preferably, a top 315 and a bottom 316. Orifice 312 allows cap 314 to fit over protruding sample well 310.

25
30

Figure 8 is an exploded view of the analytical test device shown in Figure 7 and shows the components bottom **304**, contacting surface **311**, sidewalls **343**, snaps **336**, lower plane **334** slope **332**, upper plane **322**, fluid gully **330** and locating points **324**, **326** and **328**. It can be seen that contained within housing **300** is absorbent membrane **344**, reaction membrane **342** and collecting pad **348**. Absorbent membrane **344** will be placed along lower plane **334** and along slope **332**. Preferably, absorbent membrane **344** will be affixed to a nonporous substrate **346**, such as vinyl or plastic coated paper by an adhesive or other means. Substrate **346** will extend along upper plane **322** to form a surface to which reaction membrane **342** will be affixed. Absorbent membrane **344** and reaction membrane **342** will be in contact with, but need not be affixed to each other. In another embodiment, absorbent membrane **344** will be split at its point of attachment to substrate **346** to extend above and below the substrate. This configuration serves to regulate the flow of fluid sample from absorbent membrane **344** to reaction membrane **342**. Flow control can also be enhanced by placement of a well or fluid gully **330** below the junction between absorbent membrane **344** and reaction membrane **342**.

Figure 9 is an enlarged sectional view of the analytical test device shown in Figure 7. In this view the orientation of protruding sample well **310** and contacting surface **311** can be seen. In addition, vertical bar **345** is present to hold absorbent membrane **344** down to follow the contour of slope **332**.

Figure 10 is a perspective view of another embodiment of analytical test device with a sample

aperture 410 on the top 402 of housing 400. In this embodiment, housing 400 is composed of top 402 and bottom 404. Top 402 contains display port 408.

5 **Figure 11** is an exploded view of the analytical test device of Figure 10 and shows the components, bottom 404, contacting surface 434, sidewalls 443, snaps 436, slope 432, upper plane 422, and membranes containing the reagents for carrying out the assay, as described below.

10 Contacting surface 434 is at a descending angle to slope 432. In this embodiment, absorbent membrane 444 is not disposed on contacting surface 434.

As seen in Figure 11, contained within housing

15 400 will be membranes containing the reagents necessary for carrying out the assay. In another embodiment there will be an absorbent membrane 444 and a reaction membrane 442.

20 Absorbent membrane 444 will be disposed along slope 432 to upper plane 422. Reaction membrane 442 will be disposed along upper plane 422. Absorbent membrane 444 and reaction membrane 442 will be in contact with, but need not be affixed, to each other.

25 **Figure 12** is an enlarged sectional view of the analytical test device shown in Figure 10. The orientation of contacting surface 434 can be seen.

30 **Figure 13** shows a perspective view of an embodiment of the analytical test device shown in Figure 10. In this embodiment, housing 500 is composed of top 502 and bottom 504. In addition, housing 500 has handle

518 for the convenience of the user. Top 502 contains a first display port 506 and a second display port 508 that are preferably in close proximity for ease of comparison of results.

5

Figure 14 is an exploded view of the analytical test device shown in Figure 13 and shows the components, bottom 504, contacting surface 534, sidewalls 543, snaps 536, slope 532, upper plane 522, fluid gully 530 and locating points 524, 526 and 528, and one or more membranes containing the reagents for carrying out the assay, as described below. To that end it can be seen that contained within housing 500 is absorbent membrane 544, reaction membrane 542 and collecting pad 548.

15 Absorbent membrane 544 will be placed along slope 532. Preferably, absorbent membrane 544 will be affixed to a nonporous substrate 546 such as vinyl coated paper or plastic coated paper by an adhesive or other suitable means. Substrate 546 will extend along upper plane 522

20 to form a surface to which reaction membrane 542 will be affixed. Absorbent membrane 544 and reaction membrane 542 will be in contact with, but need not be affixed to each other. In another embodiment, absorbent membrane 544 will be split at its point of attachment to substrate

25 546 to extend above and below the substrate. This configuration serves to regulate the flow of fluid sample from absorbent membrane 544 to reaction membrane 542. Flow control can also be enhanced by placement of a well or fluid gully 530 below the junction between membranes

30 544 and 542.

Figure 15 is an enlarged sectional view of the analytical test device shown in Figure 13. The

orientation of contacting surface **534** can be seen. In addition, vertical bar **540** serves to hold absorbent membrane **544** down to follow the contour of slope **532**.

5 In **Figures 16, 17, 18, 19 and 20** at point **654** on absorbent membrane **644** a diffusively bound labeled reagent, preferably an antibody and, most preferably an antibody to hCG, will be bound so as to be spatially separated from where the fluid sample first contacts the
10 absorbent membrane. In **Figures 16, 17 and 18**, analyte in the fluid sample, if present, will bind to the diffusively bound labeled reagent at point **654** on the absorbent membrane **644** to form a first complex. If desired, further fluid sample may be added up to the
15 maximum binding capacity of unlabeled reagent. The first complex and uncomplexed diffusively bound labeled reagent, if any, will be carried along with the fluid sample by capillary action ("wicking") up to the reaction membrane **642**. The position of the diffusively bound
20 labeled reagent and first complex, if any is indicated as **654** on **Figures 16, 17 and 18**. Substrate **646** is also shown.

 All first complexes carried with the fluid
25 sample will contact the reaction membrane **642**. Fluid sample will pass through the reaction membrane **642**, bringing the first complex, if any, into contact with the non-diffusively bound reagent to form a second complex composed of first complex/non-diffusively bound reagent.
30 If second complexes are formed, they will appear in the test region at bar **652** (**Figure 16**).

