



FASCICULE DE BREVET D'INVENTION

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An assay device 10 includes a first sorbent member 12 having an analyte-marker to mark an analyte, or which is configured to receive a liquid analyte-marker. The first sorbent member includes or defines a first elongate migratory flow path 14. A second sorbent member 16 is provided to receive a sample. The second sorbent member 16 includes or defines a second elongate migratory flow path 18. The second migratory flow path 18 is operatively above or below or adjacent the first migratory flow path 14 over at least a major portion of the length of the second migratory flow path 18 and is shorter in length relative to the first migratory flow path 14. A test site 24 is in migratory flow communication with both the first and the second sorbent members 12, 16 by means of the first and the second flow paths 14, 18, the test site 24 having an analyte-receptor to bind analyte in the sample.

AN ASSAY DEVICE

FIELD OF THE INVENTION

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This invention relates to an assay device, particularly to an immunochromatographic assay device for use in detecting a ligand in a biological sample.

BACKGROUND

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Various immunochemical techniques which exploit the specificity (the ability to discriminate between a desired analyte and another substance) and/or sensitivity (the ability to detect or measure a certain concentration of the analyte) of analytes for receptor molecules, or *vice versa*, are known. These techniques are particularly useful in the study of immunology and can provide quantitative assessment of the concentration of a particular analyte such as an antibody in a biological sample for use in diagnostic/prognostic techniques. Immunochemical techniques are also commonly used in other applications which can include, for example, measuring levels of environmental contaminants in biological samples.

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Several variations of basic immunochemical assay methodologies exist and are typically termed immunoassays. Some of the various well studied immunoassays include competitive binding immunoassays, immunometric assays, solid-phase immunobinding assays, enhanced immunoassays, peptide-based immunobinding assays and fluorescence- and photoluminescence-based immunoassays.

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Considerable research has taken place over the last few decades to develop user friendly, simple, accurate and precise immunoassays for providing means for diagnoses/prognoses. Such means are especially necessary in remote locations where access to advanced medical diagnostic tools is limited.

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Solid phase immunobinding assays are viewed as being particularly useful as a diagnostic/prognostic tool. Their general construction provides a robust device easily utilized by a user, typically a clinician, in both high and low technology environments. Typically, a solid phase immunobinding assay device comprises a solid support to which

either a ligand or receptor is bound. A biological test sample is then applied to the solid support whereupon a ligand-receptor interaction occurs. Various visual tests indicative of positive ligand-receptor interactions have been developed and have proven to be particularly useful in clinical settings to aid the user in diagnoses/prognoses.

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These solid phase immunobinding assays are in essence solid phase immunochromatographic assays. The most user friendly of the solid phase immunochromatographic assays are configured into self-contained kits for easy use by a user. The most common types of self-contained kits are dipstick assays, flow through assays and migratory assays, all of which are well known in the art.

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A common way to provide for a visual test is to add a marker to either a ligand or receptor such that a positive ligand-receptor interaction can be visually identified. Examples of known markers include radioactive isotopes, fluorescent molecules, gold, polystyrene and/or derivatives thereof and/or certain enzymes, to name but a few.

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Generally, when a ligand and/or receptor is labelled with a marker it is referred to as a conjugate. Often conjugates are used in addition to ligand-receptor interactions in order to enhance the sensitivity of the assay.

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For example, a first ligand will be allowed to bind with a receptor to form a ligand-receptor complex, following which a conjugate (being a second ligand or receptor having a marker) is allowed to bind to the ligand-receptor complex to form a coloured ligand-receptor-conjugate complex. Particularly, the first ligand is the analyte and binds with a receptor, the analyte-receptor, to form an analyte-receptor complex. The conjugate, a second ligand having a marker (the analyte-marker), then binds to the analyte-receptor complex to form an analyte-receptor-marker complex which is visually identifiable.

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Often, solid phase immunochromatographic assays comprise a sample loading zone onto which a sample may be loaded in use, a conjugate zone having immobilised thereon a conjugate (or analyte-marker), and a test site having immobilised thereon an analyte-receptor.

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Analyte in a sample may in use migrate along a flow path via capillary action and bind to an analyte-receptor at the test site to form an analyte-receptor complex. A portion of

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either the analyte, or receptor, comprising the analyte-receptor-complex binds to a portion of the analyte-marker to yield a visually identifiable positive test upon migration toward the test site or, alternatively, at the test site. Typically, a solid phase immunochromatographic assay also comprises a control site. Should the sample not contain the analyte of interest, the analyte-marker migrates past the test site to a control site where it interacts with a marker-receptor to form a visually identifiable control.

A disadvantage of many available solid phase immunochromatographic assays includes the fact that components in the sample, including but not limited to the analyte, impede the migration of the analyte-marker to the test site, therein increasing the time for the assay to yield a result, and also decreasing the sensitivity of the test.

Inexpensive, user friendly, accurate and precise diagnostic/prognostic test assays are needed particularly in third world and developing countries where access to, and use of, advanced medical technologies may be limited.

Life expectancy of inhabitants of third world and developing countries remain negatively affected by the failure to diagnose and/or treat curable diseases during their early stages. Such failure often results in the disease becoming untreatable resulting in unnecessary loss of life.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading cause of death resulting from a curable disease despite the availability of short-course therapy that is generally inexpensive and effective. According to the World Health Organization (WHO), tuberculosis (TB) caused an estimated 1.7 million deaths in 2009, most of which occurred in Africa. The clinical management of TB in developing countries is hampered by the unavailability of simple, accurate and precise diagnostic tools. According to an evaluation by the WHO of 19 commercial serological tests, all evaluated tests showed poor sensitivity and specificity and showed inconsistencies.

Consequently, there is a need for simple, inexpensive, accurate and precise point-of-care diagnostic/prognostic TB test assays.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided an assay device to detect an analyte or analogue thereof in a sample, the assay device including:

5 a first sorbent member having an analyte-marker to mark the analyte, or configured to receive a liquid analyte-marker, the first sorbent member including or defining a first elongate migratory flow path;

a second sorbent member to receive the sample, the second sorbent member including or defining a second elongate migratory flow path, the second migratory flow path
10 being operatively above or below or adjacent the first migratory flow path over at least a major portion of the length of the second migratory flow path and being shorter in length relative to the first migratory flow path; and

a test site in migratory flow communication with both the first sorbent member and the second sorbent member by means of the first migratory flow path and the second migratory
15 flow path respectively, the test site having an analyte-receptor to bind analyte in the sample.

In use, a user applies buffer facilitating migration of the analyte-marker to the test site, or a liquid analyte-marker which will migrate to the test site, to the first sorbent member, the user also applies the sample to the second sorbent member, analyte in the
20 sample migrating along the shorter second migratory flow path to reach the test site before the analyte-marker, the analyte binding to the analyte-receptor to form an analyte-receptor complex and the analyte-marker binding to the analyte-receptor complex to form a visually identifiable analyte-receptor-marker complex.

25 In this specification, by "major portion" is meant more than half.

Typically, the second migratory flow path is operatively above or below the first migratory flow path over at least a major portion of the length of the second migratory flow path.

30 The first sorbent member may be a composite member including more than one sorbent part joined together or arranged to be in migratory flow communication to form the first sorbent member.

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The second sorbent member may be a composite member including more than one sorbent part joined together or arranged to be in migratory flow communication to form the second sorbent member.

5 The analyte-receptor may be immobilised in or to the test site.

The assay device may further include a control site in migratory flow communication with the first sorbent member, the second sorbent member and the test site, the control site having a marker-receptor to bind analyte-marker to form a control-complex.
10 Typically, the control site, the test site, the second sorbent member and the first sorbent member are all positioned along a common linear axis, although they may be set off from the common linear axis, and the control site is located downstream of the test site in a direction of migratory flow in use. In other words, the control site may be located away from the test site in the direction of migratory flow.

