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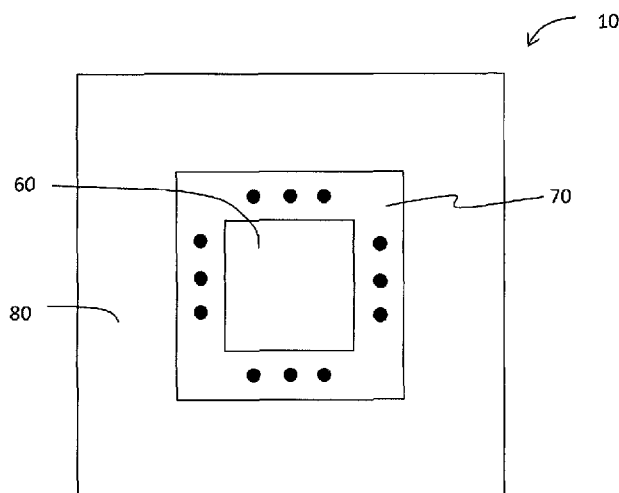


Fig. 1

(57) Abstract: A test kit comprising: a sample pad provided in a first layer and configured to receive thereon a fluid; a capture membrane provided in a second layer and configured to absorb the fluid from the sample pad and to capture at least one analyte in the fluid, the capture membrane provided around the sample pad wherein an outer periphery of the sample pad overlaps with an inner periphery of the capture membrane; and an absorbent pad configured to absorb the fluid from the capture membrane, the absorbent pad provided around the capture membrane wherein an outer periphery of the capture membrane overlaps with an inner periphery of the absorbent pad; wherein the fluid when placed on the sample pad flows radially outwards from the sample pad into the capture membrane and from the capture membrane radially outwards into the absorbent pad.



TEST KIT

FIELD OF THE INVENTION

This invention relates to a test kit, and in particular, to a disposable flow test kit for analyzing a fluid analyte.

BACKGROUND OF THE INVENTION

Test kits such as home pregnancy test kits or laboratory tests kits using bodily fluids such as urine or blood to detect or diagnose disease or other medical conditions are in common use throughout the world. Such test kits typically comprise a test strip sealed in a moisture barrier packaging before use in order to keep the test strip stable and protected during storage and transportation, to extend the shelf life of the test kit. The moisture barrier packaging typically comprises a foil bag or pouch. The test kit may additionally be encased in a plastic cassette for easier handling by the end user, in which case the test strip and the cassette are both sealed in the moisture barrier before use. Where the test kit includes a cassette, the cassette is typically provided with an open reaction window that allows one or more test lines on the test strip to be viewed during use.

A variety of test kits have been developed to allow a sample collection portion of the test kit strip to receive the fluid analyte either via a drop collection or dipstick/mid-stream collection method. A large portion of these devices detect the presence of specific components of the analyte by use of lateral flow techniques.

Lateral flow devices typically comprise a sample application area, a reaction capture membrane typically made of nitrocellulose located downstream of the sample application area and an absorption pad located downstream of the reaction capture membrane. The reaction capture membrane typically contains non-immobilized specific antibodies to the target analyte conjugated to coloured particles located in a discrete position downstream from the sample application area and then at least two discrete capture sites further downstream onto which anti-target analyte antibodies are immobilized to act as a capture zone for the specific antibody being tested for and to act as a control zone to capture the non-immobilized antibodies conjugated to coloured particles. The absorbent pad is placed furthest downstream and is designed to draw the analyte from the sample pad onto and through the reaction capture membrane and collect it by capillary action. As the analyte is drawn along the reaction pad, the specific antibody in the analyte binds to non-immobilized antibodies conjugated to coloured particles. The fluid analyte then continues to flow over or through the two capture sites and specific binding occurs. The fluid analyte

may then continue to flow to the absorbent pad. Results are obtained by observation of the capture sites for specific binding. Accumulation of the coloured particles is determined visually and is indicative of a positive test and control test result.

Lateral flow methods may be used not only for detecting a single analyte per test strip but multiple analytes may also be tested for using a lateral flow device by placing multiple capture sites downstream from each other in the path of the fluid flow. Such an arrangement of multiple capture sites in succession downstream from each other can give rise to problems. As the analyte flows on from one capture site to the next, cross-contamination can occur and this affects the sensitivity of the tests and/or can lead to false negative or false positive results. Additionally, the upstream capture sites may detrimentally remove an analyte or reduce the amount of the analyte in the fluid sample that is required for one of the subsequent reactions sites located downstream. As such, the test kits must be very sensitive as the concentration of analyte of interest in the sample fluid may be small.