 Fluid sample will continue its migration by wicking through reaction membrane **642** past the

non-diffusively bound reagent to the non-diffusively bound control reagent. Diffusively bound labeled reagent not complexed to analyte will bind with the non-diffusively bound control reagent to form a third
5 complex. The third complex will appear in the control region where bar **650** is shown (Figures 16, 17 and 18). Excess fluid sample will continue migrating to collecting pad **648**.

10 Formation of all complexes in the assay method may be by sandwich or competitive immunoassay techniques well known to those skilled in the art. Within a predetermined time period, any second complex present and the third complex will produce a detectable signal
15 mediated by the label. In another embodiment, the reaction complex will be a detectable a color change. This color change will signal either a positive (analyte present) or negative (analyte not present) reaction by providing two separate reactive regions within those
20 portions of reaction membrane visible to the user through the display ports. Other control or comparative result signals may be provided, including signals that indicate whether an invalid result is obtained, by similar means known to those skilled in the art (see, for example, the
25 signal system described in Brown et al., United States Patent No. 5,160,701, issued November 3, 1992, which is incorporated by reference herein).

As shown in **Figure 16**, a positive result is
30 indicated when color changes forming substantially similar shapes (in this instance, horizontal bars **650** and **652**) appear. In contrast, as shown in **Figure 17**, a negative result is indicated when a color change forming a distinguishable shape only appears, for example, as a

horizontal bar 650. Finally, an inconclusive result is shown in **Figure 18**, where color changes appear in both regions having substantially dissimilar shapes (i.e., horizontal bar 650 and smudge 658). In this latter
5 instance, the user would be alerted that the test should be repeated. While due to the decreased impact of leading edge effects and similar phenomena in this assay it is not expected that inconclusive results will be frequent, the assay requires such a small application of
10 fluid sample that repetition should not be problematic in most applications.

Figure 19 and **Figure 20** show perspective views of absorbent membrane 644, reaction membrane 642 and the
15 optional collecting pad 648, along with substrate 646, wherein a competitive assay is employed. Figure 19 shows a positive assay result, as indicated by no signal in reaction membrane 642. Figure 20 shows a negative assay result as indicated by the appearance of bar 658 in
20 reaction membrane 642.

EXAMPLE I

Analytical Test Device with Contacting surface Extending Outward from the Bottom

25

This example describes the method and analytical test device depicted in **Figure 7** containing the contacting surface extending outward from the bottom.

30 The assay procedure is performed using anti-hCG antibody to form the second complex and an anti-hCG-colloidal gold compound to form the first complex. A fluid sample can be collected at any time of day, but for best results, it is best to test the first

urine of the morning because it contains the highest concentration of hCG. The fluid sample is at least 1 cubic centimeter (hereinafter "cc") of urine suspected of containing hCG; the assay is performed at ambient
5 temperatures not below 15°C or exceeding 30°C. The 1 cc of urine is added to protruding sample well **310** by pipette. The fluid sample contacts contacting surface **311**. The analytical test device is then tilted so that fluid sample enters entry port **340** and contacts absorbent
10 membrane **344**. Within 3 minutes visible color changes, (from pure white to pink) through substantially the center of display ports **306** and **308** on reaction membrane **342**, are faintly visible. By the end of five minutes, a vividly pink bar symbol has appeared through
15 substantially the center of each display port, indicating the presence of hCG in the sample. All other visible areas of the reaction membrane visible through the second display port remain white, which is the normally visible color of the reaction membrane. This assay may be
20 performed at any time after a suspected conception to determine the presence of hCG and, therefore, pregnancy.

EXAMPLE II

25 Analytical Test Device with Sample Aperture on the Top

This example describes the method and analytical test device depicted in **Figure 13** containing a sample aperture on the top.

30

The assay procedure is performed using anti-hCG antibody to form the second complex and an anti-hCG-colloidal gold compound to form the first complex. A fluid sample can be collected at any time of

day, but for best results, it is best to test the first urine of the morning because it contains the highest concentration of hCG. The fluid sample is at least 1 cubic centimeter (hereinafter "cc") of urine suspected of
5 containing hCG; the assay is performed at ambient temperatures not below 15°C or exceeding 30°C. The 1 cc of urine is added to the sample aperture **510** on the top by pipette. The fluid sample contacts contacting surface **534**. The analytical test device is then tilted so that
10 fluid sample contacts absorbent membrane **544** located at slope **532**. Within 3 minutes visible color changes, (from pure white to pink) through substantially the center of display ports **506** and **508** on reaction membrane **542**, are faintly visible. By the end of five minutes, a vividly
15 pink bar symbol has appeared through substantially the center of each display port, indicating the presence of hCG in the sample. All other visible areas of the reaction membrane visible through the second display port remain white, which is the normally visible color of the
20 reaction membrane. This assay may be performed at any time after a suspected conception to determine the presence of hCG and, therefore, pregnancy.

Although the invention has been described with
25 reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

I claim:

1. An analytical test device, comprising:

(a) a top having one or more display ports, and
5 optionally, a sample aperture, an entry port and a
vertical bar;

(b) a bottom having an upper plane, a slope and
a contacting surface attached to the slope at a
descending angle, optionally, a lower plane between the
10 slope and contacting surface and, optionally, an entry
port; and

(c) a strip comprising an absorbent membrane,
a reaction membrane having one or more reagents that form
a reaction complex with an analyte in a fluid sample and,
15 optionally, a collecting pad,

wherein the absorbent membrane, the reaction
membrane and the collecting pad, when present, are in
fluid flow contact with one other; the strip is placed on
the bottom such that the reaction membrane is visible
20 through the display port and the absorbent membrane is on
the slope and on the lower plane when present; and the
fluid sample contacts the absorbent membrane by first
contacting the contacting surface when the top and bottom
are assembled.