15 In one embodiment of the assay device, the second migratory flow path is operatively above the first migratory flow path and above an operative level of the test site over at least a major portion of the length of the second migratory flow path, the second migratory flow path having a descending portion dropping towards said operative level of
20 the test site.

The first migratory flow path may be linear when viewed in the direction of at least one axis of a Cartesian coordinates system.

25 The first migratory flow path may be linear when viewed in the direction of all three axes of a Cartesian coordinates system.

The second migratory flow path may be linear when viewed in the direction of at least one axis of a Cartesian coordinates system.

30 The second migratory flow path may be linear when viewed in the direction of two of the axes of a Cartesian coordinates system.

The first migratory flow path and the second migratory flow path may be operatively parallel when viewed in the direction of at least one axis of a Cartesian coordinate system.

- 5 Preferably, the test site and the first migratory flow path is at the same level, i.e. in the same horizontal plane when the first migratory flow path is held horizontally.

The marker-receptor may be immobilised in or to the control site.

- 10 The second sorbent member may include a receiving zone distal the test site to receive sample when in use, and a migration zone defining said second elongate migratory flow path proximal the test site, the migration zone extending away from the receiving zone toward the test site descending in position relative to the receiving zone as it extends. The receiving zone and migration zone may be of different materials.

- 15 Thus, in one embodiment of the assay device, the second sorbent member includes or defines a receiving zone distal the test site in use to receive sample, and a migration zone defining said second elongate migratory flow path proximal the test site, the migration zone extending away from the receiving zone toward the test site, the receiving zone and the migration zone being of different sorbent materials.
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- The assay device may further include a sorbent wick operatively downstream of the test site, e.g. extending away from the control site in the direction of migratory flow, to sorb, in use, excess sample, buffer, analyte-marker and/or analyte or analogues thereof.

- 25 In one embodiment of the assay device, the assay device includes a sorbent wick operatively downstream of the test site to sorb, in use, excess sample, buffer, analyte-marker and/or analyte or analogues thereof, the first sorbent member, the second sorbent member, the test site and the sorbent wick all being positioned along a common linear axis although not all necessarily in the same horizontal plane. Thus, at least one of these features may be set off from the common linear axis. Typically, as hereinbefore indicated, at least the second sorbent member is not in the same horizontal plane as at least the test site.
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The first and/or second sorbent members may be received and secured onto a support, preferably of a synthetic plastics or polymeric material.

5 In one embodiment of the assay device, the first sorbent member and the second sorbent member are supported on a common elongate linear support, the elongate linear support being operatively below and in register with both the first elongate migratory flow path and the second elongate migratory flow path.

10 The assay device may further include a housing to operatively accommodate the first and second sorbent members, alternatively, to operatively accommodate the first and second sorbent members which are secured to the support.

The housing may be generally cylindrical, e.g. elongate and parallelepipedal.

15 The housing may have two major opposed parallel faces with opposed parallel sides extending between the faces, and with ends of the housing being closed. In short, the housing may resemble a flat bar.

20 The housing may include a base and lid, the base operatively receiving the first and second sorbent member, alternatively, the first and second sorbent members secured to the support. The base and lid may each include or define complementary shaped attachment formations operatively to engage one another so as to securely close the housing from an outside environment.

25 The housing or the lid may be configured to define a sample opening, a buffer or liquid analyte-marker opening and a viewing opening.

30 The sample opening may be superposingly positioned above the receiving zone, the buffer or liquid analyte-marker opening may be superposingly positioned above a portion of the first sorbent member, and the viewing opening may be superposingly positioned above at least the test site, preferably above the test and control sites, when the control site is present. The viewing opening may be covered by a clear or transparent material, preferably glass or a transparent synthetic plastics or polymeric material.

In one embodiment of the assay device, the device includes a housing which houses at least the first sorbent member and the second sorbent member and the test site and which encloses the first migratory flow path and the second migratory flow path, the housing defining a sample opening above, i.e. operatively above, and in fluid communication with, the second sorbent member, a buffer or liquid analyte-marker opening above, i.e. operatively above, and in fluid communication with, the first sorbent member and a viewing opening or viewing window above, i.e. operatively above the test site, the housing being generally cylindrical.

The housing or the lid may define a further buffer opening adjacent to the sample opening and in fluid communication with the second sorbent member, in use, to receive buffer to facilitate the migration of sample along the second elongate migratory flow path toward the test site.

The second sorbent member may be operatively supported at least partially on top of the first sorbent member, with a liquid impermeable barrier member being located or sandwiched between the first sorbent member and the second sorbent member.

Typically, the second sorbent member, over at least a portion of the second elongate migratory flow path, is spaced from and not supported by the first sorbent member.

In one embodiment of the invention, the second sorbent member, over a portion of the second elongate migratory flow path between the receiving zone and the test site, is spaced from the first sorbent member by an air gap.

The assay device may be used for detecting anti-MTB-38 antibodies in biological samples of patients suspected of having tuberculosis. Thus, in one embodiment, the assay device is an anti-MTB-38 antibodies detection device wherein the analyte-receptor is TB-6-14-38 and the analyte-marker is colloidal gold-protein-A-conjugate.

In another embodiment, the assay device is an anti-MTB-38 antibodies detection device wherein the analyte-receptor is TB-6-14-38, the analyte-marker is colloidal gold-protein-A-conjugate and the marker-receptor is anti-protein-A.

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According to a second aspect of the invention there is provided a method of determining presence of an analyte in a sample, the method including:

applying buffer to a first sorbent member having an analyte-marker to mark the analyte, or applying a liquid analyte-marker to a first sorbent member configured to receive a liquid analyte-marker;

allowing the analyte-marker to migrate along a first elongate migratory flow path to a test site having an analyte-receptor to bind analyte in the sample;

applying said sample to a second sorbent member;

allowing the sample to migrate along a second elongate migratory flow path to said test site, the second migratory flow path being operatively above or below or adjacent the first migratory flow path over at least a major portion of the length of the second migratory flow path and being shorter in length relative to the first migratory flow path;

allowing any analyte present to bind to the analyte-receptor to form an analyte-receptor complex; and

allowing the analyte-marker to bind to any analyte-receptor complex to form a visually identifiable analyte-receptor-marker complex.

The method may be used to assay a biological sample.

The biological sample may be obtained from a living or a dead organism. The method is thus an ex vivo method, with the sample, when containing biological material obtained from a living organism, being assayed outside of the living organism.

The method may include ensuring that the sample reaches the test site before the analyte-marker reaches the test site. This may include using a sorbent material for the second migratory flow path of selected dimensions and migratory flow characteristics and using a sorbent material for the first migratory flow path of selected dimensions and migratory flow characteristics to allow the sample to reach the test site before the analyte-marker, when the sample and the analyte marker are applied substantially simultaneously respectively to the second sorbent member and the first sorbent member.

The first sorbet member, the second sorbent member and the test site may form part of an assay device in accordance with the first aspect of the invention.

In one embodiment of the method of the invention, the second migratory flow path is operatively above the first migratory flow path and above an operative level of the test site over at least a major portion of the length of the second migratory flow path, the second migratory flow path having a descending portion dropping towards said operative level of the test site.

The method may further include applying buffer to the second sorbent member to facilitate ease of sample migration along the second migratory pathway toward the test site. The buffer may be applied to the second sorbent member at a location further from the test site than the sample was applied.

