(12) UK Patent Application (19) GB (11) 2617194 (13) A

(43) Date of A Publication 04.10.2023

(21) Application No: 2204764.1

(22) Date of Filing: 01.04.2022

(71) Applicant(s):
Mint Diagnostics Ltd
Unit 190 Kent Science Park, Carver Drive, Sittingbourne, Kent, ME9 8NP, United Kingdom

(72) Inventor(s):
Richard White
Cedric Hurth

(74) Agent and/or Address for Service:
Marks & Clerk LLP
62-68 Hills Road, CAMBRIDGE, CB2 1LA, United Kingdom

(51) INT CL:
B01L 3/00 (2006.01) ***G01N 33/543*** (2006.01)

(56) Documents Cited:
GB 2593199 A
WO 2018/187535 A1
WO 2014/035167 A1
WO 2022/036346 A1
WO 2018/152296 A1
US 20190118178 A1

(58) Field of Search:
INT CL **B01L, G01N**
Other: **WPI, EPODOC**

(54) Title of the Invention: **Microfluidic collection and test**
Abstract Title: **Integrated Fluid Sample Test Strip**

(57) An integrated fluid sample test strip 200 comprising a first layer 224, an inlet 202 for receiving a series of solutions including a fluid sample and a substrate solution, a test chamber 206 and a second layer 222. The test chamber incubates and performs biosensing tests on one or more solutions received from the inlet and is functionalized with one or more bioreceptors for binding to a target analyte wherein the test chamber comprises a plurality of test electrodes 216 to perform at least part of a biosensing test and is configured to receive the solutions from the inlet. The second layer comprises a cavity corresponding to the test chamber, wherein the cavity is recessed along and under at least part of a sidewall of the test chamber, the second layer comprising a hydrophilic surface adjacent to the first layer. The second layer may comprise a channel for guiding a solution from the inlet to the test chamber wherein the channel is formed in the hydrophilic surface of the second layer. The strip may also comprise a capillary pump 210 and vent holes 212. Ideally, the strip is used to perform ELISA or ELONA tests for hormones on saliva.