25

2. The analytical test device of claim 1,
further comprising the top, the bottom or the assembled
top and bottom having the optional sample entry port and
the contacting surface extending outward from the bottom
30 and the absorbent membrane is in fluid flow contact with
the contacting surface through the entry port.

3. The analytical test device of claim 1, wherein the top has the vertical bar and wherein the vertical bar deflects the absorbent membrane to the follow the contour of the slope.

5

4. The analytical test device of claim 2, wherein the lower plane is present.

5. The analytical test device of claim 2,
10 further comprising a cap placed over the contacting surface.

6. The analytical test device of claim 1, wherein the collecting pad is present and in fluid flow
15 contact with the reaction membrane.

7. The analytical test device of claim 1, wherein the sample aperture is present and the contacting surface is below the sample aperture.

20

8. The analytical test device of claim 1, further comprising a diffusively bound labeled reagent bound to the absorbent membrane at a point spatially separated from the point where the fluid sample first
25 contacts the absorbent membrane.

9. The analytical test device of claim 8, wherein bound to the reaction membrane is:

(a) a non-diffusively bound reagent
30 complementary to an analyte in the fluid sample at a point beneath the display port; and

(b) a non-diffusively bound control reagent complementary to the diffusively bound labeled reagent, wherein the non-diffusively bound control reagent is
35 bound at a point beneath the display port that is

spatially separated from the point at which the non-diffusively bound reagent is bound.

10. The analytical test device of claim 9,
5 further comprising a first display port and a second display port, wherein the non-diffusively bound reagent is positioned beneath the first display port and the non-diffusively bound control reagent is positioned beneath the second display port.

10

11. The analytical test device of claim 8,
wherein bound to the reaction membrane at a point beneath the display port is a non-diffusively bound reagent, wherein the analyte and the diffusively bound labeled
15 reagent compete for binding to the non-diffusively bound reagent.

12. A method for detecting an analyte in a fluid sample, comprising the steps of:
20 (a) contacting the fluid sample to the contacting surface of the analytical test device of claim 1; and

(b) detecting the analyte by observing the reaction complex through the display port.

25

13. The method of claim 12, wherein the reaction complex is visible.

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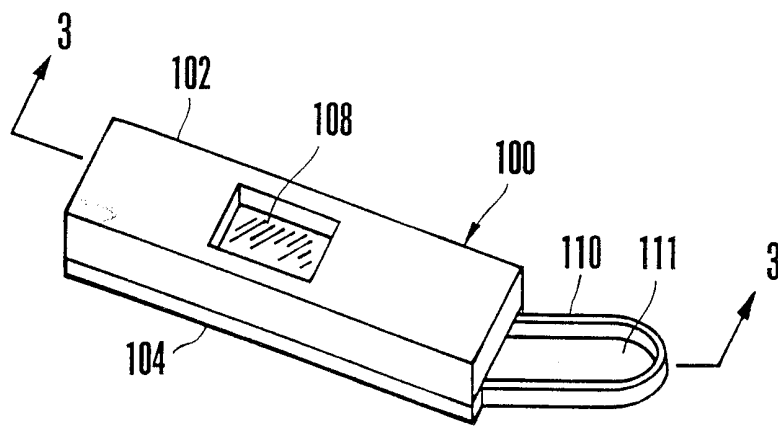
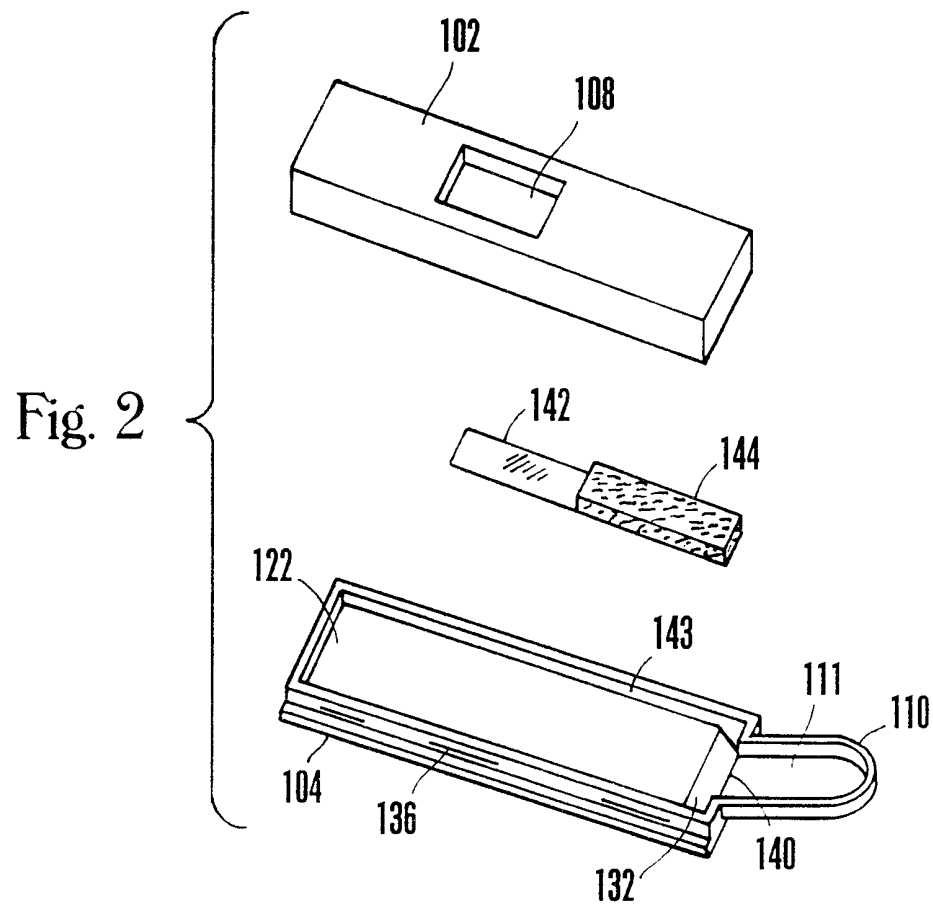