There is thus a need to provide a rapid, sensitive method for detecting multiple analytes in a fluid sample that minimizes or avoids the problem of cross-contamination that exists in currently available test kits for multiple analytes.

SUMMARY OF INVENTION

There is provided a test kit comprising: a sample pad provided in a first layer and configured to receive thereon a fluid; a capture membrane provided in a second layer and configured to absorb the fluid from the sample pad and to capture at least one analyte in the fluid, the capture membrane provided around the sample pad wherein an outer periphery of the sample pad overlaps with an inner periphery of the capture membrane; and an absorbent pad configured to absorb the fluid from the capture membrane, the absorbent pad provided around the capture membrane wherein an outer periphery of the capture membrane overlaps with an inner periphery of the absorbent pad; wherein the fluid when placed on the sample pad flows radially outwards from the sample pad into the capture membrane and from the capture membrane radially outwards into the absorbent pad.

The second layer may be provided below the first layer. A central portion of the second layer under the sample pad may be non-absorbent.

Alternatively, the second layer may be provided above the first layer. A portion of the first layer under the capture membrane may be non-absorbent.

The absorbent pad may be provided in the first layer at an outer periphery of the first layer and spaced apart from the absorbent pad. The first layer may comprise a non-absorbent spacer provided around the sample pad between the sample pad and the absorbent pad.

Alternatively, the absorbent pad may be provided in a third layer below the second layer. A central portion of the third layer under the capture membrane may be non-absorbent.

Alternatively, the absorbent pad may be provided in a third layer above the second layer. An outer portion of the second layer under the absorbent pad may be non-absorbent.

The capture membrane may be provided around the sample pad as a continuous band.

Alternatively, the capture membrane may comprise a number of membrane portions provided around the sample pad, the number of membrane portions spaced apart from each other by non-absorbent portions provided around the sample pad.

The absorbent pad may be provided around the capture membrane as a continuous band.

Alternatively, the absorbent pad may comprise a number of absorbent portions provided around the capture membrane, the number of absorbent portions spaced apart from each other by non-absorbent portions provided around the capture membrane.

The capture membrane may comprise a number of discrete capture sites spaced apart from each other around the sample pad.

The sample pad may comprise a plurality of layers.

BRIEF DESCRIPTION OF FIGURES

In order that the invention may be fully understood and readily put into practical effect there shall now be described by way of non-limitative example only exemplary embodiments of the present invention, the description being with reference to the accompanying illustrative drawings.

Fig. 1 is a top view of a disposable test kit.

Fig. 2 is a schematic cross-sectional view of a first exemplary embodiment of the test kit of Fig. 1.

Fig. 3 is a schematic cross-sectional view of a second exemplary embodiment of the test kit of Fig. 1.

Fig. 4 is a schematic cross-sectional view of an alternative second exemplary embodiment of the test kit of Fig. 1.

Fig. 5 is a schematic cross-sectional view of a further alternative configuration of the test kit of Fig. 1.

Fig. 6 is a schematic cross-sectional view of a third exemplary embodiment of the test kit of Fig. 1.

Fig. 7 is a schematic cross-sectional view of a fourth exemplary embodiment of the test kit of Fig. 1.

Fig. 8 is a top view of a fifth exemplary embodiment of the test kit.

Fig. 9 is a top view of a sixth exemplary embodiment of the test kit.

DETAILED DESCRIPTION

Exemplary embodiments of the test kit 10 will be described below with reference to Figs. 1 to 9. The same reference numerals are used throughout the figures to denote the same or similar parts among the various embodiments.

As shown in Figs. 1 to 9, the test kit 10 comprises a sample pad 60 configured to receive a fluid thereon, a capture membrane 70 provided around the sample pad 60 and configured to capture at least one analyte in the fluid and, and an absorbent pad 80 provided around the capture membrane 70 and configured to absorb the fluid that has passed through the capture membrane 70.