The method may include allowing unbound analyte-marker to migrate from the first sorbent member along the first migratory pathway, past the test site to a control site and to bind to a marker-receptor at the control site to form a control-complex which is visually identifiable.

The method may include using a sorbent wick operatively downstream of the test site, e.g. extending away from the control site in direction of migratory flow, to sorb, in use, excess sample, buffer, analyte-marker and/or analyte or analogues thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described, by way of example only, with reference to the accompanying diagrammatic drawings in which:

Figure 1 shows a three-dimensional view of an assay device external a housing in accordance with one embodiment of the invention;

Figure 2a shows a three-dimensional view of the housing of the assay device;

Figure 2b shows a sectional side view of the assay device;

Figure 3a shows a diagrammatic representation of the biochemistry of the assay device in use when analyte binds to an analyte-receptor to form an analyte-receptor-complex;

Figure 3b shows a diagrammatic representation of the biochemistry of the assay device in use when analyte-marker binds to an analyte-receptor-complex to form an analyte-receptor-marker complex;

Figure 3c shows a diagrammatic representation of the biochemistry of the assay device in use when analyte-marker binds marker-receptor to form a control-complex;

Figure 4a shows test and control strips of individual devices after samples containing varying concentrations of analyte have been assayed;

5 Figure 4b shows quantitative analysis of the device using a ESE-Quanti lateral flow reader;

Figure 5a shows the device after sample containing analyte has been assayed; and

Figure 5b shows the device after sample not containing analyte has been assayed.

10 DETAILED DESCRIPTION OF THE DRAWINGS

Referring to the accompanying diagrammatic drawings, Figure 1 shows one embodiment of an assay device 10 in accordance with the invention for use in detecting an analyte or analogue thereof in a sample. The assay device 10 comprises a first sorbent member 12 having an analyte-marker 15 and which forms part of a first elongate substantially linear migratory flow path 14, and a second sorbent member 16 to receive the sample and which forms part of a second elongate migratory flow path 18. The second migratory flow path 18 is above the first migratory flow path and shorter in length relative to the first migratory flow path 14. The migratory flow path 18 is superimposed over the migratory flow path 14 over at least a portion of the length of the migratory flow path 14, with a portion of the migratory flow paths 14, 18 being common to both. Essentially, the first sorbent member 12 is a pad of sorbent material having partially immobilised thereon and/or therein, e.g. by impregnation, an amount of analyte-marker 15. The first sorbent member 12 is supported on a support strip 20 which provides rigidity to the components of the assay device 10.

Abutting against both the first sorbent member 12 and the support strip 20 is a control and test strip 22 being a sorbent material and having a test site 24 and a control site 26. As the control and test strip 22 also abuts against the support strip 20 further rigidity is provided to the assay device 10. The first sorbent member 12 and the control and test strip 22 may be integrally formed from the same material. It is important to note that there may be more than 1 test sites (or test lines).

The test site 24 has immobilised thereon and/or therein an amount of an analyte-receptor 25 which binds analyte and/or analogues thereof. The analyte-receptor 25 is

permanently immobilised to the sorbent material of the test site 24 and is not soluble or only sparingly soluble in a buffer used for example for the analyte-marker 15 or for the analyte/sample, or in the sample. The control and test strip 22 and the first sorbent member 12 are in migratory flow communication with each other along the first elongate migratory flow path 14. The control site 26 is located away from the test site 24 in a direction 27 of migratory flow along the control and test strip 22. In other words, the test site 24 is between the first sorbent member 12 and the control site 26.

The second sorbent member 16 comprises a receiving zone 28 and a migration zone 30. The receiving zone 28 is located at a position at least partially superimposed over the first sorbent member 12 and, in use, receives sample. The migration zone 30 is defined by a nitrocellulose membrane strip which extends away from the receiving zone 28 in the direction 27 of flow and depends at a predefined angle toward the control and test strip 22 until a distal end 32 of the migration zone 30 adjoins the control and test strip 22. The receiving zone 28, migration zone 30 and control and test strip 22 are all in migratory flow communication with one another and define the second migratory flow path 18.

The first migratory flow path 14 thus extends from the first sorbent member 12 to the test site 24 and the control site 26 along the control and test strip 22, which is also in the form of a nitrocellulose membrane strip.

The second migratory flow path 18 extends from the receiving zone 28, along the migratory zone 30 and a portion of the control and test strip 22 to the test site 24 and the control site 26. The second migratory flow path 18 extends from the receiving zone 28, being at a higher elevation relative to the first sorbent member 12, and descends along the migratory zone 30 to run along the test and control strip 22 to the test site 24 and the control site 26.

The second migratory flow path 18 is shorter relative to the first migratory flow path 14, but shares an end portion with the first migratory flow path 14. Typically, flow of a substance along the second migratory flow path 18 will take less time relative to the first migratory flow path 14 for at least two reasons: firstly, the second migratory flow path 18 is shorter in length relative the first migratory flow path 14, and secondly, the effect of gravity on a substance may increase rate of flow in the second flow path 18 relative to the first flow path 14, since in use the second migratory flow path 18 descends along an elevation

gradient whereas the first migratory flow path 14 is substantially horizontal. It is to be understood that a person reasonably skilled in the art could devise of additional ways to ensure the rate of flow of a substance is faster along the trajectory of the second flow path 18 relative to the first flow path 14, by for example, constructing the receiving zone 28 and migratory zone 30 from a material which would allow for a greater rate of flow compared to material used to construct the first sorbent member 12.

In a preferred embodiment of the invention as shown in Figure 1, the first sorbent member 12 and the receiving zone 28 are spaced by a separator, typically a fluid impermeable barrier member, such as a strip 34 of a liquid impermeable synthetic plastics or polymeric material. The strip 34 prevents the sample and/or any analyte or analogues thereof from contacting the first sorbent member 12 which contains the analyte-marker 15. It is known that in many instances components of the sample including the analyte or analogues thereof impede the flow of analyte-marker 15 when bound to or interacting with the analyte-marker 15, causing a delay in the time taken by the assay device 10 to yield a result and decreasing the sensitivity of the assay. It is desired to limit direct contact by and between the receiving zone 28 and the first sorbent member 12.

In use, a sample to be tested for the analyte is applied to the receiving zone 28 and a liquid buffer is applied at the same time to the second sorbent member 16. Liquid buffer is then applied to the first sorbent member 12 a minute later after the application of the liquid buffer to the second sorbent member 16. The analyte-marker 15 is immobilised to the first sorbent member 12 but becomes demobilised upon the application of the buffer onto the first sorbent member 12. The analyte-marker 15 then traverses the first migratory path 14 toward the test site 24 and control site 26. The analyte, if present in the sample, will migrate from the receiving zone 28 toward the test site 24 at a faster rate relative the analyte-marker 15 and will reach the test site 24 sooner than the analyte marker 15 during assay. Therefore, the analyte will bind to the analyte-receptor 25 to form an analyte-receptor-complex. After formation of the analyte-receptor-complex the analyte-marker 15 reaches the test site 24 and binds with the analyte-receptor-complex to form an analyte-receptor-marker complex. This analyte-receptor-marker complex is coloured and provides a visual test for the presence of an analyte in a sample.

The control site has a marker-receptor 35 immobilised thereon and/or therein. The marker-receptor 35, in use, binds analyte-marker 15 to form a control-complex. The

control-complex shows the user of the assay device 10 that the analyte-marker 15 has migrated along the first migratory flow path 14 past the test site 24 and to the control site 26. The immobilised marker-receptor 35 is not soluble or only sparingly soluble in the buffer and/or in the sample.