Fig. 1



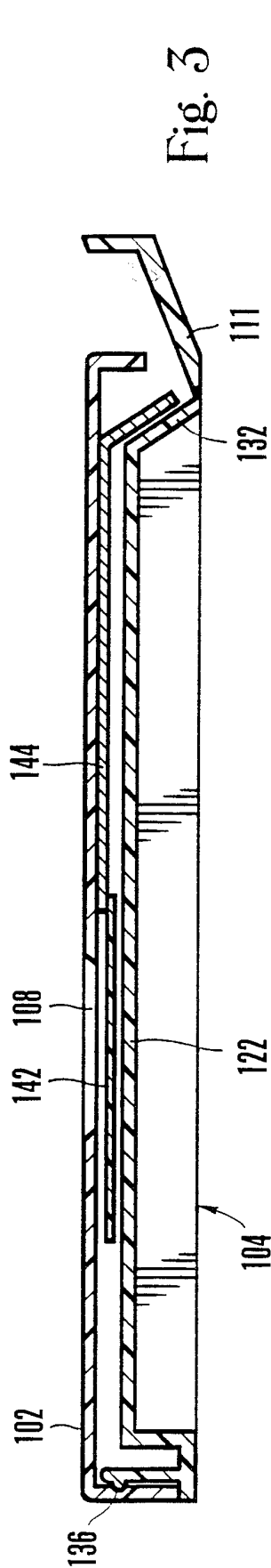


Fig. 3

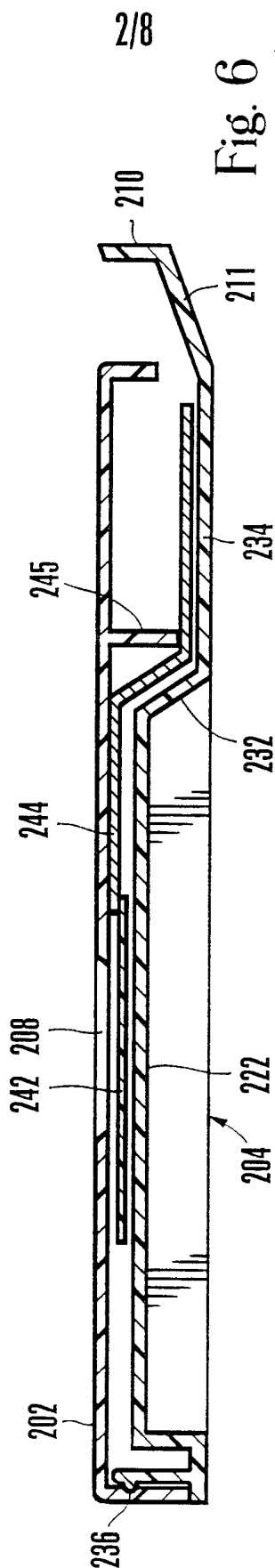


Fig. 6

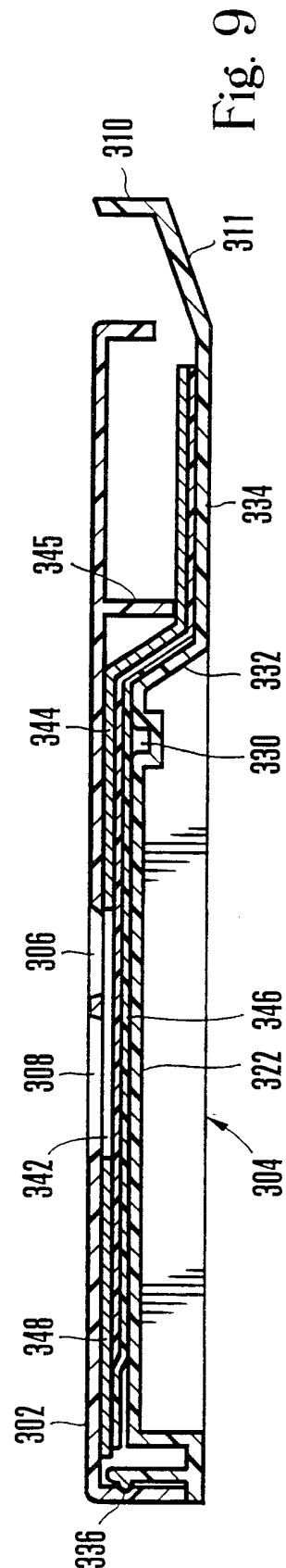


Fig. 9

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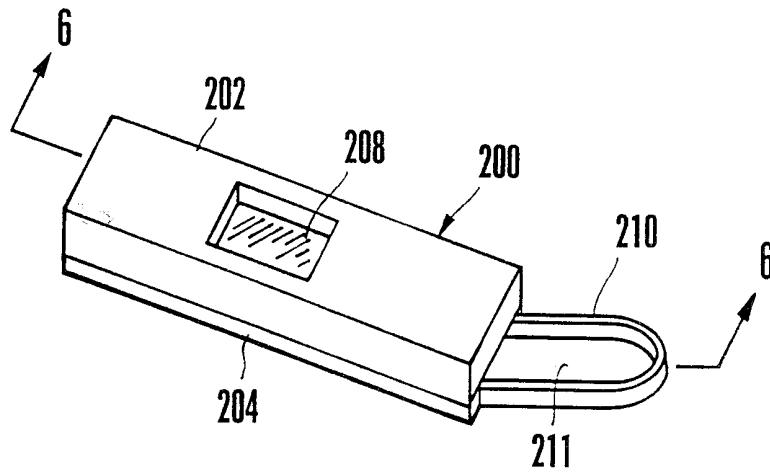