In a first embodiment as shown in Fig. 2, the sample pad 60, the capture membrane 70, and the absorbent pad 80 are provided in individual layers 61, 71, 81 respectively. The three layers 61, 71, 81 are preferably three concentric layers of a same shape of increasing size respectively. The second layer 71 is provided below the first layer 61 and the third layer 81 is provided below the second layer 71. The first layer 61 preferably further comprises a conjugate pad (not shown) adjacent the sample pad 60 for a coloured conjugate to bond with the at least one analyte in the fluid. The second layer 71 comprises a non-absorbent central portion 73 under the sample pad 60. The central portion 73 of the second layer is surrounded by the capture membrane 70 which is provided as a

continuous band at the outer periphery of the second layer 71. The central portion 73 has a smaller area than the area of the sample pad 60 so that an outer periphery of the sample pad 60 overlaps with an inner periphery of the capture membrane 70 to form a first interface 67 between the sample pad 60 and the capture membrane 70.

The third layer 81 comprises a non-absorbent central portion 83 under the capture membrane 70. The central portion 83 of the third layer is surrounded by the absorbent pad 80 which is provided as a continuous band at the outer periphery of the third layer 81. The central portion 83 of the third layer 81 has a smaller area than the area covered by the capture membrane 70 and the central portion 73 of the second layer 71 so that an outer periphery of the capture membrane 70 overlaps with an inner periphery of the absorbent pad 80 to form a second interface 78 between the capture membrane 70 and the absorbent pad 80. The sample pad 60, the capture membrane 70, and the absorbent pad 80 are thus configured to be in fluid communication with each other through the first and second interfaces 67 and 78. Both the central portions 73, 83 of the second and third layers 70, 80 respectively may be a void or any suitable non-absorbent material.

In the first embodiment, the sample pad 60, the capture membrane 70, and the absorbent pad 80 are in three different planes in three layers 61, 71, 81 respectively. At the first interface 67, a lower surface of the sample pad 60 is in contact with an upper surface of the capture membrane 70. At the second interface 78, a lower surface of the capture membrane 70 is in contact with an upper surface of the absorbent pad 80.

The first embodiment also preferably comprises a waterproof backing layer 90 under the third layer 81.

In a second embodiment as shown in Fig. 3, the sample pad 60 and the absorbent pad 80 are provided in a same first layer 68, and the capture membrane 70 is provided in a second layer 71 under the first layer 68. The two layers 68, 71 are preferably two concentric layers of a same shape of decreasing size respectively. The second layer 71 is provided below the first layer 68. The first layer 68 comprising the sample pad 60 and the absorbent pad 80 preferably further comprises a conjugate pad (not shown) adjacent the sample pad 60 for a coloured conjugate to bond with the at least one analyte in the fluid.

The first layer 68 may optionally comprise a non-absorbent spacer 683 that is provided as a continuous band around the sample pad 60. The absorbent pad 80 is provided as a

continuous band around the spacer 683 at the outer periphery of the first layer 681. The spacer 683 thus separates the sample pad 60 from the absorbent pad 80 such that fluid cannot flow directly from the sample pad 60 to the absorbent pad 80.

The second layer 71 comprises a non-absorbent central portion 73 surrounded by the capture membrane 70 that is provided as a continuous band around the non-absorbent central portion 73. The non-absorbent central portion 73 of the second layer has a smaller area than the area of the sample pad 60 so that an outer periphery of the sample pad 60 overlaps with an inner periphery of the capture membrane 70 to form a first interface 67 between the sample pad 60 and the capture membrane 70. The central portion 73 may be a void or any suitable non-absorbent material.

The second layer 71 may further comprise an outer portion of non-absorbent material 75 around the capture membrane 70 to support the absorbent pad 80 on the first layer 68 above the second layer 71.

The area covered by the capture membrane 70 and the central portion 73 of the second layer 71 is larger than the area covered by the sample pad 60 and the spacer 683 of the first layer 681, so that an outer periphery of the capture membrane 70 overlaps with an inner periphery of the absorbent pad 80 to form a second interface 78 between the capture membrane 70 and the absorbent pad 80. The sample pad 60, the capture membrane 70, and the absorbent pad 80 are thus configured to be in fluid communication with each other through the first and second interfaces 67 and 78 respectively.

In the second embodiment, the sample pad 60 and the absorbent pad 80 are in a same plane in the layer 68 and the capture membrane 70 is in a different plane in the layer 71. At the first interface 67, a lower surface of the sample pad 60 is in contact with an upper surface of the capture membrane 70. At the second interface 78, an upper surface of the capture membrane 70 is in contact with a lower surface of the absorbent pad 80.