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The assay device 10 further comprises a sorbent wick 36 extending away from the control site 26 such that the wick 36 is the last region to where sample and/or dissolved components of the assay device and/or buffer can flow to when the assay device 10 is in use. The wick 36 serves to sorb excess sample, buffer, analyte-marker 15, analyte and/or analogues thereof, and/or any other dissolved components of the assay device 10 when in use.

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The assay device 10 further comprise an elongate rectangular parallelepipedal housing 38 to operatively accommodate the first and second sorbent members 12, 16 as shown in Figures 2a and 2b. The housing 38 is thus substantially a rectangular cuboid and typically comprises a base 40 and a lid 42. The support strip 20 is received onto the base 40 of the housing and secured in place using an adhesive substance. The base 40 and lid 42 each have complementary shaped attachment formations (not shown) to operatively secure the lid 42 and the base 40, providing for a substantially tamper proof assay device 10.

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Figure 2b shows a side sectional view through the assay device 10. The lid 42 is configured to define a sample opening 44, a buffer opening 46 (which can instead be used for the application of a liquid analyte-marker) and a viewing opening 48; the sample opening 44 being superposingly positioned above the receiving zone 28, the buffer opening 46 being superposingly positioned above a portion of the first sorbent member 12, and the viewing opening 48 being superposingly positioned above the test and control sites 24, 26. The viewing opening 48 may be covered by a clear material, preferably glass or plastic (not shown), defining a viewing window. Viewing windows are often important to avoid any contamination on the test site 24 and/or control site 26 which may lead to the assay device 10 not functioning properly. Each opening is defined by a bevelled edge of an upper surface 50 of the lid portion 42. The bevelled edge is cosmetic and ergonomic in nature, and it is to be understood that many variations may be readily conceivable by a person reasonably skilled in the art.

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In a preferred embodiment of the invention, as shown in Figure 2a, the lid 42 further defines a second buffer opening 52 adjacent to but upstream of the sample opening 44 and in fluid communication with the receiving zone 28. In use, buffer may be applied to the sample received onto the receiving zone 28 in order to facilitate migration of sample (which may or may not contain analyte) along the second migratory flow path 18 to the test site 24.

In one embodiment of the invention described the analyte-marker 15 is a gold-protein-A conjugate. It is well known in the art to utilise colloidal gold conjugates in immunochromatographic techniques to assist in providing a clear coloured positive and/or negative test result. The marker-receptor 35 which is immobilised at the control site 26 is anti-protein-A which will bind to any amount of gold-protein-A which did not bind to the analyte at the test site 24. This embodiment of the assay device 10 is used to test for anti-MTB-38 in a sample which if present in a sample should, in use, bind to the analyte-receptor 25 which is receptor protein TB-6-14-38 immobilised to the test site 24.

The analyte may be an antigen or an antibody and the analyte-receptor may be an antigen or an antibody, depending on the whether the analyte is an antigen or antibody.

Figures 3 a-c show the biochemistry taking place when the assay device 10 is in use. The analyte 45 (anti-MTB-38) reaches the test site 24 well before the analyte-marker 15 (gold-protein-A conjugate) to form analyte-receptor-complex 55 by binding to the analyte-receptor 25 (receptor protein TB-6-14-38). This is due to the fact that the second migratory path 18 is shorter in length relative the first migratory path 14, and because of the effect of gravity on the sample the analyte 45 migrates at a faster rate toward the test site 24 relative to the analyte-marker 15. Once the analyte-marker 15 reaches the analyte-receptor-complex 55 it binds to form the analyte-receptor-marker complex 65. All unbound analyte-marker 15 migrates to the control site 26 and binds with marker-receptor 35 (anti-Protein-A) to form a control-complex 75.

It is to be understood that the first and second sorbent members 12 and 14, the test site 24 and the control site 26 can be loaded with a wide variety of different analyte-markers, analytes, analyte-receptors and marker-receptors respectively. It is perfectly plausible to alter and/or vary the assay device 10 of the preferred embodiment herein

described such that the analyte is a protein and/or amino acid and/or another biological ligand, or even an inorganic substance, for example.

The assay device 10, as illustrated, provides for a user friendly, accurate and precise means to identify analyte in a sample for use by clinicians in diagnoses/prognoses of patients.

EXAMPLES

A. Materials and Methods:

i. The assay device

Analyte-marker 15 was prepared by conjugating gold nanoparticles with protein-A. The analyte-marker was then immobilised onto the first sorbent member 12 which comprises a Millipore glass fibre conjugate pad obtained from Diagnostic Consulting Network (DCN), USA.

Marker-receptor 35 in the form of anti-protein-A was immobilised onto the control and test strip 22 at the control site 26. The marker-receptor 35 is substantially permanently immobilised onto the control site 26. The control and test strip 22 comprises Nitrocellulose Whatman AE 98 fast purchased from Diagnostic Consulting Network (DCN), USA which is composed of 100% nitrocellulose.

Analyte-receptor 25 in the form of TB-6-14-38 kDa fusion protein was immobilised onto the test site 24 of the control and test strip 22. The TB-6-14-38 kDa fusion protein was bought from CTK Biotech, USA.

ii. Purified analyte test examples

In order to determine the sensitivity of the lateral flow assay device 10, various dilutions of analyte, anti-MTB-38 kDa monoclonal antibody, were prepared as the sample to be assayed. 100ng/ml; 80 ng/ml, 60 ng/ml; 40 ng/ml; 20 ng/ml; 10 ng/ml and 5ng/ml of anti-MTB-38 kDa monoclonal antibody in PBS were prepared. 10 µl of each sample concentration was then applied to the receiving zone 28 via the sample opening 44 and the sample was allowed to migrate along the second migratory flow path 18 to the test site 24. After 1 minute, Phosphate Buffered Saline was applied to the first sorbent member 12

through the buffer opening 46. The results were visually identifiable and inspected by a user of the device 10 within 10 minutes from the time the sample was applied to the receiving zone 28. For each different sample concentration test examples a fresh, never before used, assay device 10 was utilized.

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iii. Whole blood or serum matrix test examples

To further test the performance of the device 10, blood or serum matrix known to have the analyte anti-MTB-38 kDa monoclonal antibody was used as sample. 5 µl of whole blood or serum was applied on the receiving zone 28 via the sample opening 44, and immediately 2 drops (40 µl) of buffer were applied to the receiving zone 28 via the second buffer opening 52, and 1 minute later 2 drops of buffer were applied to the first sorbent member 12 via the buffer opening 46. The results were visually identifiable and inspected by a user of the device 10 within 10 minutes from the time the sample was applied to the receiving zone 28.

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In all tests that were conducted the buffer utilized was 1M Phosphate Buffered Saline (137mM NaCl, 2.7mM KCl, 8mM Na₂Cl, 1.46mM KH₂PO₄) bought from Sigma Aldrich.