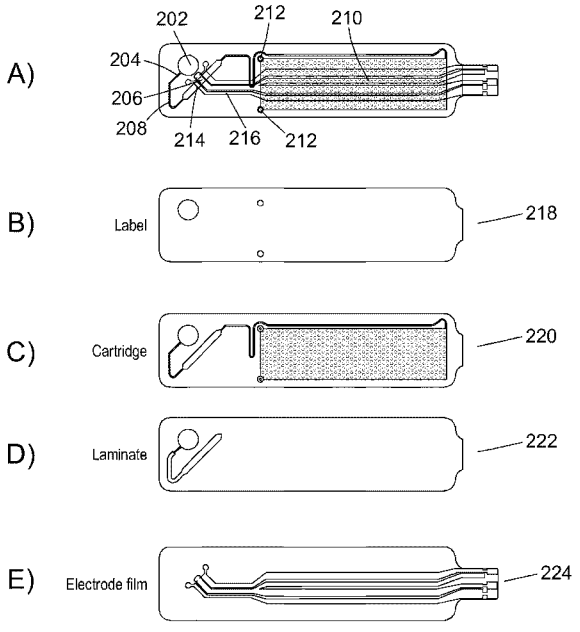

FIG. 2

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

GB 2617194 A

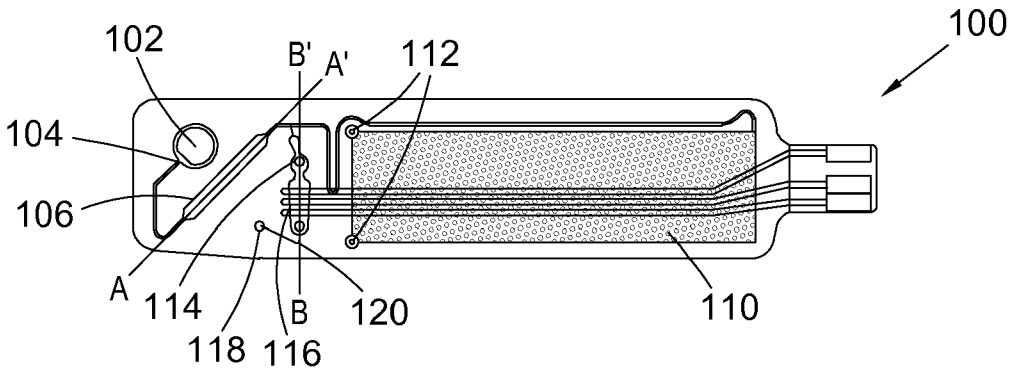

FIG. 1

30 03 23

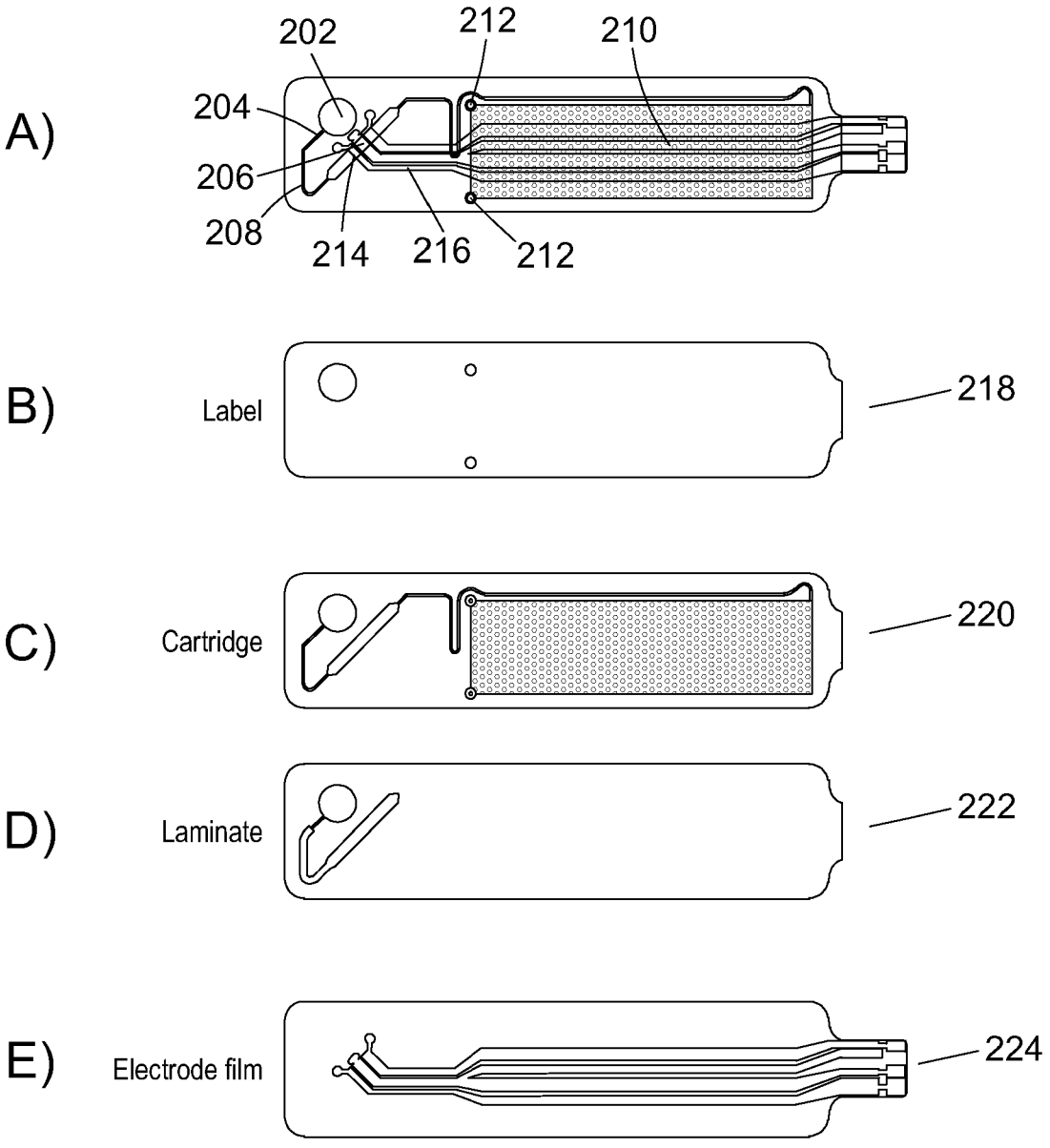

FIG. 2

30 03 23

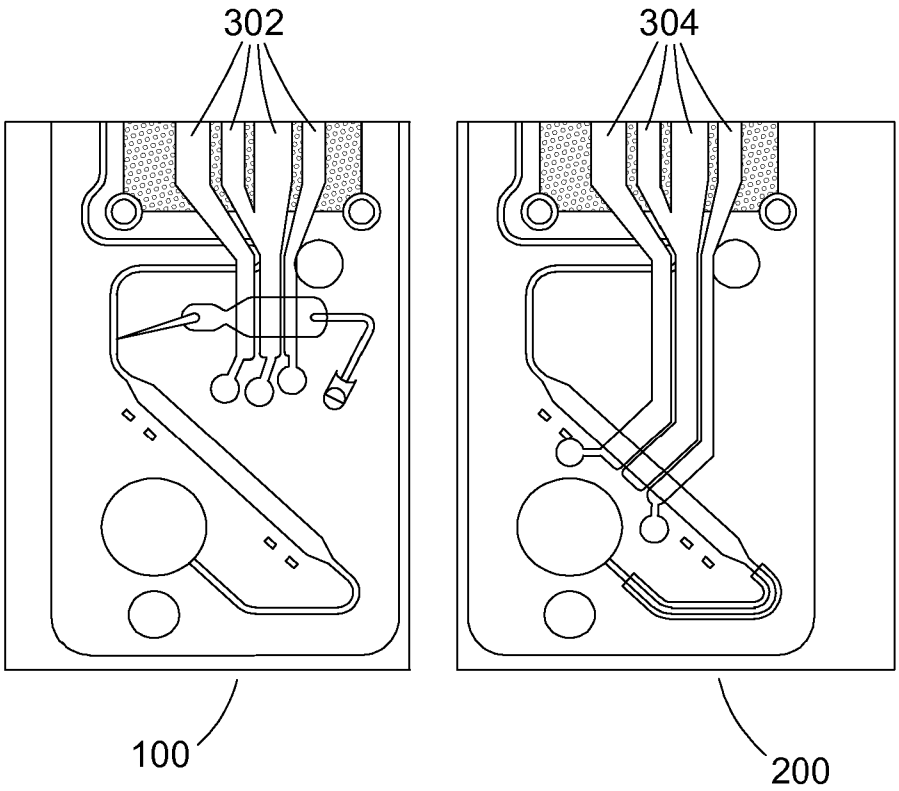

FIG. 3

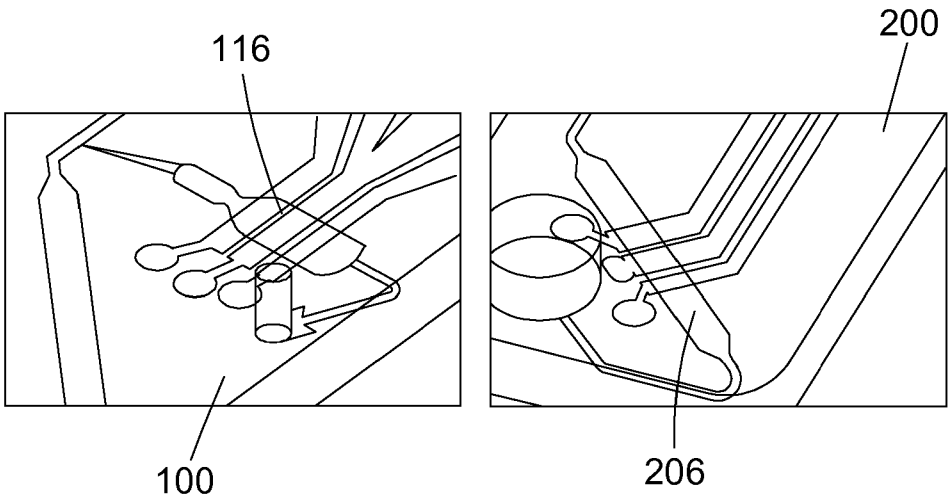

FIG. 4

30 03 23

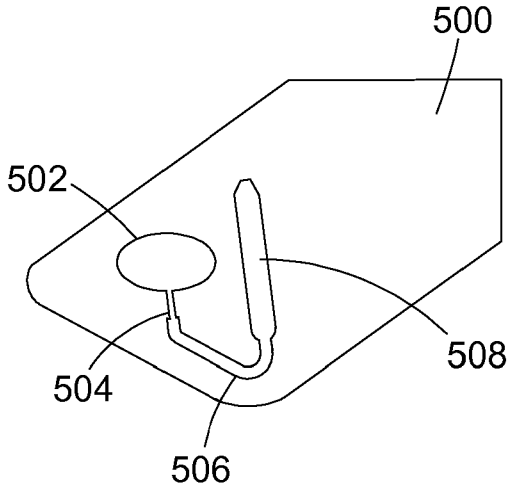

FIG. 5

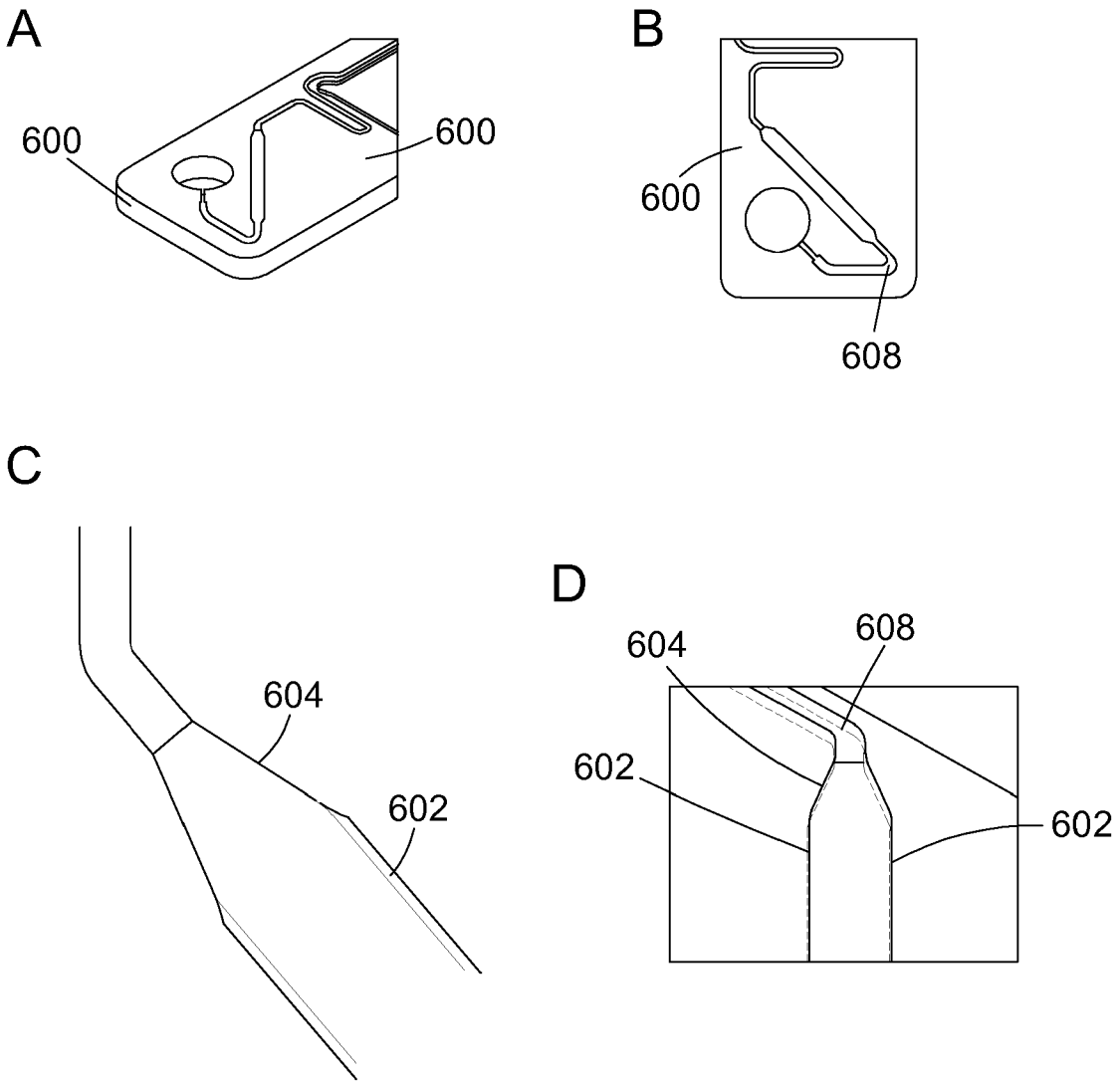

FIG. 6

30 03 23

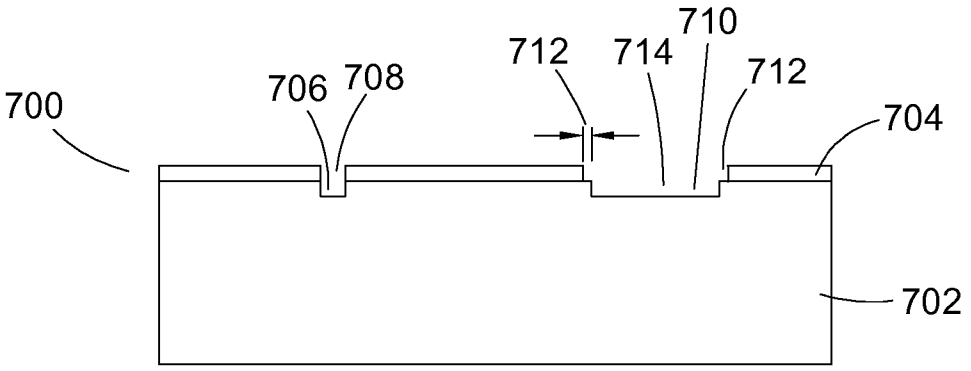

FIG. 7

30 03 23