The second embodiment also preferably comprises a waterproof backing layer 90 under the second layer 71.

In an alternative configuration of the second embodiment as shown in Fig. 4, no non-absorbent spacer is provided in the first layer 68. Instead, the absorbent pad 80 may

simply be provided on the second layer 71 in a spaced apart arrangement from the sample pad 60.

In a further alternative configuration of the test kit 10 as shown in Fig. 5, the sample pad 60 may comprise a plurality of layers 60-1, 60-2. The plurality of layers 60-1, 60-2 of the sample pad 60 may be made of a same or different materials.

In a third embodiment of the test kit 10 as shown in Fig. 6, the second layer 71 comprising the capture membrane 70 is provided above the first layer 61 comprising the sample pad 60 so that an outer periphery of the sample pad 60 overlaps with an inner periphery of the capture membrane 70 to form a first interface 67 between the sample pad 60 and the capture membrane 70. An outer portion 65 of the first layer 61 is non-absorbent and provided under the capture membrane 70 to support the capture membrane 70. The third layer 81 comprising the absorbent pad 80 is provided above the second layer 71 so that an outer periphery of the capture membrane 70 overlaps with an inner periphery of the absorbent pad 80 to form a second interface 78 between the capture membrane 70 and the absorbent pad 80. An outer portion of the second layer 71 that is non-absorbent is provided under the absorbent pad 80 to support the absorbent pad 80.

In the third embodiment, at the first interface 67, an upper surface of the sample pad 60 is in contact with a lower surface of the capture membrane 70. At the second interface 78, an upper surface of the capture membrane 70 is in contact with a lower surface of the absorbent pad 80. The sample pad 60, the capture membrane 70, and the absorbent pad 80 are thus configured to be in fluid communication with each other through the first and second interfaces 67 and 78 respectively.

The third embodiment also preferably comprises a waterproof backing layer 90 under the first layer 61.

In a fourth embodiment of the test kit 10 as shown in Fig. 7, the second layer 71 comprising the capture membrane 70 is provided as a continuous band above the first layer 61 comprising the sample pad 60 so that an outer periphery of the sample pad 60 overlaps with an inner periphery of the capture membrane 70 to form a first interface 67 between the sample pad 60 and the capture membrane 70. A non-absorbent portion 65 around the sample pad 60 is provided in the first layer 61 under the capture membrane 70 to support the capture membrane 70.

The absorbent pad 80 is provided as a continuous band at an outer periphery of the first layer 61, spaced apart from the sample pad 60, around the non-absorbent portion 65, so that an outer periphery of the capture membrane 70 overlaps with an inner periphery of the absorbent pad 80 to form a second interface 78 between the capture membrane 70 and the absorbent pad 80.

In the fourth embodiment, at the first interface 67, an upper surface of the sample pad 60 is in contact with a lower surface of the capture membrane 70. At the second interface 78, a lower surface of the capture membrane 70 is in contact with an upper surface of the absorbent pad 80. The sample pad 60, the capture membrane 70, and the absorbent pad 80 are thus configured to be in fluid communication with each other through the first and second interfaces 67 and 78 respectively. The sample pad 60 and absorbent pad 80 are in the same plane while the capture membrane is in a different plane 70.

The fourth embodiment also preferably comprises a waterproof backing layer 90 under the first layer 61.

In a fifth embodiment as shown in Fig. 8, the capture membrane may comprise a number of membrane portions 79 provided around the sample pad 60, the membrane portions 79 being spaced apart from each other by non-absorbent portions 97 provided around the sample pad 60. Similarly, the absorbent pad may comprise a number of absorbent portions 89 provided around the capture membrane 70, the absorbent portions 89 being spaced apart by non-absorbent portions 98 provided around the capture membrane 70.

Alternatively, in a sixth embodiment as shown in Fig. 9, the absorbent pad 80 may continue to be provided as a continuous band around the membrane portions 79 of the capture membrane and the non-absorbent portions 97 between the membrane portions 79.