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B. Results:

i. Purified analyte test examples

The analyte, anti-MTB-38 kDa monoclonal antibody, was used to evaluate the sensitivity of the test. Assays were run utilizing varying concentrations of anti-MTB-38 kDa monoclonal antibody, ranging from 0-100 ng/ml, diluted in buffer as explained above. The assay device 10 indicated positive results with all the different concentrations. The intensity of the coloured positive test provided an indication as to the concentration of analyte in the sample as shown in Figure 4a. The data was confirmed using the ESE-Quanti lateral flow reader as shown in Figure 4b. The intensities of the test and control lines of the test strips were also measured using a ESE quanti lateral flow assay strip reader to determine the intensity of the test and control lines. The intensity of the test and control lines is associated with the magnitude of the area of the resulting peaks in Figure 4b. An increase in the peak area is correlated to an increase in the intensity of the lines. At 0 ng/ml, the test line appears to have some intensity which has been treated as the background and was

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used as a blank by subtracting its value from the areas of the peaks from other concentrations. The area of the peaks increased when the concentration of the MTB 38 kda antibody was increased although some concentrations do not show much of a difference in their peak areas. The difference in the peak areas from 100ng/ml and 80ng/ml is minute because the difference between the concentrations is not that huge to elicit a great difference in peak areas from closely related concentrations, such that 80 ng/ml has a higher peak area than 100 ng/ml. It was determined that the minimum analyte concentration that the assay device was able to detect and provide a coloured positive test for was 5ng/ml.

ii. Whole blood or serum matrix test examples

Analyte (anti-MTB-38 antibody) free blood was obtained from the South African National Blood Service and used in a control test example, and blood spiked with 5ng/ml of analyte (anti-MTB-38 antibody) was used in a real test example. In each control and real test examples a fresh, never before used, assay device 10 was utilized. Figures 5 a-b show photographs of each of the two assay devices 10 after completing the real and control test examples respectively. The real test (Figure 5 a) having been conducted with an anti-MTB-38 antibody rich sample correctly reflects a visually identifiable positive result. The control test (Figure 5 b) having been conducted with anti-MTB-38 free sample correctly reflects a visually identifiable negative result.

The positive result of Figure 5a shows that the test strip 24 having analyte-receptor (TB-6-14-38 fusion protein) successfully bonded to the analyte (anti-MTB-38 antibody) and to the analyte-marker (gold conjugated protein-A) to form a visually identifiable analyte-receptor-marker complex 65. The control site 26 clearly indicates that the flow mechanics of the device 10 operated correctly since unbound analyte-marker 15 (gold conjugated protein-A) has successfully migrated from the first sorbent member 12 to the control site 26 and bonded with the marker-receptor (anti-protein-A) to form a visually identifiable control complex 75. In Figure 5b the test site remains uncoloured since there is no bound analyte for the analyte-marker to bind to, and in consequence, the analyte marker migrates further along the control and test strip 22 to bind at the control site 26 with the marker-receptor.

Free blood was obtained from the South African National Blood Service and used in a control test example, and blood spiked with 5ng/ml of analyte (anti-MTB-38 antibody) was used in a real test example. 5 μ l of the blood samples was applied on the receiving zone 28 via the sample opening 44, and immediately 2 drops (40 μ l) of buffer were applied to the receiving zone 28 via the second buffer opening 52. One minute later 2 drops of buffer were applied to the first sorbent member 12 via the buffer opening 46. The results were visually identifiable and inspected by a user of the device 10 within 10 minutes from the time the sample was applied to the receiving zone 28.

The above examples demonstrate the use of an accurate, precise and user friendly assay device 10. The assay device 10, as illustrated, has improved sensitivity due to the fact that the analyte migrates independently of the analyte-marker, since it binds first to the test site 24 prior to reacting with the analyte-marker owing to the mechanical construction of the device 10. Therefore, there is more effective binding of the analyte to the test site 24 and hence increased sensitivity of the test. It was also observed that the device 10 has enhanced background clearance due to improved uniformity in the migration of analyte-marker in the absence of the sample analyte and therefore, it is easier to interpret the results of the assay device 10, in use. The assay device 10, as illustrated, being strip-like or bar-like, is compact and easy to transport stacked or bound together with like devices in a bundle. The assay device 10, as illustrated, is also easy to manufacture and assemble, and requires limited skills to use.



06/05/2013

CLAIMS:

1. An assay device to detect an analyte or analogue thereof in a sample, the assay device including:

5 a first sorbent member having an analyte-marker to mark the analyte, or configured to receive a liquid analyte-marker, the first sorbent member including or defining a first elongate migratory flow path;

10 a second sorbent member to receive the sample, the second sorbent member including or defining a second elongate migratory flow path, the second migratory flow path being operatively above or below or adjacent the first migratory flow path over at least a major portion of the length of the second migratory flow path and being shorter in length relative to the first migratory flow path; and

15 a test site in migratory flow communication with both the first sorbent member and the second sorbent member by means of the first migratory flow path and the second migratory flow path respectively, the test site having an analyte-receptor to bind analyte in the sample.

20 2. The assay device according to claim 1, which includes a control site in migratory flow communication with the first sorbent member, the second sorbent member and the test site, the control site having a marker-receptor to bind analyte-marker to form a control-complex, the control site, the test site, the second sorbent member and the first sorbent member all being positioned along a common linear axis and the control site being located downstream of the test site in a direction of migratory flow in use.

25 3. The assay device according to claim 1 or claim 2, in which the second migratory flow path is operatively above the first migratory flow path and above an operative level of the test site over at least a major portion of the length of the second migratory flow path, the second migratory flow path having a descending portion dropping towards said operative level of the test site.

30 4. The assay device according to any of claims 1 to 3, in which the second sorbent member is operatively supported at least partially on top of the first sorbent member, with a liquid impermeable barrier member being located or sandwiched between the first sorbent member and the second sorbent member.

5. The assay device according to any of claims 1 to 4, in which the second sorbent member includes or defines a receiving zone distal the test site in use to receive sample, and a migration zone defining said second elongate migratory flow path proximal the test site, the migration zone extending away from the receiving zone toward the test site, the receiving zone and the migration zone being of different sorbent materials.

6. The assay device according to any of claims 1 to 5, in which the first sorbent member and the second sorbent member are supported on a common elongate linear support, the elongate linear support being operatively below and in register with both the first elongate migratory flow path and the second elongate migratory flow path.

7. The assay device according to any of claims 1 to 6, which includes a housing which houses at least the first sorbent member and the second sorbent member and the test site and which encloses the first migratory flow path and the second migratory flow path, the housing defining a sample opening above, and in fluid communication with, the second sorbent member, a buffer or liquid analyte-marker opening above, and in fluid communication with, the first sorbent member and a viewing opening or viewing window above the test site, the housing being generally cylindrical.

8. The assay device according to claim 7, in which the housing defines a further buffer opening adjacent to the sample opening and in fluid communication with the second sorbent member, in use, to receive buffer to facilitate the migration of sample along the second elongate migratory flow path toward the test site.

9. The assay device according to any of claims 1 to 8, which includes a sorbent wick operatively downstream of the test site to sorb, in use, excess sample, buffer, analyte-marker and/or analyte or analogues thereof, the first sorbent member, the second sorbent member, the test site and the sorbent wick all being positioned along a common linear axis although not all necessarily in the same horizontal plane.

10. The assay device according to any of claims 1 to 9 which is an anti-MTB-38 antibodies detection device wherein the analyte-receptor is TB-6-14-38 and the analyte-marker is colloidal gold-protein-A-conjugate.

11. The assay device according to claim 2 which is an anti-MTB-38 antibodies detection device wherein the analyte-receptor is TB-6-14-38, the analyte-marker is colloidal gold-protein-A-conjugate and the marker-receptor is anti-protein-A.