Fig. 4

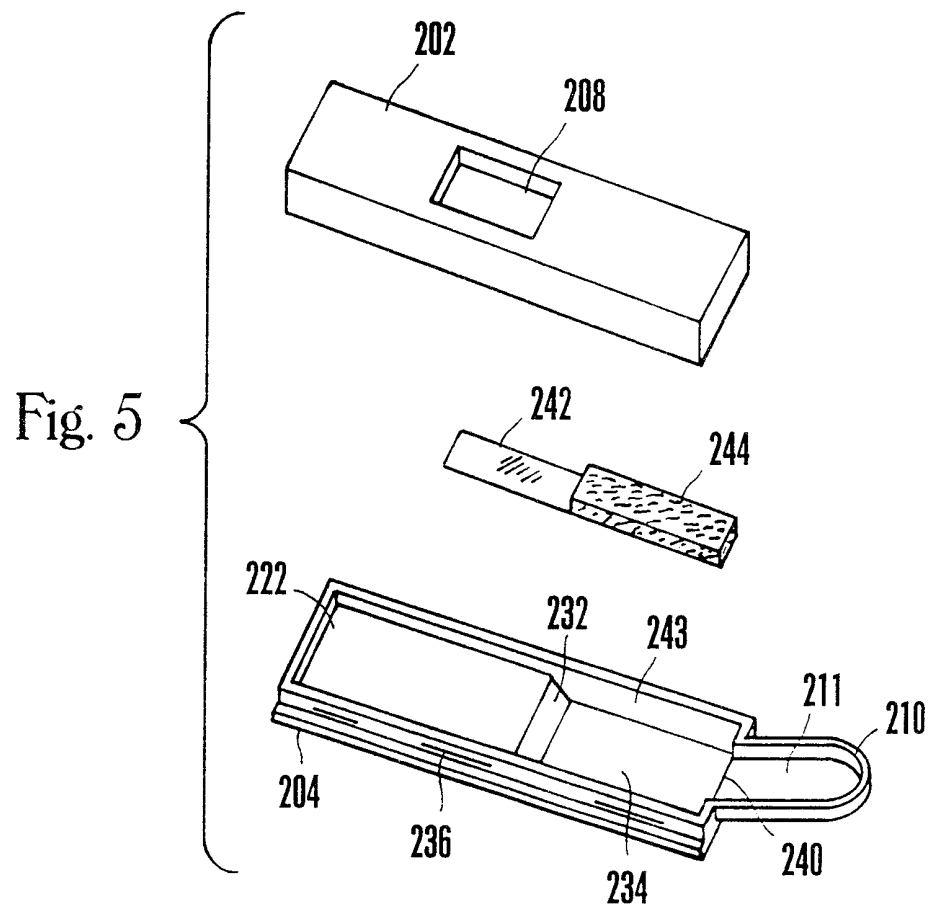


Fig. 5

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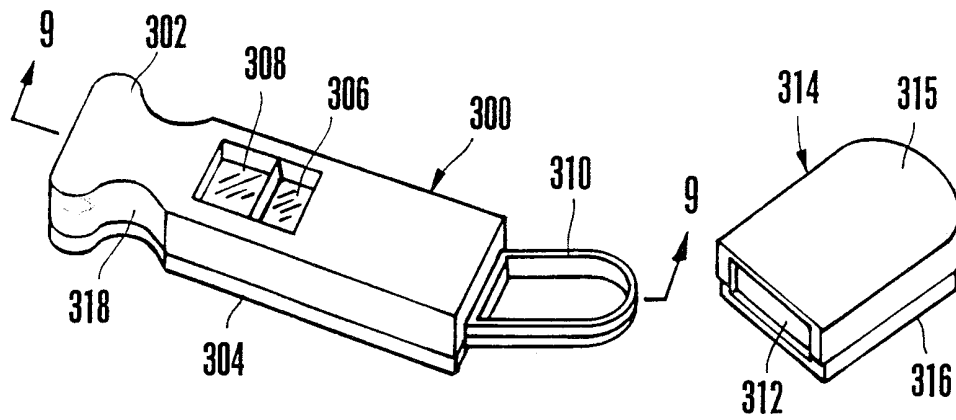
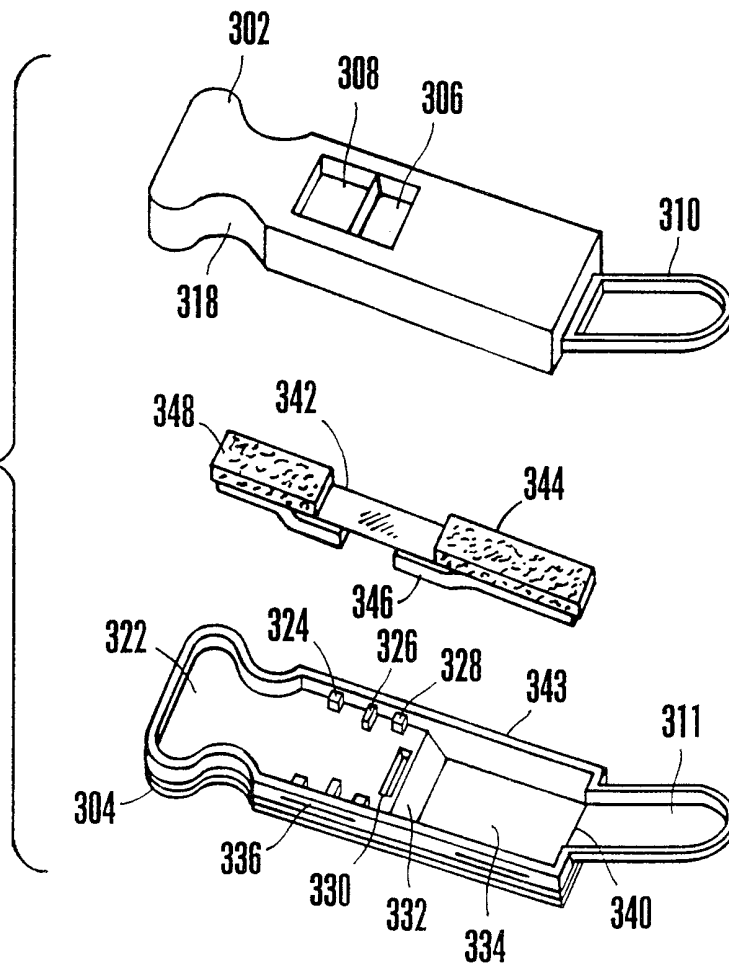


Fig. 7

Fig. 8



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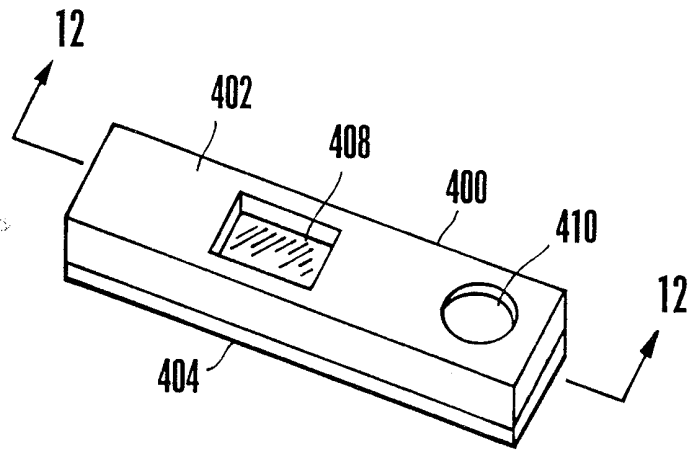