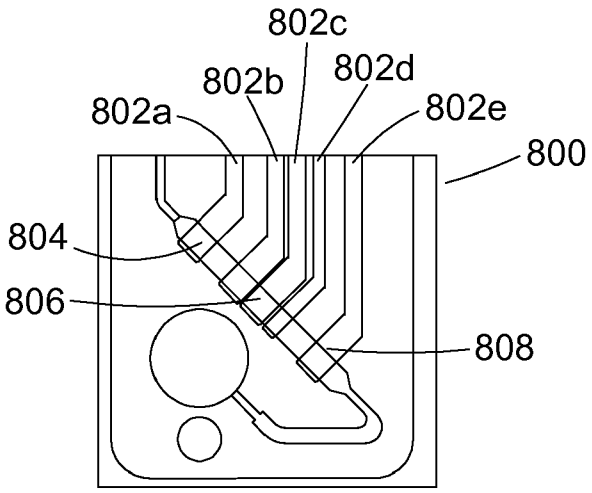

FIG. 8

30 03 23

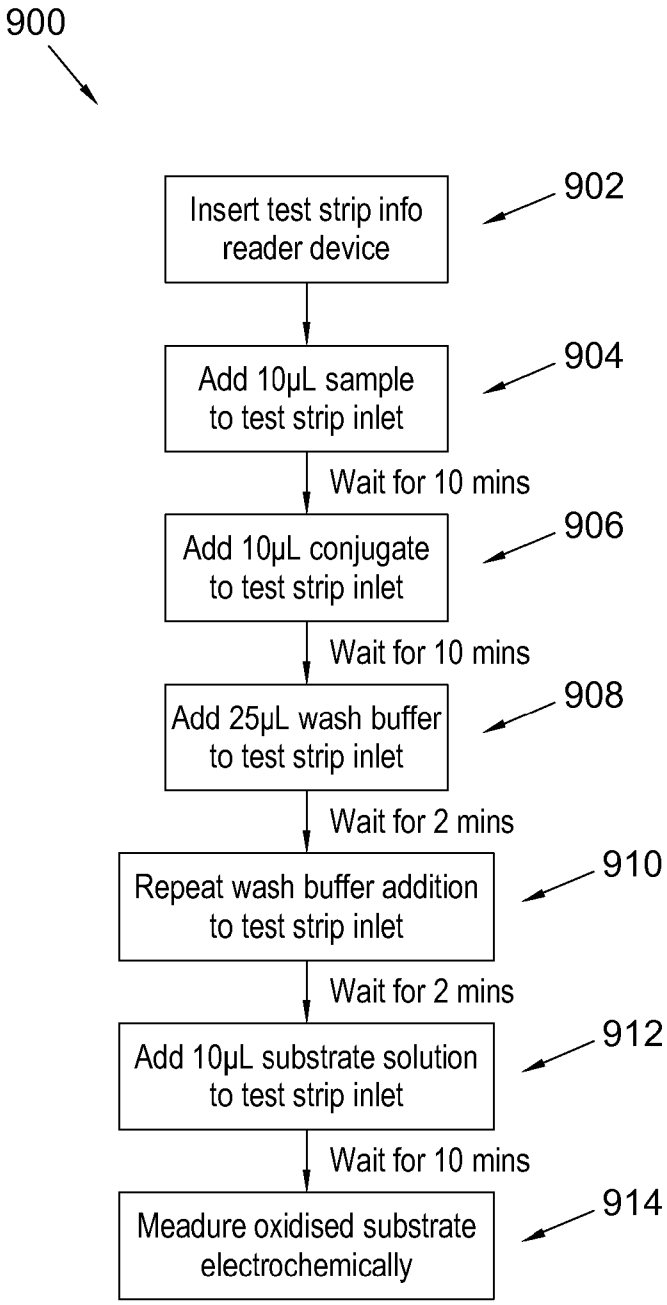
900
Insert test strip info reader device
902
Add 10µL sample to test strip inlet
904
Wait for 10 mins
Add 10µL conjugate to test strip inlet
906
Wait for 10 mins
Add 25µL wash buffer to test strip inlet
908
Wait for 2 mins
Repeat wash buffer addition to test strip inlet
910
Wait for 2 mins
Add 10µL substrate solution to test strip inlet
912
Wait for 10 mins
Meadure oxidised substrate electrochemically
914