5 12. A method of determining presence of an analyte in a sample, the method including:

applying buffer to a first sorbent member having an analyte-marker to mark the analyte, or applying a liquid analyte-marker to a first sorbent member configured to receive a liquid analyte-marker;

10 allowing the analyte-marker to migrate along a first elongate migratory flow path to a test site having an analyte-receptor to bind analyte in the sample;

applying said sample to a second sorbent member;

15 allowing the sample to migrate along a second elongate migratory flow path to said test site, the second migratory flow path being operatively above or below or adjacent the first migratory flow path over at least a major portion of the length of the second migratory flow path and being shorter in length relative to the first migratory flow path;

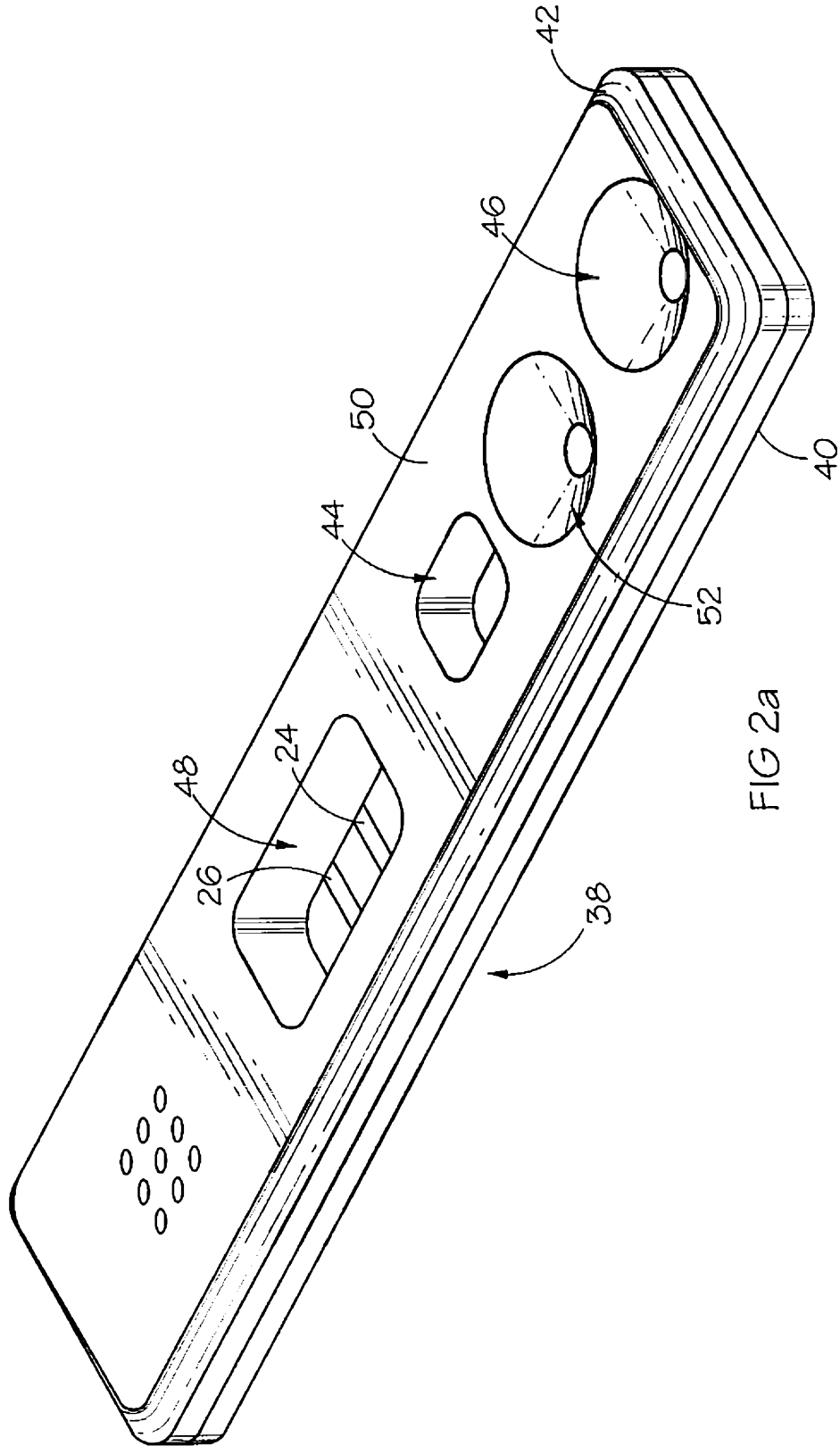
allowing any analyte present to bind to the analyte-receptor to form an analyte-receptor complex; and

20 allowing the analyte-marker to bind to any analyte-receptor complex to form a visually identifiable analyte-receptor-marker complex.

13. The method according to claim 12, which includes ensuring that the sample reaches the test site before the analyte-marker reaches the test site.

25 14. The method according to claim 13, in which the second migratory flow path is operatively above the first migratory flow path and above an operative level of the test site over at least a major portion of the length of the second migratory flow path, the second migratory flow path having a descending portion dropping towards said operative level of the test site.

30 15. The method according to any of claims 12 to 14, which includes applying buffer to the second sorbent member to facilitate ease of sample migration along the second migratory pathway toward the test site, the buffer being applied to the second sorbent member at a location further from the test site than the sample was applied.