In all embodiments, a fluid when placed on the sample pad 60 flows radially outwards from the sample pad 60 into the capture membrane 70 and from the capture membrane 70 radially outwards into the absorbent pad 80. In use, a sample of a fluid suspected to contain at least one analyte is placed on the sample pad 60. The sample pad 60 having a porous structure allows the fluid to diffuse through it and flow radially outwards from the point of application. The sample pad 60 being in direct contact with the capture membrane 70 at the interface 67 allows the fluid to flow radially outwards from the sample pad 60 into

the capture membrane 70. The capture membrane 70 comprising of a wholly or partially porous material, for example nitrocellulose, allows the fluid to flow through the capture membrane 70 and through the number of discrete capture sites 74 in the capture membrane 70. The capture membrane 70 being in direct contact with the absorbent pad 80 at the interface 78 allows the fluid to continue to flow radially outwards from the capture membrane 70 into the absorption pad 80.

In all embodiments, the capture membrane 70 comprises a number of discrete capture sites (depicted for illustration purposes only as black dots) that are spaced apart from each other around the sample pad 60, as shown in in Figs. 1, 4 and 5. Present at each capture site 74 is an immobilized reagent or antibody capable of binding with a specific analyte in the fluid that flows through the capture membrane 70. Due to the outwards radial flow of the fluid from the point of application of the sample of the fluid on the sample pad 60, each of the number of discrete capture sites 74 in the capture membrane 70 receives its own portion of the fluid that has not come into contact with any other capture site 74 in the capture membrane 70. In this way, contamination and cross-talk between capture sites 74 is avoided.

It should be noted that in Figs. 1, 4 and 5 where the capture sites have been depicted as black dots, these are only for illustration purposes as the capture sites 74 would normally be invisible before use of the test kit 10. Furthermore, although the capture sites have been depicted as black dots, the actual mark that appears on each of the capture sites may be any desired colour and shape, e.g., a line, a spot, that appears during use of the test kit if an analyte that the test kit is configured to test for is present in the fluid being tested. The capture sites may be located at any appropriate location on the capture membrane as may be desired besides the locations shown in the figures. The capture sites may also comprise one or more control sites where at least one mark of any desired shape and colour appears during use of the test kit to indicate that the test valid.

The disposable test kit preferably further comprises a base sheet (not shown) provided as a bottom-most layer of the test kit, a sealing sheet having a sample collection opening (not shown) provided above the layer having the sample pad, and a top sheet (not shown) configured to be attached to the sealing sheet before use of the test kit to keep the sample collection opening sealed. The top sheet is further configured to be at least partially detached from the sealing sheet to open the sample collection opening during use of the test kit. The sample collection opening is preferably sealed by a cover (not shown) before

use of the test kit. The cover is attached to the top sheet such that at least partially detaching the top sheet from the sealing sheet detaches the cover from the sealing sheet to open the collection opening. The cover is preferably integral with or part of the sealing sheet before use of the test kit. A transparent reaction window may be provided in the sealing sheet to allow the capture sites to be viewed. The top sheet may be only partially layered over the sealing sheet without covering the reaction window so no transparent portion needs be provided in the stop sheet. Where the top sheet is fully layered over the sealing sheet, the top sheet should accordingly be provided with a transparent portion over the reaction window.

It is envisaged that the disposable test kit 10 may be provided as part of a microfluidic device wherein the sample pad 60 of the disposable test kit 10 is in fluid connection with a microfluidic channel of the microfluidic device such that a fluid sample from the microfluidic device may be directly applied to the sample pad 60.

Whilst there has been described in the foregoing description exemplary embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations in details of design, construction and/or operation may be made without departing from the present invention. For example, although the layers of the disposable test kit have been illustrated as being concentric squares or circles, the layers may have any other shapes such as triangles or hexagons, etc. Although the layers of the disposable test kit have been depicted as concentric layers of various size, the layers may be of the same size so long as an outer periphery of the sample pad overlaps with an inner periphery of the capture membrane and an outer periphery of the capture membrane overlaps with an inner periphery of the absorbent pad. In the embodiments where the capture membrane and/or the absorbent pad are provided as a number of membrane portions and/or a number of absorbent portions, the size, shape and quantity of the membrane portions and/or absorbent portions may be varied from the depictions in Figs. 8 and 9 in any way that may be desired according to test or tests that that the test kit is configured for.