FIG. 9

30 03 23

A) Sample addition

B) Competition reaction

C) Washing step

D) Substrate reaction

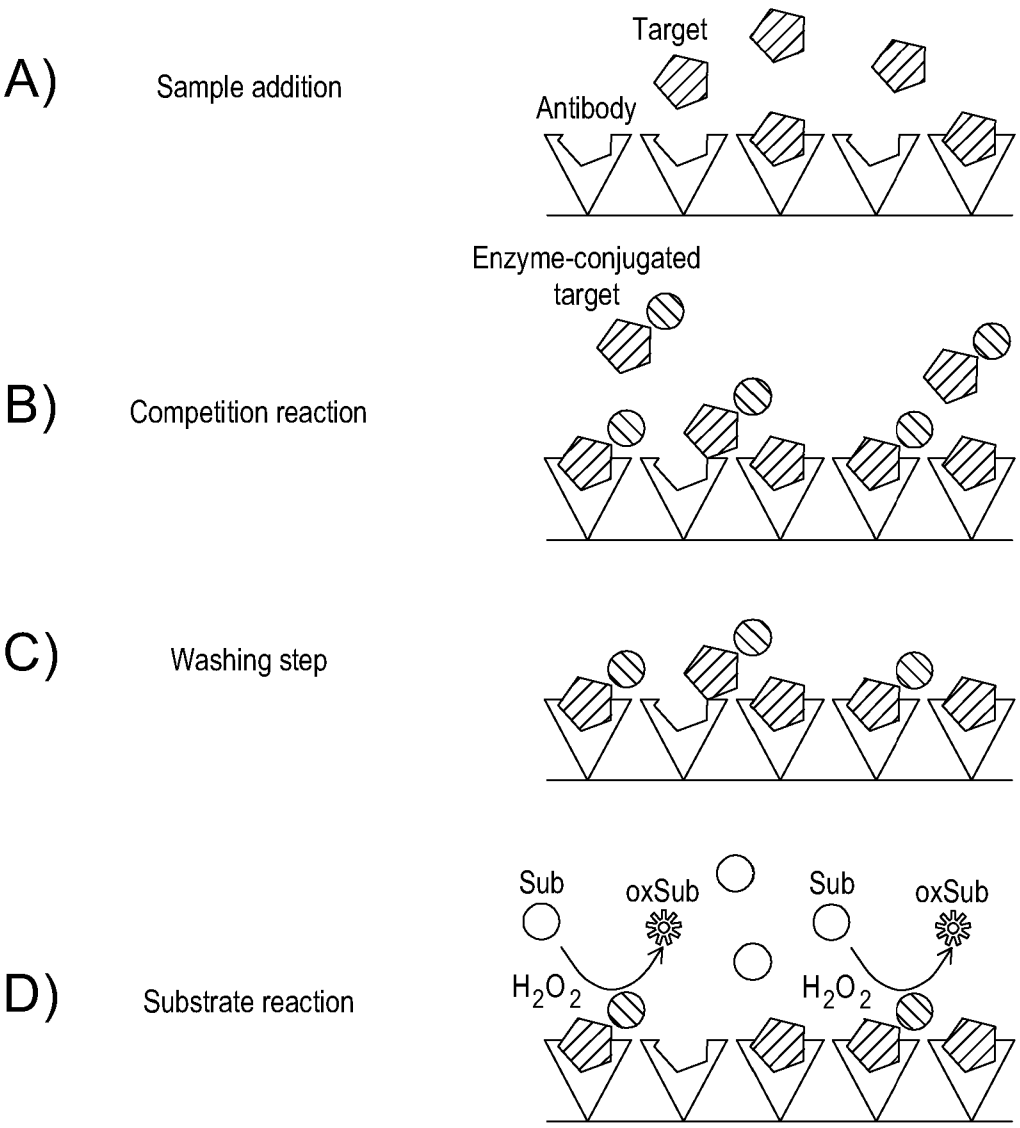

FIG. 10

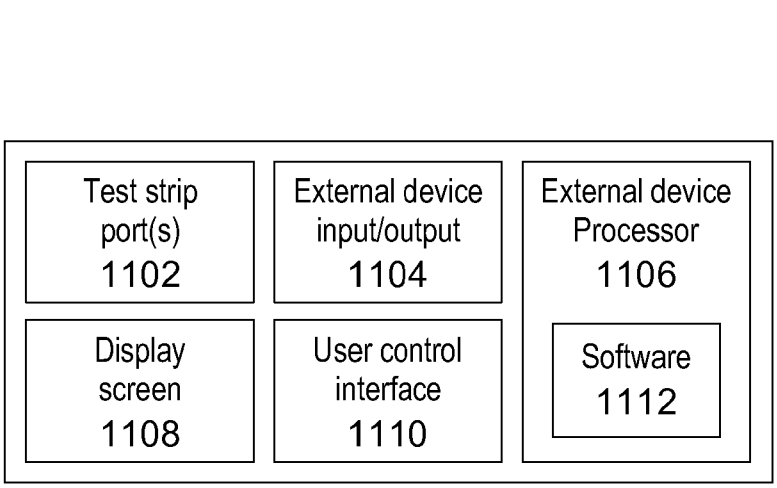

FIG. 11

30 03 23

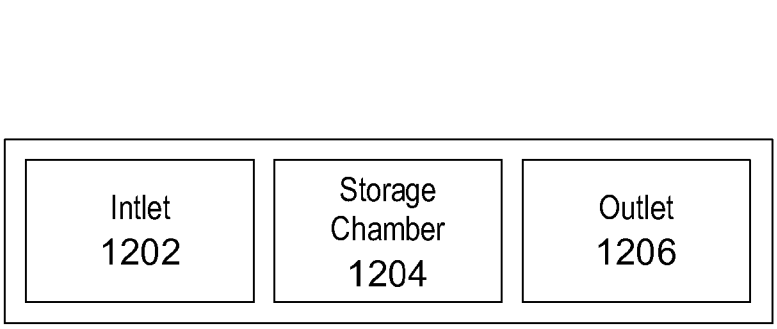

FIG. 12

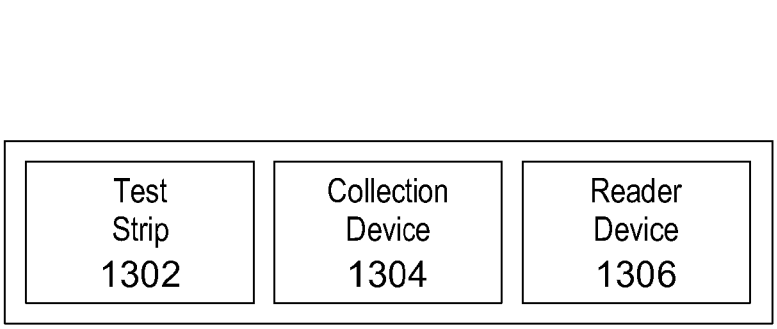

FIG. 13

30 03 23

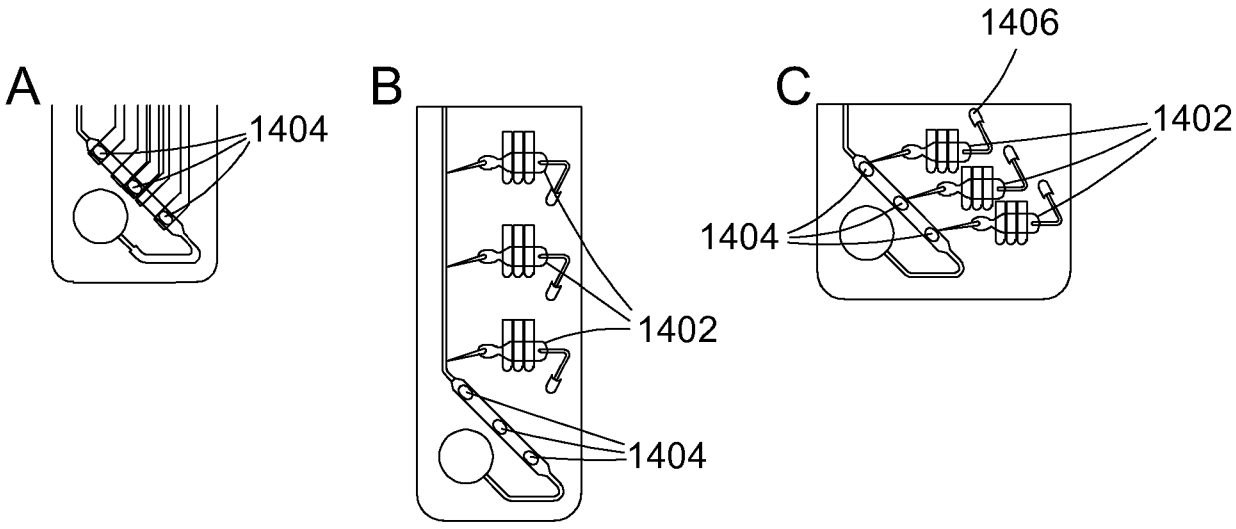

FIG. 14

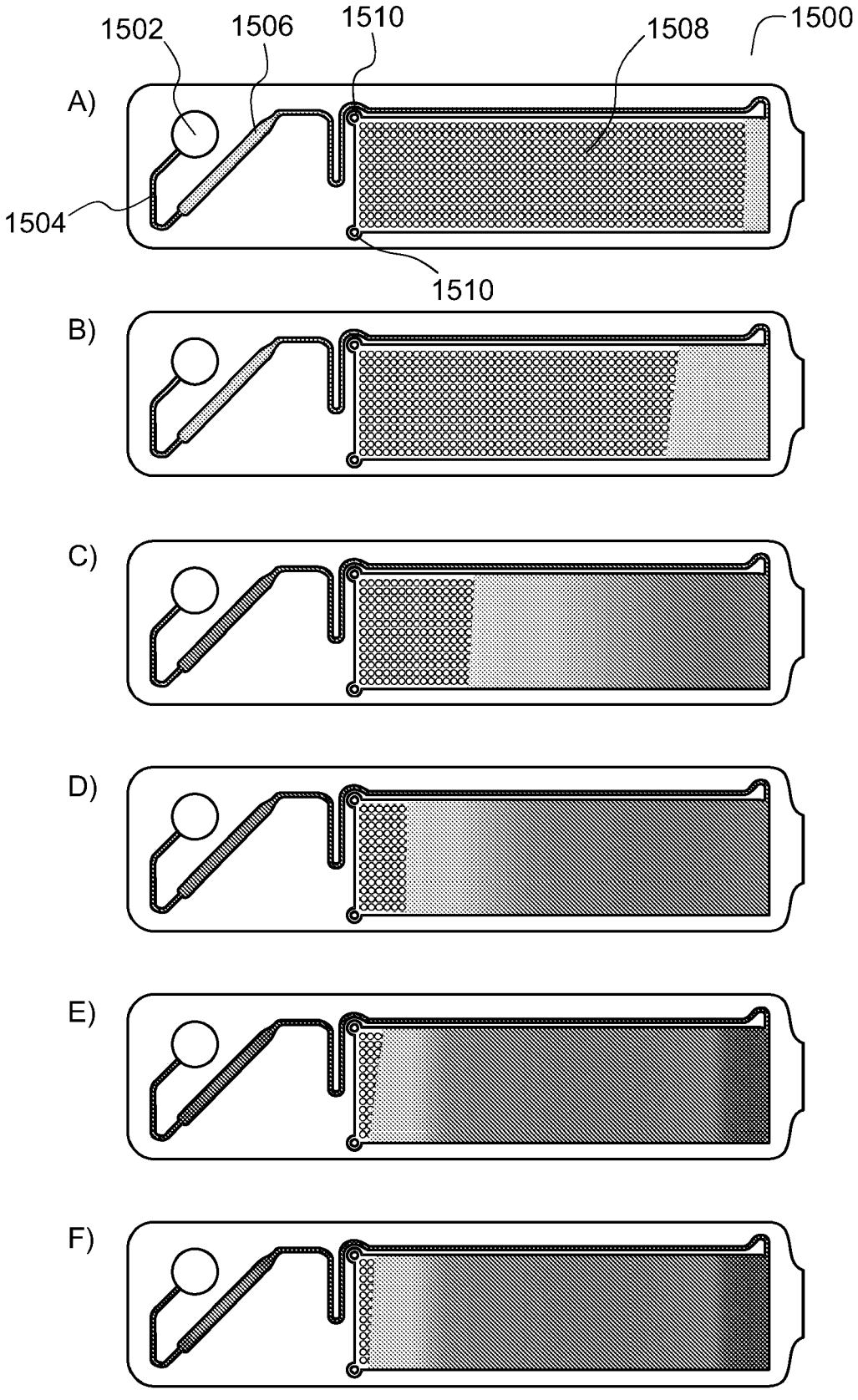

FIG. 15

30 03 23

Microfluidic Collection and Test

FIELD OF THE INVENTION

The present invention generally relates to an integrated fluid sample test strip, a fluid sample test system, and use of the test strip or test system to perform an ELISA or ELONA test. Preferably the fluid sample comprises saliva. In particular, preferred embodiments measure analyte levels in saliva, for example for hormone testing or monitoring.

BACKGROUND OF THE INVENTION

Hormones regulate many processes within the body including metabolism, digestion, reproduction, our ability to access energy reserves, our mood and emotions, sleep to name a few. Understanding our endocrinology can help us optimise our health, wellness and/or fitness. For example, cortisol is a stress hormone that is generally released in the human body as a result of physical or psychological stressors. Where the stress response is dysregulated, this can lead to broad health problems and/or performance deterioration, with regard to a person's mental or physical state.

Using for example saliva to test for hormones may be desirable. However, analyte concentrations in saliva can be extremely low. For example, the hormone oestradiol may be present at below picogram/mL levels. Detecting such low concentrations of analytes often requires an assay approach involving amplification. Enzyme-linked immunosorbent assays (ELISA) employ enzymatic amplification to oxidise a substrate species. In the most common form of ELISA the oxidized substrate species are coloured and the intensity of the colour is indicative of the analyte concentration. This approach is known as a colorimetric assay. It is also possible to measure the oxidized substrate species electrochemically to allow greater quantification and sensitivity.

However, to obtain reliable quantitative results from analysis of saliva using existing methods, a sample is generally prepared and controlled prior to analysis. For example, to carry out an ELISA test for detecting cortisol concentration of saliva, the sample is centrifuged and the pH checked before analysis. Similarly, for a salivary lateral flow test, the saliva is diluted in buffer solutions before analysis.

Such existing methods are unsuitable for home saliva testing. Ideally, biosensor devices should be suitable for use by an untrained consumer in their own home. Any requirement for multiple reagents and/or sample pre-treatment generally add complexity to the testing protocol and may introduce significant sources of error, and thus are example barriers to home diagnostic tests becoming more prevalent.

A further barrier is that, to obtain reliable quantitative results from analysis of saliva using existing methods, relatively large volumes of saliva need to be collected. Saliva collection methods such as passive drooling are required with long collection times, e.g., 15 minutes.

Attempts have been made to address several of these issues. For example, GB2593199 (A) generally provides an integrated test strip device for monitoring biomarker levels. However, the architecture of the