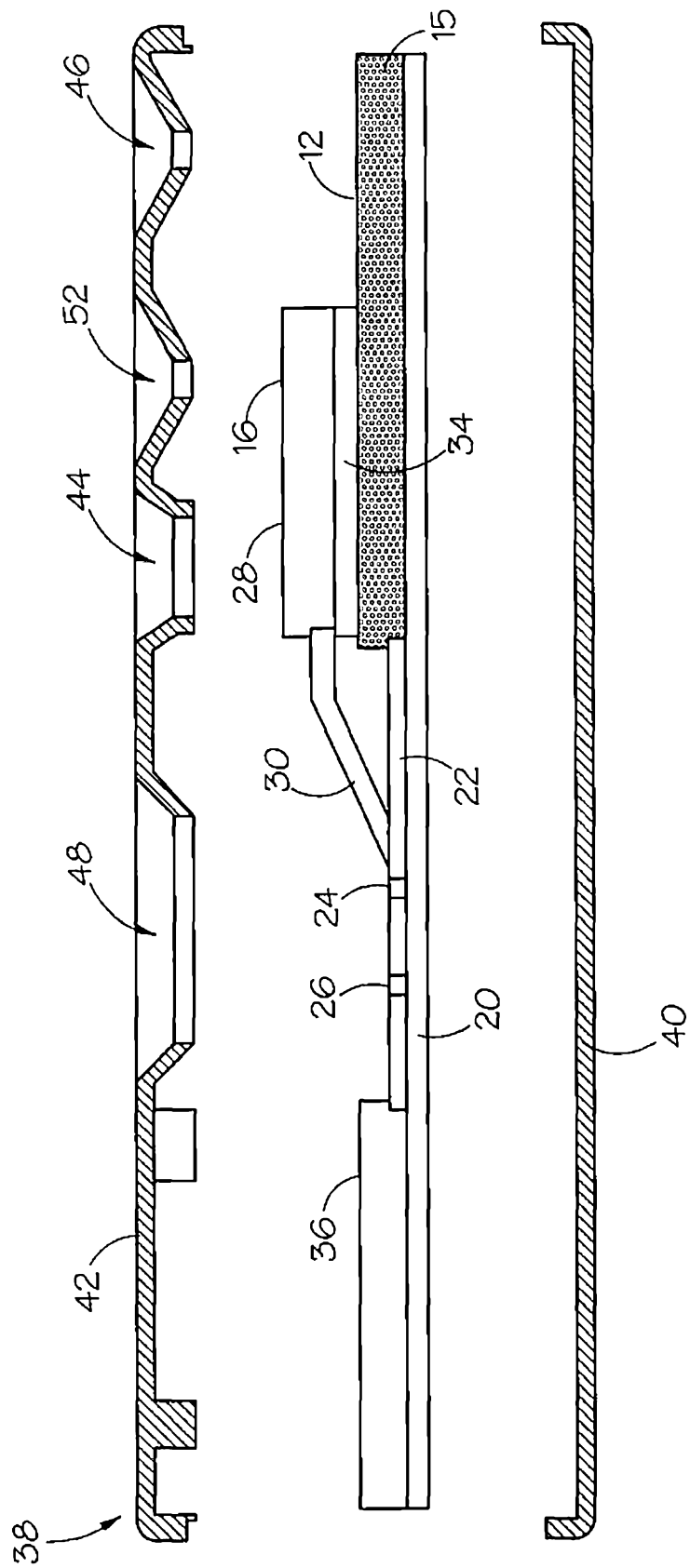


FIG 2b

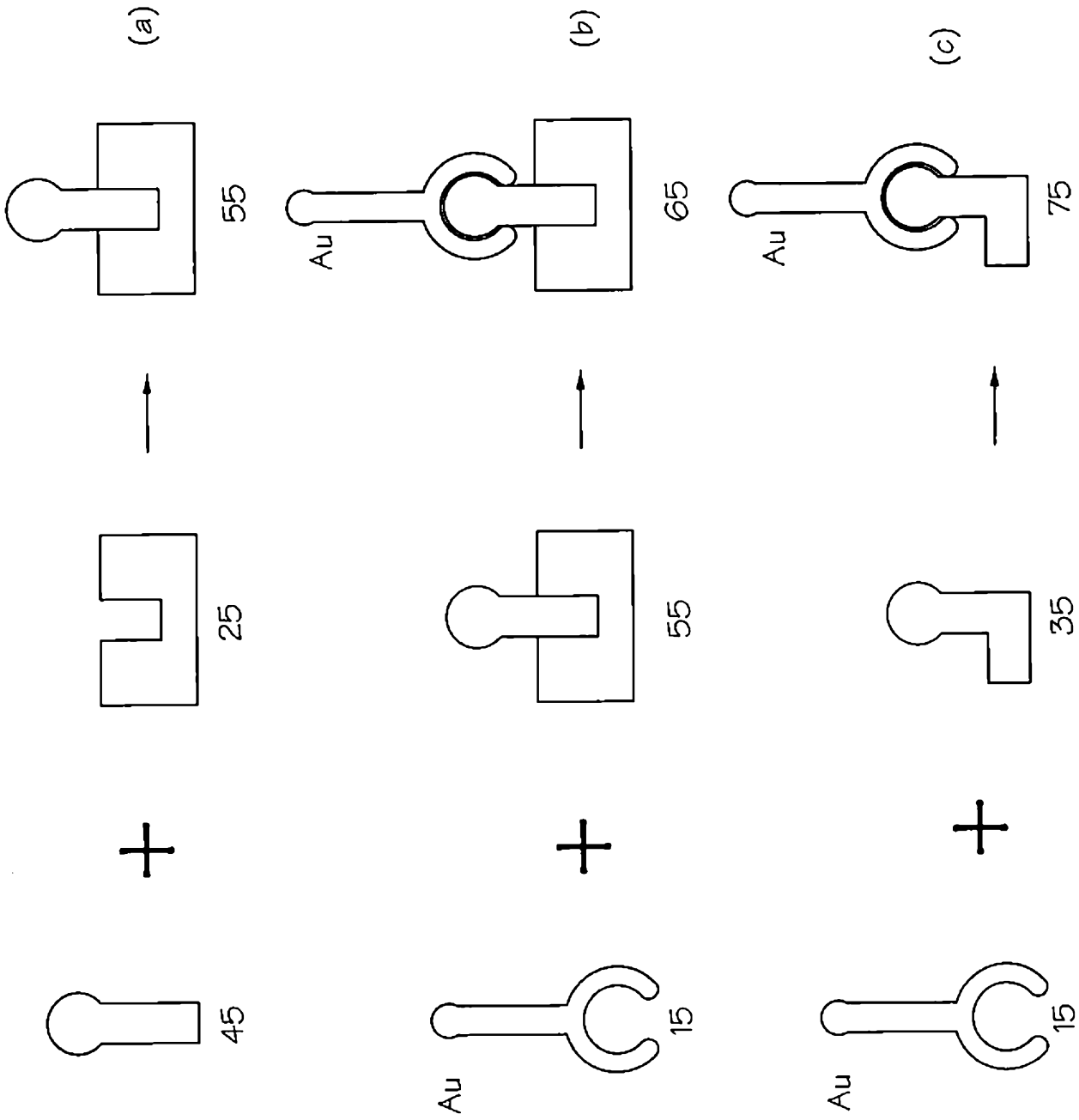


FIG 3

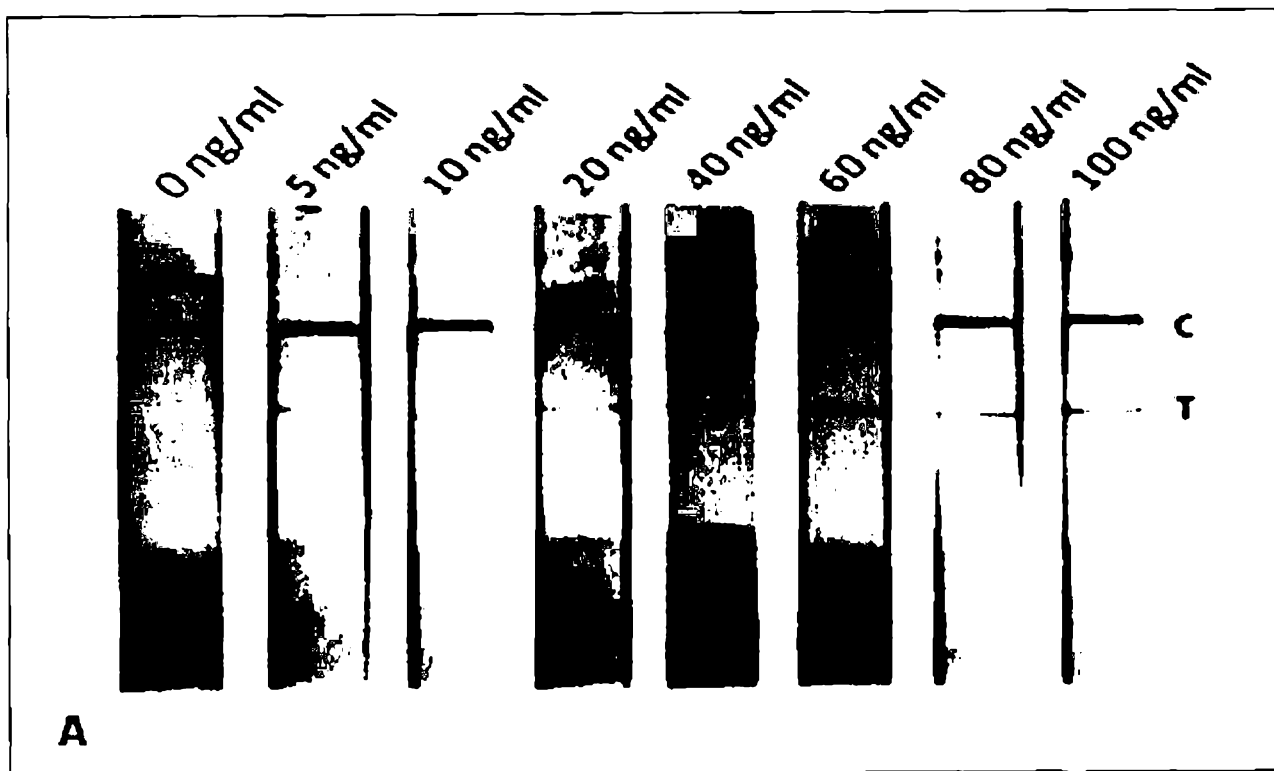