Fig. 10

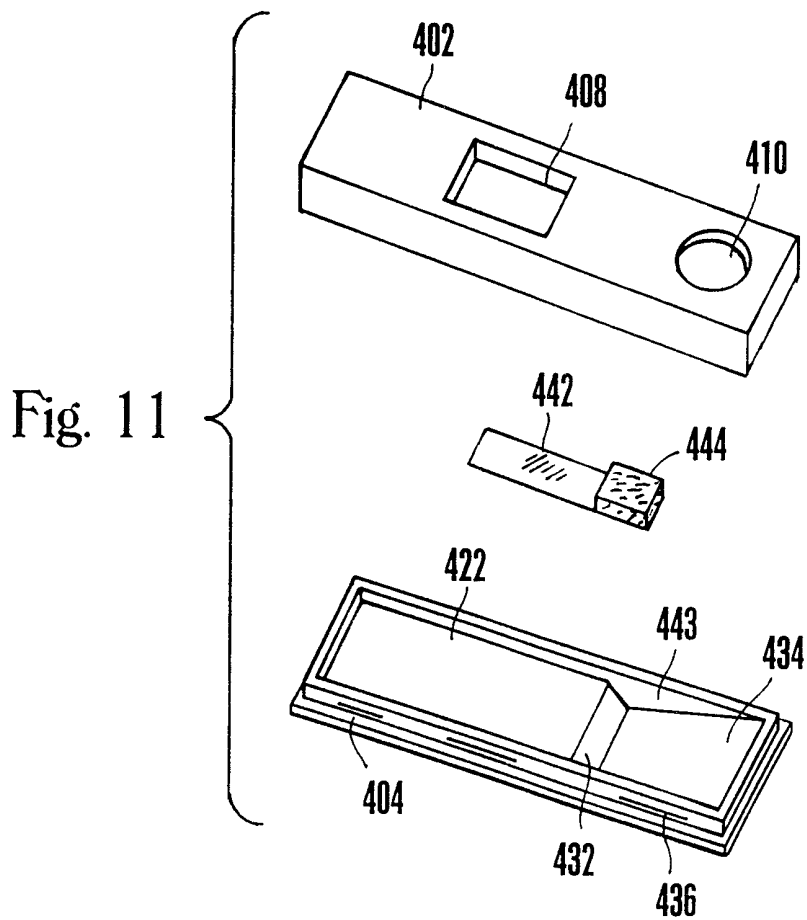


Fig. 11

Fig. 12

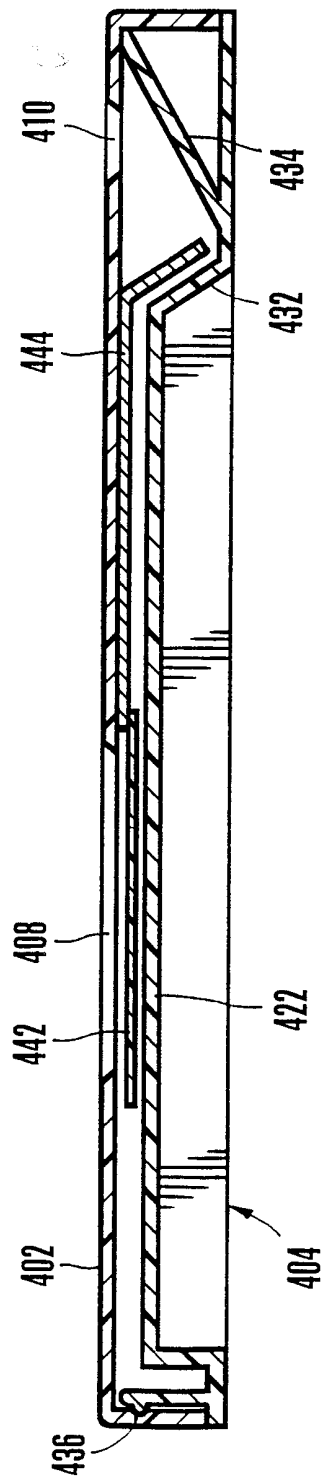
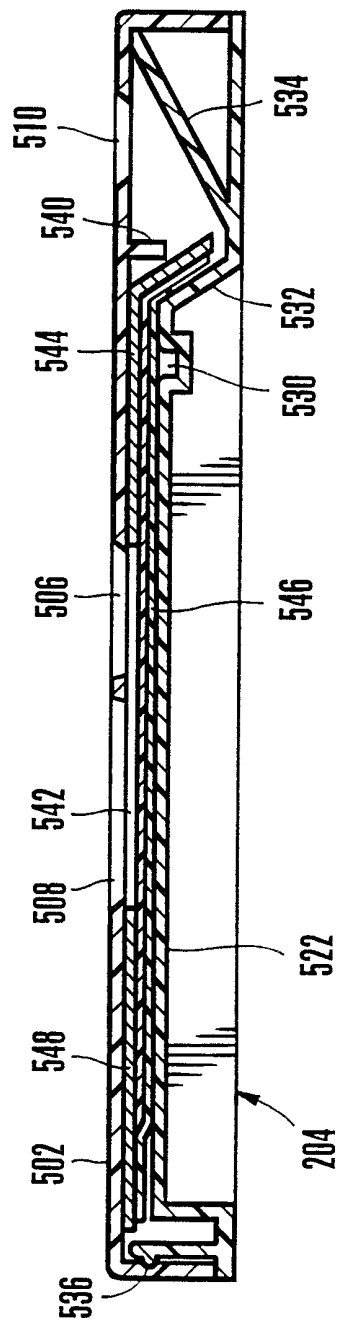