test strip device of GB2593199 (A) may introduce a level of uncertainty to the measurement results due to, for example, systematic variability resulting from the wash buffer potentially diluting the substrate to be measured between the incubation and test chamber.

The field of fluid sample testing therefore needs an improved method or device to monitor the levels of chosen biomarker(s), e.g., the field of saliva testing needs an improved method or device to monitor such levels in an individual's saliva. Such an improved method may allow, e.g., a compact and/or portable test apparatus, quantitative measurement, greater convenience for the user, speed, accuracy, sensitivity and/or reliability, and preferably without any requirement of a lab environment, trained professionals and/or sample pre-treatment. It is desirable that the testing needs minimum user intervention or input at any stage after the sample collection and during analysis.

For use in understanding the present invention, the following disclosures are referred to:

- WO2017/132565 A1: "Saliva Glucose Measurement Devices and Methods" (Labelle et al; published 3.8.17);

- US2017/0226557 A1: "Strip-based Electrochemical sensors for Quantitative Analysis of Analytes" (Wang et al; published 10.8.17); and

- US2009/0306543 A1: "Specimen Sample Collection Device and Test System" (Slowey et al, published 10.12.09);

- US6248598 B1: "Immunoassay that provides for both Collection of Saliva and Assay of Saliva for one or more Analytes with Visual Readout" (Bogema; published 19.6.01)

- US9223855 B1: "Method and System for Training Athletes based on Athletic Signatures and a Classification thereof" (Wagner; published 29.12.15);

- US20100206748 A1: "Stress Measurement Kit and Stress Measurement Method" (Morita et al; published 19.8.10);

- WO2011030093 A1: "Glucose Measurement Method and System" (McColl et al; published 17.3.11);

- US2007015286 A1: "Diagnostic Strip Coding System and Related Methods of use" (Neel et al; published 18.1.07);

- US2011174616 A1: "Methods for Measuring Physiological Fluids" (Roberts et al; 21.7.11);

- US2016313313 A1: "Lateral Flow Assay Apparatus and Method, and Sensor Therefor" (Love et al; published 27.10.16);

- JPWO2014181753 A1: "Measuring Device" (Shigeru; published 13.11.14);

- US2011108440 A1: "Underfill Recognition System for a Biosensor" (Wu et al; published 12.5.11);

- "The lab-on-PCB approach: tackling the μTAS commercial upscaling bottleneck" (Moschou et al; Lab Chip, 2017, 17, 1388. DOI: 10.1039/c7lc00121e);

- "ELISA-type assays of trace biomarkers using microfluidic methods" (Dong et al; WIREs Nanomed Nanobiotechno. 2017, 9:e1457. DOI: 10.1002/wnan.1457);

- "Materials for Microfluidic Immunoassays: A Review" (Mou et al; Adv. Healthcare Mater. 2017, 6, 1601403. DOI: 10.1002/adhm.201601403);

- "A Novel Microfluidic Point-of-Care Biosensor System on Printed Circuit Board for Cytokine Detection" (Evans et al; Sensors 2018, 18, 4011; DOI:10.3390/s18114011);

- WO2017025921A1: "Aptamer Biosensors useful for detecting hormones, hormone mimics, and metabolites thereof" (Kumar, published 16.2.17);

- US 9,207,244B2: 'System and methods for detection and quantification of analytes' (Khatak et al; published 21.5.15);

- Zimmerman et al, LabChip, 2007, 7, 119–125, "Capillary pumps for autonomous capillary systems".