Figure 4a

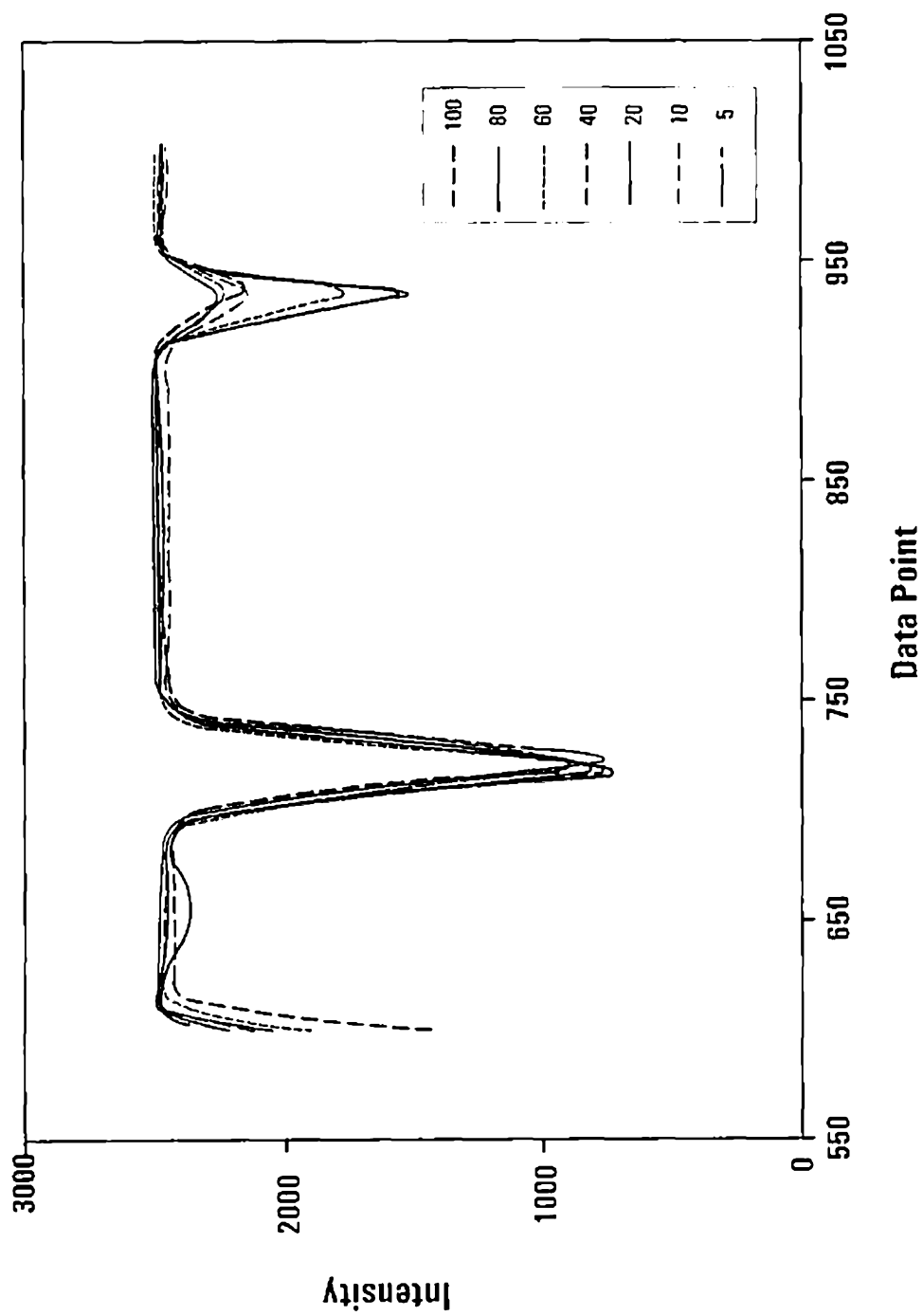


Figure 4b

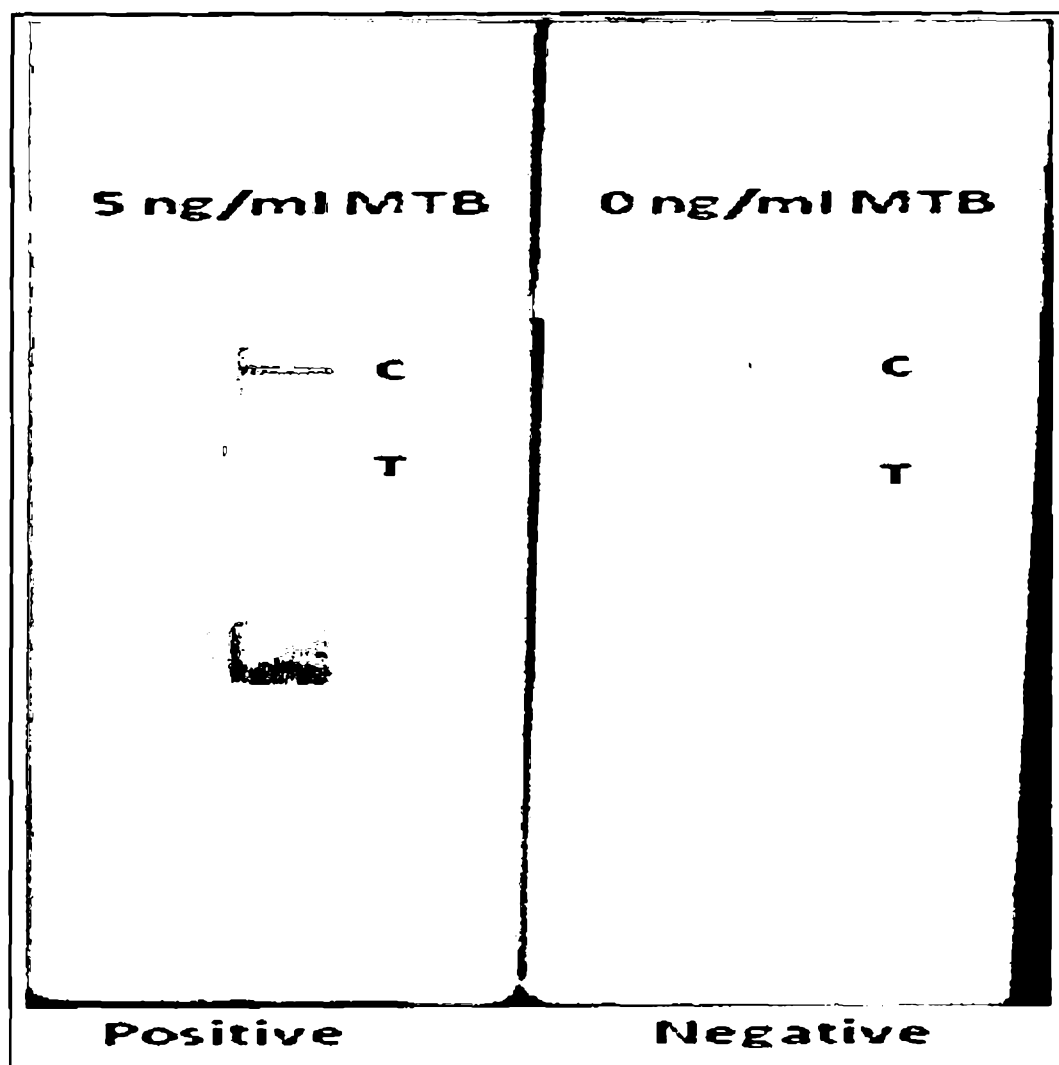


Figure 5a and b