CLAIMS

1. A test kit comprising:
 - a sample pad provided in a first layer and configured to receive thereon a fluid;
 - a capture membrane provided in a second layer and configured to absorb the fluid from the sample pad and to capture at least one analyte in the fluid, the capture membrane provided around the sample pad wherein an outer periphery of the sample pad overlaps with an inner periphery of the capture membrane; and
 - an absorbent pad configured to absorb the fluid from the capture membrane, the absorbent pad provided around the capture membrane wherein an outer periphery of the capture membrane overlaps with an inner periphery of the absorbent pad;
 - wherein the fluid when placed on the sample pad flows radially outwards from the sample pad into the capture membrane and from the capture membrane radially outwards into the absorbent pad.
2. The test kit of claim 1, wherein the second layer is provided below the first layer.
3. The test kit of claim 2, wherein a central portion of the second layer under the sample pad is non-absorbent.
4. The test kit of claim 1, wherein the second layer is provided above the first layer.
5. The test kit of claim 4, wherein a portion of the first layer under the capture membrane is non-absorbent.
6. The test kit of any preceding claim, wherein the absorbent pad is provided in the first layer at an outer periphery of the first layer and spaced apart from the sample pad.
7. The test kit of claim 6, wherein the first layer comprises a non-absorbent spacer provided around the sample pad between the sample pad and the absorbent pad.
8. The test kit of claim 2 or claim 3, wherein the absorbent pad is provided in a third layer below the second layer.
9. The test kit of claim 8, wherein a central portion of the third layer under the capture membrane is non-absorbent.

10. The test kit of claim 4 or claim 5, wherein the absorbent pad is provided in a third layer above the second layer.
11. The test kit of claim 10, or any one of claims 6 and claim 7 when dependent on claim 2 or claim 3, wherein an outer portion of the second layer under the absorbent pad is non-absorbent.
12. The test kit of any preceding claim, wherein the capture membrane is provided around the sample pad as a continuous band.
13. The test kit of any one of claims 1 to 11, wherein the capture membrane comprises a number of membrane portions provided around the sample pad, the number of membrane portions spaced apart from each other by non-absorbent portions provided around the sample pad.
14. The test kit of any preceding claim, wherein the absorbent pad is provided around the capture membrane as a continuous band.
15. The test kit of any one of claims 1 to 13, wherein the absorbent pad comprises a number of absorbent portions provided around the capture membrane, the number of absorbent portions spaced apart from each other by non-absorbent portions provided around the capture membrane.
16. The test kit of any preceding claim, wherein the capture membrane comprises a number of discrete capture sites spaced apart from each other around the sample pad.
17. The test kit of any preceding claim, wherein the sample pad comprises a plurality of layers.

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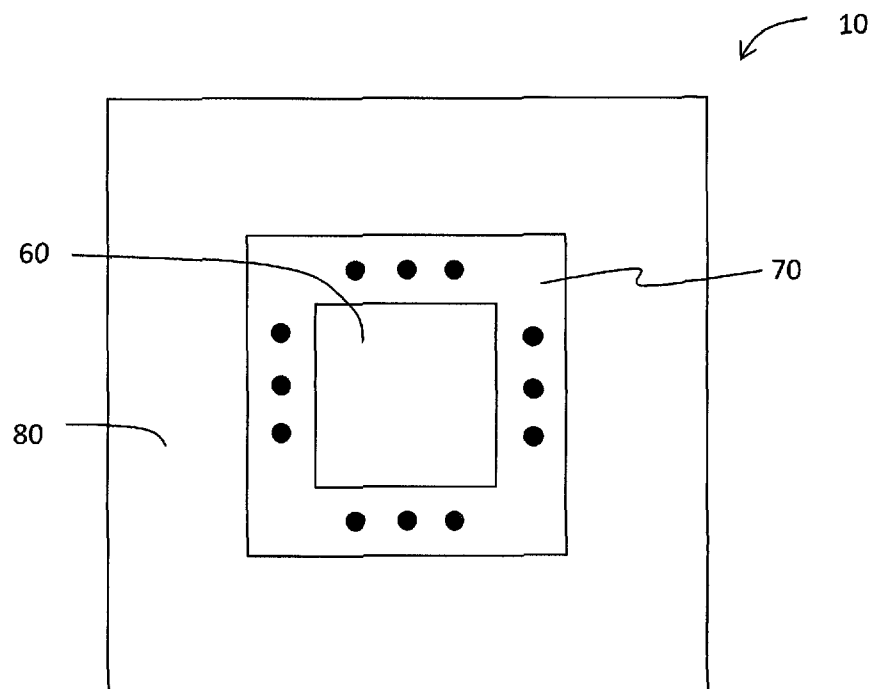