Fig. 15



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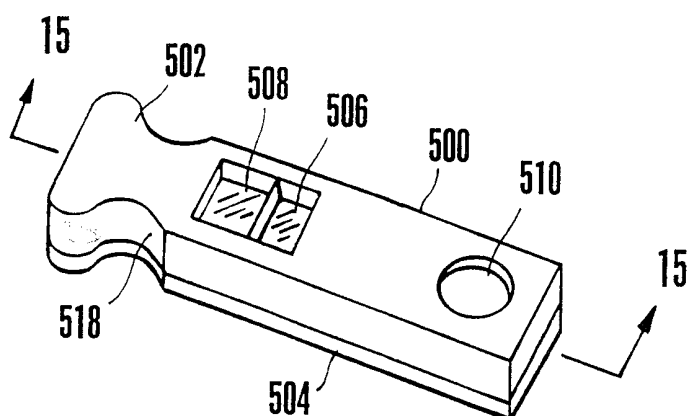
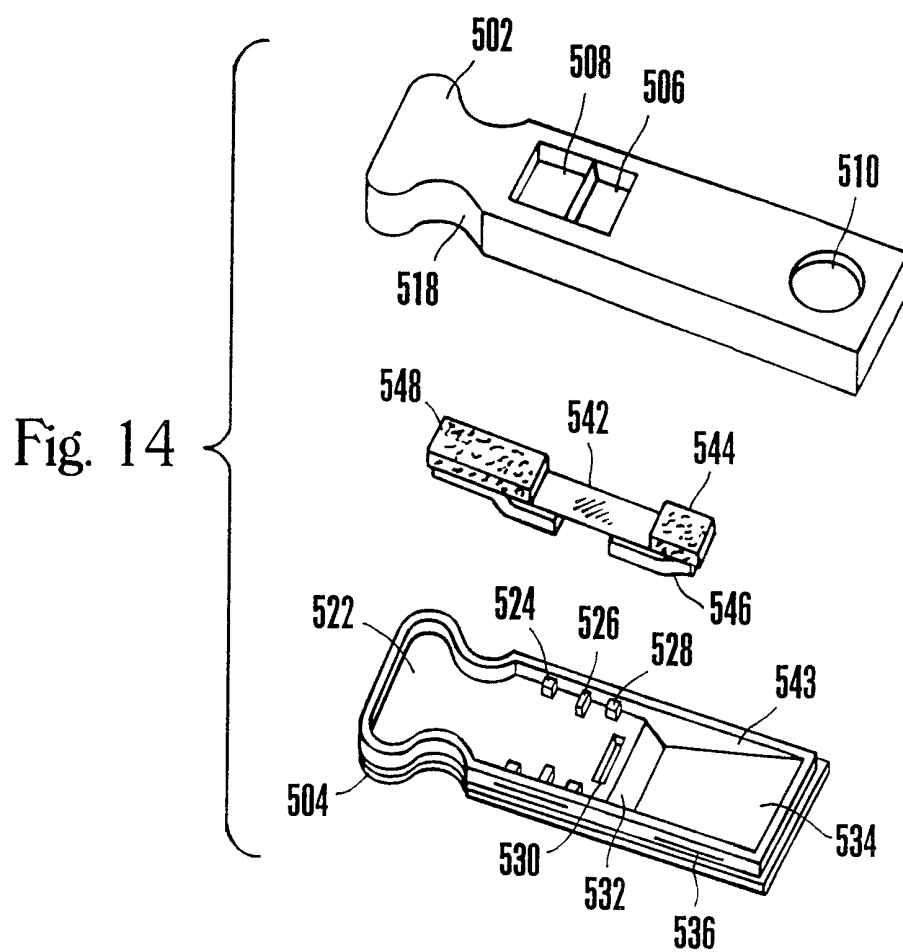