- G. Volpe et al, Analyst, June 1998, Vol. 123 (1303–1307).

- GB2593199 (A): "Microfluidic point-of-care assay" (Vasilakis et al; published 22.09.21).

SUMMARY

According to the present invention, there is provided an integrated fluid sample test strip comprising:

a first layer;

an inlet for receiving a series of solutions, said solutions comprising at least a fluid sample and a substrate solution;

a test chamber for incubating and performing biosensing tests on one or more solutions received from the inlet, the test chamber formed in the first layer and configured to receive the solutions from the inlet, the test chamber functionalized with one or more bioreceptors for binding to a target analyte, the test chamber comprising a plurality of test electrodes to perform at least part of a biosensing test of the substrate solution; and

a second layer comprising a cavity that is adjacent to the test chamber, wherein the cavity is recessed along and under at least part of a sidewall of the test chamber, the second layer comprising a hydrophilic surface adjacent to the first layer.

Preferred embodiments may provide advantages such as accuracy, sensitivity and/or reliability of detection or measurement of a target analyte, e.g., biomarker, that may be in received saliva. This is desirable in various fields of activity, such as health or wellness programs for elite sports training and/or precision medicine to allow drugs administration tailored according to a patient, e.g., to their individual hormone levels. Preferably, the integrated nature of the strip allows all of the features to be provided to the user within a one-piece device that is preferably portable, compact and/or disposable.

While the present disclosure generally refers throughout to assays for testing saliva, e.g., salivary hormone tests, embodiments may additionally or alternatively be suitable for use with one or more other matrices. Such other matrices which may comprise bodily fluids such as blood and/or urine. Further example matrices comprise non-bodily fluids, e.g., water, for example for use in environmental monitoring. Therefore, references to saliva within the present disclosure are generally interchangeable with references to any one or more of such other fluids.

The test strip may enable incubation of a sample and a competing conjugate with bioreceptor molecules (such as an antibody or aptamer) followed by a subsequent electrochemical measurement in a single combined incubation and test chamber. The incubation of the sample and competing conjugate may be simultaneous or sequential. The architecture may enable precise control of liquid within the microfluidic network, allowing the measurement of analyte levels in an, e.g., saliva, sample in a relatively small amount of time (for example, in less than 1 hour). Embodiments may not require a laboratory setting for operation of the strip and/or for preparation of the saliva sample, and therefore may advantageously allow analysis in a field or point-of-care environment.

The inlet may allow solutions and other fluids to be introduced to the test strip (noting that the term 'fluid' is used herein to refer to 'liquid', e.g. a solution). Preferably (i.e., optionally), the inlet is configured to have an external aperture cross-section that is sufficiently wide to ease the introduction, yet is of small enough width (e.g., diameter) to allow dispensed fluids to wet preferably an entire bottom surface of the inlet. Advantageously, a large inlet width/diameter may minimise capillary effects near the external aperture of the inlet, while allowing preferably the entire bottom of the inlet to be wetted by introduced fluids. This may ensure that preferably all of the fluids will enter the microfluidic network of the test strip. The width (e.g., diameter) and/or volume of the inlet may therefore vary between different embodiments, depending on the volume of fluids required for a particular assay. The inlet volume may be determined such that an expected combined volume of introduced fluids is greater than the combined internal volume for holding fluid in the test strip downstream of the retention valve. This may allow one or more later introduced fluids to be retained in the inlet. Advantageously, such retention of fluid in the inlet may provide additional hydrostatic pressure.

The inlet may optionally comprise a retention valve which may reduce (e.g., prevent) air flow into downstream components of the strip, e.g., into the reaction and/or test chamber. This may improve the accuracy, sensitivity and/or reliability of testing. The retention valve may be active or passive, wherein a passive retention valve may have the greatest capillary pressure in substantially (preferably entirely) the whole capillary system of the test strip, e.g., not just higher relative to (immediately) adjacent microfluidic features. In embodiments, the retention valve generally pins the dewetting meniscus. An example passive valve generally comprises a fluid flow path having a constriction, and/or a narrow path relative to at least directly adjacent features from which the fluid flows in and out of the valve, and/or preferably has a smaller cross sectional area for fluid through-flow than any other microfluidic features of the test strip. The valve may be configured to regulate flow of liquid through the valve by means of capillary force.

More specifically, the retention valve may ensure fluidic flow with reduced (preferably complete absence of) air bubbles downstream in the test strip (e.g., in any fluid in flow paths between the retention valve and the capillary pump or the combined incubation and test chamber), and/or may reduce or prevent 'dead' volumes where non-specific reactions or binding events (which may be other than those between the analyte of interest and the bioreceptor molecules (antibody and/or aptamer)) may otherwise take place in the test strip. Non-specific reactions or binding events are generally undesirable and may be due to species sticking to the channel surfaces or solution getting trapped in a non-preferred location. Such dead volumes may increase the noise in measurement values, decreasing the accuracy and/or reliability of the test results. Preferably, the inlet retention valve is configured to have the greatest capillary pressure in the test strip. This may be enabled, for example, by the retention valve having a smaller cross-sectional area than any of the other components of the test strip. This may result in the valve rarely (preferably never) becoming empty of fluid during use of the test strip. (Noting that the term 'cross-section' is used herein to refer to an area through which can flow).

In embodiments, at least one biosensing test may comprise at least two stages: biorecognition; and an electrochemical transduction. The biorecognition and electrochemical transduction may both occur in the combined incubation and test chamber (alternatively referred to herein as a test chamber, incubation chamber, reaction chamber, measurement chamber and/or sensing chamber). The combined incubation and test chamber may be pre-functionalised with bioreceptor molecules during manufacture of the test strip. Such bioreceptor molecules may bind with a target analyte in fluids such as solutions introduced into the test strip. This binding may occur due to the biorecognition between the bioreceptor molecule and the target analyte. This may allow test strips to be designed to detect levels of specific target analytes, reduce the number of steps required by the user, and/or improve the reliability of the results in point of care use.

The combined incubation and test chamber may be configured to expose solutions to one or more test electrodes. Such test electrode(s) may be controllable to perform the electrochemical transduction and detect a level of the target analyte in a sample input into the test strip. In this regard, the test strip may be used for sequential and/or simultaneous competition immunoassays. In such embodiments, the electrochemical transductions may be performed on one or more later input fluids (e.g., solutions), rather than directly on a test (e.g., saliva) sample. Additionally or alternatively to sequential and/or simultaneous competition immunoassays, the test strip may be used for other assay formats. For steroid hormones, which are small molecules, a competitive assay may be preferred, for example because each hormone molecule generally only has one epitope.

Generally, it is advised to separate incubation chambers and testing chambers, as the use of a single chamber for both stages of an assay may increase the background (noise) signal due to non-specific adsorption and/or because of the deposition of passivating substances from earlier fluids (such as the sample onto the sensor electrodes). However, the Applicants have found that the architecture of the present invention enables the combination of the test and incubation chambers without resulting in a

significant increase in the non-specific signal. It has further been found that the sensors were not significantly passivated by substances present in the sample(s) or the reagents, for example because they may be reduced or removed from the sensors by a wash buffer before the measurement occurs.

The test electrodes may enable an electrochemical test of a substrate solution. This may facilitate extremely sensitive quantification of, e.g., salivary, hormone concentrations.

The integrated test strip may optionally comprise a capillary pump which may allow waste solution(s) to flow through the test strip and away from the microfluidic elements involved in performing the biosensing test, e.g., the reaction and test chamber. The capillary pump may comprise one or more vent holes to allow air in the test strip to evacuate the capillary pump as input fluids flow through the strip. Advantageously, such vent hole(s) may reduce, e.g., prevent, any increase in air pressure in the test strip. An increase in air pressure (due to, for example, trapped air) may reduce the flow rate of the input fluids and/or prevent the fluids from flowing through the test strip.