Fig. 1

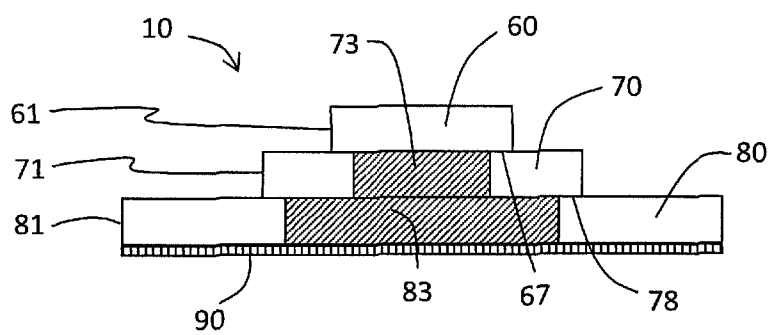


Fig. 2

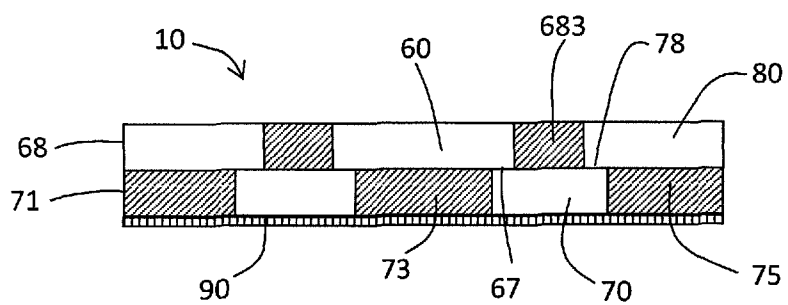


Fig. 3

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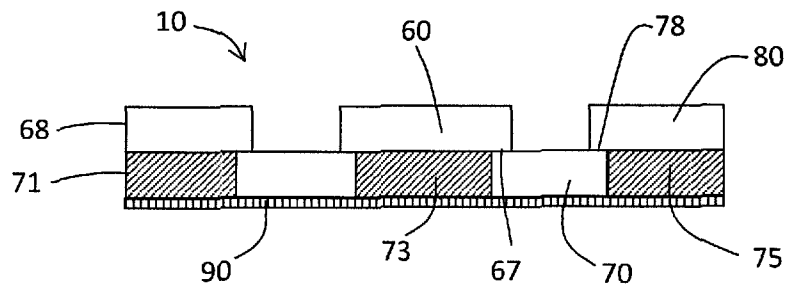