Fig. 13



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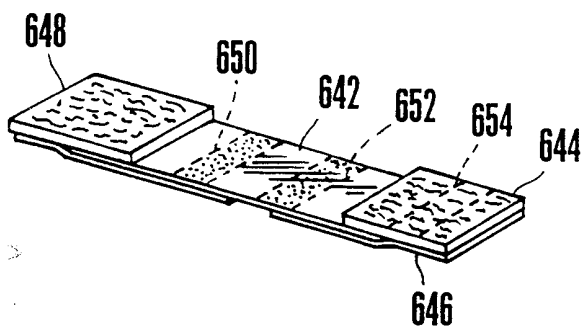


Fig. 16

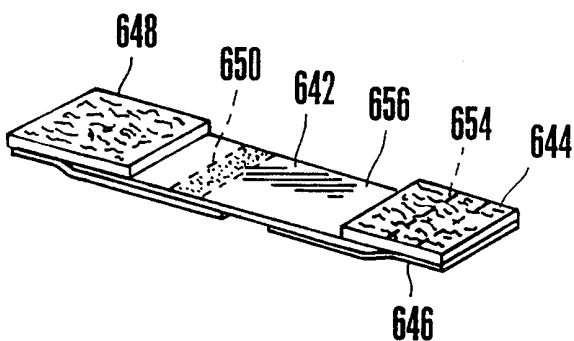


Fig. 17

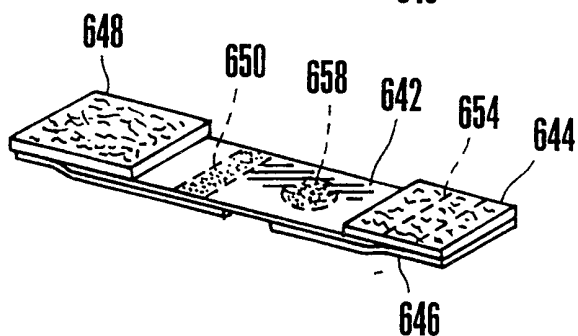


Fig. 18

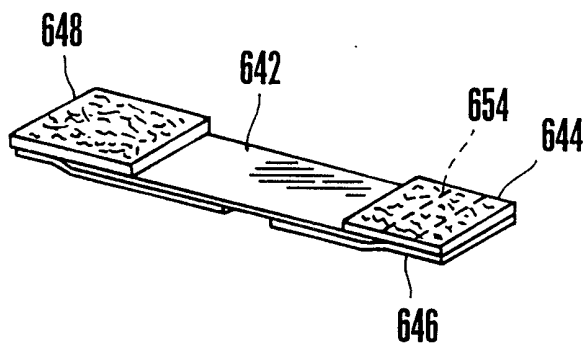


Fig. 19

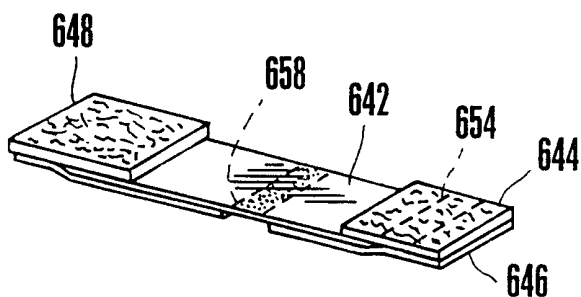


Fig. 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27698

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/543, 33/558

US CL : 436/518, 514, 501

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)

U.S. : 436/518, 514, 501

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)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| Y | US 5,602,040 A (MAY et al.) 11 February 1997, see entire patent. | 1-13 |
| Y | US 5,559,041 A (KANG et al.) 24 September 1996, see entire patent, especially col. 11-14. | 1-13 |
| Y | US 4,943,522 A (EISINGER et al.) 24 July 1990, see entire patent. | 1-13 |

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

☐ See patent family annex.

| | |
|---|--|
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Date of the actual completion of the international search

01 APRIL 1999

Date of mailing of the international search report

06 MAY 1999

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