The test strip comprises of at least a first (e.g. polymer) layer and a second (e.g. adhesive) layer with at least one hydrophilic surface; the second layer thus may be referred to as a 'hydrophilic layer'. The combined incubation and test chamber may be formed in at least the polymer layer, preferably also in an adjacent cavity of the second layer, further preferably such that the entire surface of the second layer that is a surface of the chamber is hydrophilic. (In embodiments, a hydrophilic surface may extend across part(s) or substantially an entirety of the first layer's interface with the second layer). A recess or undercut may be provided in the second layer around at least part of the combined incubation and test chamber. Advantageously, the provision of the recess may decrease the flow rate of the fluid though the combined incubation and test chamber, and thereby increase the interaction time between the solution and the bioreceptor molecules disposed in the combined incubation and test chamber. In some implementations, it has been found that, without this recess, a cut edge of the hydrophilic layer around the combined incubation and test chamber may interact with the fluid in the combined incubation and test chamber. As a result, and due to the hydrophilic nature of the cut edge, some or all of the fluid may flow along the edge of the combined incubation and test chamber. Depending on the specific placement of the bioreceptor molecules within the combined test and incubation chamber, this may potentially result in at least a portion of the fluid bypassing (and therefore not interacting with) some, most or all of the bioreceptor molecules. In embodiments, the captured antibodies are generally at the centre of the chamber, but without the recess the fluid may bypass the center of the chamber. In this case, the actual flow rate may decrease, however this may be mostly because the actual cross-section used by the fluid is significantly reduced. Thus, in implementations without the recess, the interaction between the solution and the bioreceptor molecules may be drastically reduced, thereby resulting in a significant reduction in the accuracy of measurement results.

In addition, fluid flowing along the edge of the combined incubation and test chamber typically flows through a reduced cross-section of the combined test and incubation chamber. Therefore,

implementations without this a recess may have a reduced the flow rate of fluid through the combined incubation and test chamber. This in turn may result in a greater level of uncertainty regarding the time required for each step of the assay process, and/or a further potential reduction in the accuracy of measurement results.

The hydrophilic layer may be recessed by at least 50μm, for example by 65μm or 100μm along at least part of a sidewall of the combined incubation and test chamber. Preferably, the hydrophilic layer may be recessed by a distance that is less than a thickness of the hydrophilic layer, in order to assist in reducing the risk of fluids bypassing the test chamber via the recess and/or becoming trapped in the recess. For example, in a test strip with a second (e.g. adhesive and/or hydrophilic) layer that has a thickness of 200μm, the recessed may be less than 200μm along the whole or part of the sidewall of the combined incubation and test chamber.

In implementations, the combined incubation and test chamber may comprise a tapered or narrowed end for receiving solutions from the inlet. The recess size around the tapered end may be less than the recess size around the rest of the combined incubation and test chamber. The presence of a smaller recess may enable or increase the interaction between the fluids in the combined incubation and test chamber and the edge of the hydrophilic layer, and thereby assist the flow of the fluids into the combined incubation and test chamber. For example, the recess may be less than 50μm along sidewall of the tapered end, and/or the hydrophilic layer may not be recessed at all along the sidewall of the tapered end.

Additionally or alternatively, a channel for guiding solution from the inlet to the combined incubation and test chamber may be formed in at least the hydrophilic surface of the second layer. For example, the channel may be formed in the first and layer and second layers, e.g. such that the channel comprises a formation in first (polymer) layer and a corresponding cavity, cutting or formation in the second (adhesive) layer. The Applicants have found that a potential barrier between the channel and the combined incubation and test chamber may result in a variable delay in the time taken for the fluid to enter the combined incubation and test chamber from the inlet. By forming the channel in the hydrophilic surface, the potential barrier may be reduced (as e.g. the fluid in the channel is no longer in contact with the hydrophilic surface). Beneficially, this may significantly reduce the variability in the time taken for the fluid to enter the combined incubation and test chamber. In implementations, the variable delay may be reduced to negligible levels, such as under 1 second.

Therefore, according to the present invention, there is further provided an integrated fluid sample test strip comprising:

a first layer;

a second layer comprising a hydrophilic surface adjacent to the first layer;

an inlet for receiving a series of solutions, said solutions comprising at least a fluid sample and a substrate solution;

a test chamber for incubating and performing biosensing tests on one or more solutions received from the inlet, the test chamber, the test chamber configured to receive the solutions from the inlet, the test chamber functionalized with one or more bioreceptors for binding to a target analyte, the test chamber comprising a plurality of test electrodes to perform at least part of a biosensing test of the substrate solution; and

a channel for guiding a solution from the inlet to the test chamber, wherein the channel is formed in the hydrophilic surface of the second layer.

In embodiments, the entire surface of the second layer that is a surface of the chamber is hydrophilic. Preferably, the hydrophilic surface may extend across part(s) of or substantially an entirety of the first layer's interface with the second layer.

In some implementations, the combined incubation and test chamber may comprise a plurality of test regions, with each test region comprising one or more of the plurality of test electrodes. The plurality of test regions may be separated by a distance greater than the diffusion distance of the solution over the incubation period, to reduce mixing of reagents in the test regions resulting from diffusion. Preferably, the separation distance may be at least twice the diffusion distance to reduce mixing of the reagents between the test regions. In implementations, the incubation period may be 15 minutes, however it will be understood that this period is provided as an example, and other incubation periods may be used. Suitable separation distances may include, for example, at least about 0.9mm, or by at least about 1.8mm.

Additionally or alternatively, the combined incubation and test chamber may comprise reduced width sections between some or all of the test regions. The reduced width sections may increase the time required for samples in the fluid to diffuse between different test regions. This in turn may allow the test regions to be more closely spaced, resulting in e.g. a smaller test strip.

The provision of multiple separated test regions may allow much easier multiplexing of the measurement results. This is because the fluid may flow in the test strip at a relatively laminar regime (e.g. with a low Reynolds number) given the flow rates involved (e.g. typically 10 – 100 μL/min). This may therefore assist in avoiding turbulent areas with high mixing rates.

Nevertheless, there may still be some mixing at the interface of two laminar fluids progressing at the same velocity. Therefore, multiplexing may also be enabled by the provision of multiple separated test chambers. Thus, there is further provided an integrated fluid sample test strip comprising:

i. an inlet for receiving a series of solutions, said solutions comprising at least a fluid sample and a substrate solution, wherein the inlet comprises a retention valve for temporarily retaining each said solution to thereby reduce air flow through the retention valve;
ii. a reaction chamber to receive the solutions from the inlet via the retention valve, the reaction chamber functionalized with one or more bioreceptors for binding to a target analyte;

iii. a capillary pump to receive from the reaction chamber at least one of the solutions including at least the fluid sample, the capillary pump comprising at least one vent hole to allow any air to escape from the capillary pump and thereby reduce pressure in the capillary pump;

iv. a plurality of test chambers to receive the substrate solution from the reaction chamber, the plurality of test chambers each comprising test electrodes to perform at least part of a biosensing test of the substrate solution;

v. hydrophobic vent holes coupled to each of the test chambers to allow a flow of solution from the reaction chamber into the test chamber when the vent hole is unsealed and to allow a flow of solution from the reaction chamber to the capillary pump and/or other test chambers when the vent hole is sealed.

There may further be provided a test strip wherein the capillary pump comprises at least one capillary channel defined by an array of micropillars. Such a channel may provide capillary pressure, which may enhance or enable flow of solutions into and/or through the capillary pump.

In some embodiments, at least one said micropillar may comprise a substantially diamond-shaped cross section. Such a shape may be exactly diamond shaped, or may for example have curved corners. A diamond shape may be substantially (e.g., exactly) rhombic.

There may further be provided a test strip wherein the capillary pump comprises an internal bypass channel along at least part of a perimeter of the capillary pump, wherein a smallest cross-sectional width of the bypass channel is greater than a smallest separation between adjacent said micropillars. Bypassing flows may be reduced in such an embodiment. Such a bypass channel in the form of a peripheral clearance between a boundary of the capillary pump and the array of micropillars may reduce or prevent bypassing flows along the frame of the micropillar array.