Fig. 4

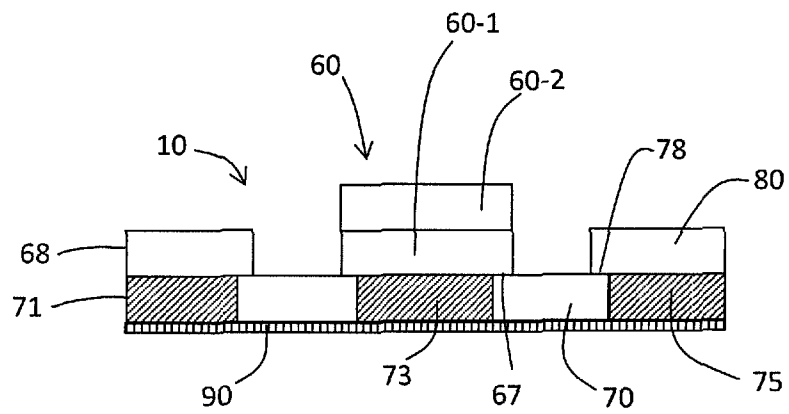


Fig. 5

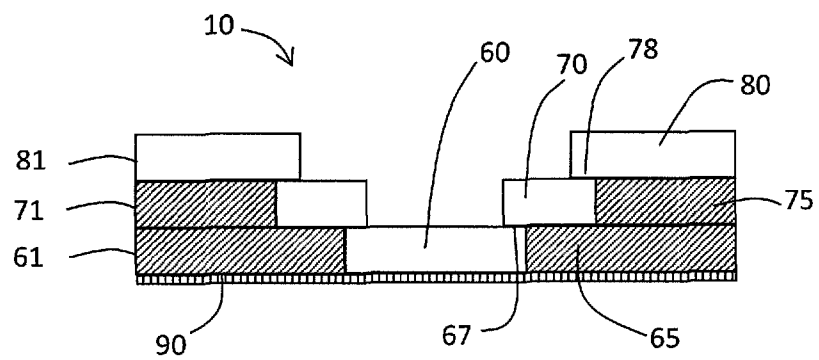


Fig. 6

3 / 3

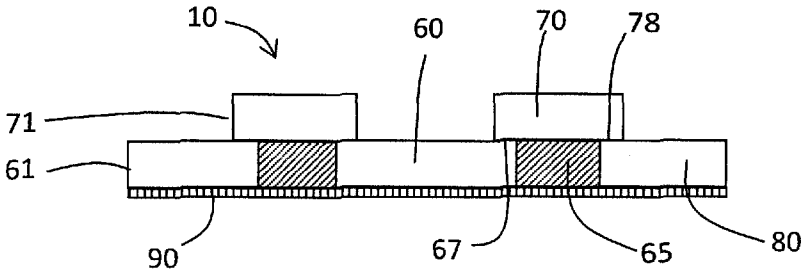


Fig. 7

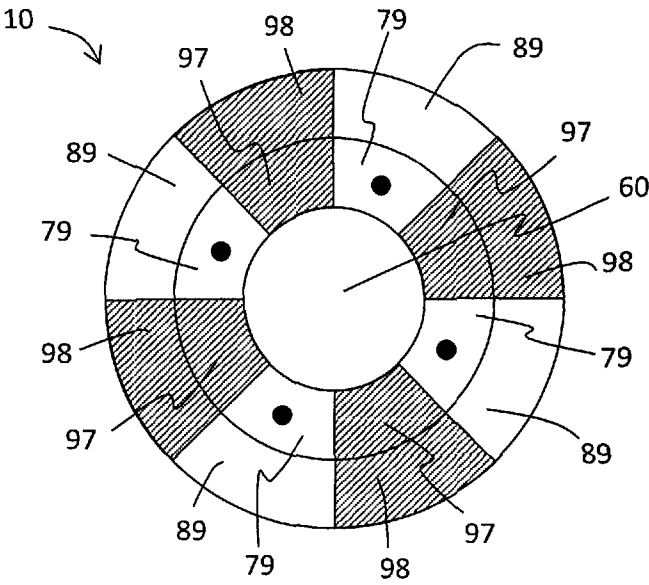


Fig. 8

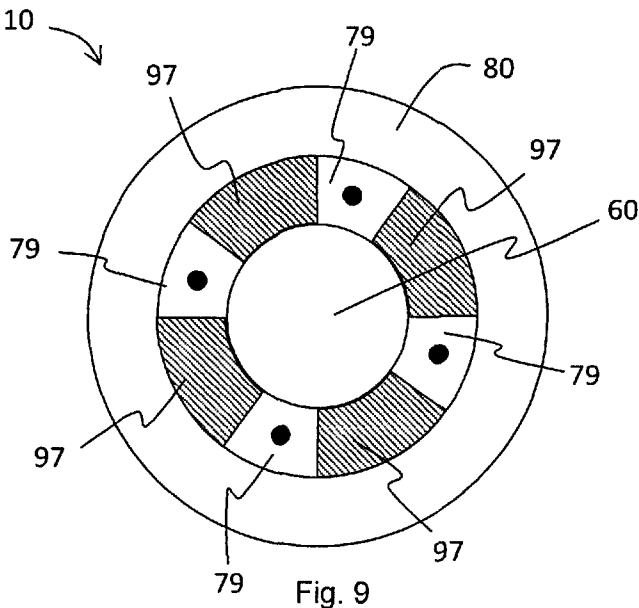


Fig. 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/SG2016/050257

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/52
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, COMPENDEX, EMBASE, FSTA, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 853 894 A1 (INMUNOLOGIA Y GENETICA APLIC S A [ES]) 1 April 2015 (2015-04-01) claims paragraphs [0061], [0071], [0076]; figure 1 -----	1-17
X	WO 00/14535 A1 (AMIRA MEDICAL [US]) 16 March 2000 (2000-03-16) page 19, line 17 - page 20, line 21; figures 1, 2, 4, 5 -----	1-17
X	EP 0 443 231 A1 (ENVIRONMENTAL DIAGNOSTICS INC [US]) 28 August 1991 (1991-08-28) claims column 7, line 3 - line 42; figures 1-3 column 8, line 52 - line 54 column 3, lines 7-11 column 4, line 54 - column 5, line 8 column 9, line 12 - line 19 -----	1-17



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

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25/01/2017

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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