There may further be provided a test strip wherein a smallest separation between adjacent said micropillars is less than a smallest width of a solution flow path from the reaction chamber to the capillary pump. In embodiments this may provide a greater capillary pressure in the capillary pump compared to the flow path between the reaction chamber and capillary pump. Advantageously, this may provide a more robust flow of fluids into the capillary pump.

There may further be provided the test strip, wherein the capillary pump has an inlet comprising a constriction. The introduction of a bypass channel may result in a gap between the micropillar array and the pump inlet, and this gap may reduce or prevent flow into the pump. The constriction may reduce the gap between the capillary pump inlet and the micropillar array, and may thereby maintain a capillary pressure at the inlet of the capillary pump and thus aids flow into the pump.

There may further be provided a test strip wherein:

- a channel for guiding solution from the test chamber to the capillary pump is formed in at least the first layer;
- the inlet is formed at in least the first layer;
- the capillary pump is formed in at least the first layer;
- the first layer comprises a polymer layer; and/or
- the second layer comprises an adhesive layer.

Thus, features of the test strip may in some embodiments be formed within the above-mentioned hydrophilic and/or polymer layers.

The test strip may be configured to measure levels of the analyte, wherein the analyte is a hormone. More generally, the analyte may comprise, e.g., be, a hormone and/or biomarker. The test strip may be configured to perform the measurement by performing an ELISA or ELONA (Enzyme-Linked Oligonucleotide Assay) test, and/or other tests.
There may further be provided the test strip, wherein the fluid sample comprises saliva, blood or urine.
According to another aspect of the invention, there is provided a fluid sample test system comprising the fluid sample test strip and at least one of:

- a fluid sample collector device for collecting the fluid sample and inputting the fluid sample into the inlet; and
- a reader device for controlling at least one of the test electrodes to perform the at least part of the biosensing test, and to output a result of the biosensing test.

Such a reader device may be used to determine and/or output analyte levels from raw test result data. The reader device may be configured to multiplex measurement results received from one or more test regions; Additionally or alternatively, a fluid sample collector device may be configured to aid in the collection of, and/or input into the inlet of, the fluid sample by the user.

According to a further aspect of the invention, there is provided a use of the fluid sample test strip or the fluid sample test system, to perform an ELISA or ELONA test.

There may further be provided the use, comprising:

(i) receiving the fluid sample in the inlet; and/or

(ii) receiving the substrate solution in the inlet.

There may further be provided the use, comprising:

(iii) receiving a solution comprising an enzyme-conjugate in the inlet; and/or

(iv) receiving one or more wash-buffer solutions in the inlet.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the invention and to show how the same may be carried into effect, reference will now be made, by way of example, to the accompanying drawings, in which:

Fig. 1 shows an example schematic of a prior art saliva test strip;

Figs. 2A-E show an example schematic of a saliva test strip and various example layers according to an embodiment of the invention;

Fig. 3 shows a comparison between example electrode structures of the test strips of Figs. 1 and 2.

Fig. 4 shows a comparison between the test chamber of the test strip of Fig. 1 and the Combined Incubation and Test chamber of Fig. 2.

Fig. 5 shows a diagram of an example second layer of the test strip according to an embodiment of the invention.

Fig. 6 shows illustrative diagrams example Combined Incubation and Test chambers of a test strip according to an embodiment of the invention.

Fig. 7 shows an example cross section of a test strip according to an embodiment of the invention.

Fig. 8 shows schematically an example electrode configuration of a test strip according to an embodiment of the invention.

Fig. 9 shows a flow chart of an example method of measuring analyte concentrations in a saliva sample according to an embodiment of the invention;

Fig. 10 illustrates an example competitive assay comprising steps a-d.

Fig. 11 shows a block diagram of an example reader device;

Fig. 12 shows a block diagram of an example collector device; and

Fig. 13 shows a block diagram of an example test system.

Fig. 14 shows schematically further example electrode configuration of test strips according to an additional aspects of the invention.

Fig. 15a-f illustrate an example flow of fluid through an embodiment of the invention during the method of Fig. 9.

DETAILED DESCRIPTION OF EMBODIMENTS

Embodiments generally provide a microfluidic apparatus for performing an assay, such as an enzyme-linked immunosorbent Assay. The assay is preferably performed at a point of care, which is generally a location that is convenient to the user and thus preferably not in a dedicated a laboratory.

In general, a competitive immunoassay is one where the analytes (e.g. hormone molecules) in a sample (e.g. saliva, blood, urine) and a fixed amount of a labelled analyte analogue (e.g. the "conjugate"; analyte conjugated with a radioisotope, fluorescent or enzyme label) compete for the binding sites on a film with a known amount of immobilised antibody. Once the sample, conjugate and antibodies have been incubated together and the competition has taken place, the amount of analyte is determined by measuring the amount of conjugate that has bound to the antibody (or alternatively, that remains free in solution).

In some embodiments of the invention, a conjugate comprising the hormone of interest and an enzyme such as horseradish peroxidase (HRP) is used. In the presence of a substrate solution (such as hydrogen peroxide with tetramethylbenzidiene (TMB)), HRP may oxidise the TMB. At lower analyte concentrations in the sample, the antibodies bind to a higher proportion of conjugates. As a result, when the substrate is incubated with this antibody film the TMB will oxidise to a greater extent. Oxidised TMB is typically measured colorimetrically (for example, TMB may turn blue or yellow depending on the degree of oxidisation). However, embodiments of the present invention may measure the TMB electrochemically in order to increase the sensitivity of test. (see ref. *Analyst, June 1998, Vol. 123 (1303–1307) by G. Volpe et al.*). (For further detail regarding TMB, it is noted that a blue product of a HRP/H2O2 + TMB reaction is generally a one-electron oxidation product of TMB. A two-electron oxidation product is generally coloured yellow. After the HRP reaction with TMB/H2O2, the reaction may be stopped by using a strong acid, which may further oxidise the one-electron oxidation products and/or stabilise the system preferably to allow more accurate measurement in a spectrophotometer or plate reader. However, use of a stop solution in embodiments of the present invention may displace reacted TMB from the reaction chamber. Preferably, embodiments do not use a stop solution and/or measure the one-electron oxidation product).

Generally, there are two approaches that can be employed for a flow-based competitive immunoassay, either sequential or simultaneous competition. In simultaneous addition, the sample is mixed with the conjugate solution and then they are incubated with the antibody film simultaneously. In sequential addition, the sample is introduced to the antibody film first and has the first opportunity to bind with the antibody binding sites and then the conjugate solution is introduced afterwards to react with the remaining binding sites. Embodiments of the present invention are suitable for either approach. However, in some embodiments sequential addition is preferred as this approach generally enables a higher sensitivity and doesn't require the preparation of a sample/conjugate solution with precise volumes mixing prior to the testing procedure.

Fig. 10 shows an example of a sequential addition competitive immunoassay in an embodiment. In step A, a sample (for example, saliva) containing a target analyte (for example, a hormone) binds with some of the bioreceptor molecules (for example, an antibody). In step B, an enzyme conjugate (for example, HRP) binds with some or all of the remaining bioreceptor molecules. In step C, a buffer solution is used to remove

the remaining unbound enzyme conjugate from the vicinity of the bioreceptor molecules. In step D, a substrate solution (hydrogen peroxide with tetramethylbenzidiene (TMB)) reacts with the bound enzyme conjugate. In general, the amount of reacted or oxidised substrate solution may be related to the amount of bound enzyme conjugate. As a result, in this example, the greater the measured oxidation of the TMB the lower the hormone analyte concentration in the saliva sample.

Fig. 9 shows an example process 900 for performing a sequential addition competitive immunoassay, such as an ELISA test, using a test strip according to an embodiment. In step 902 the test strip is inserted into an electronic reader device (however this step could alternatively occur as a final or intermediary step in the process). The reader device may be used to, for example, control the test electrodes and/or provide the test results to the user.

In step 904 a sample containing a target analyte is inserted into the test strip via the inlet. This sample may be, for example, a 10μL saliva sample from the user. The volume of the sample may vary depending on the particulars of e.g. the immunoassay and target analyte.

In step 906 a conjugate solution is inserted into the test strip via the inlet. In alternative embodiments, for example embodiments of the test strip which utilise a simultaneous competition immunoassay, the conjugate may be mixed with the sample and inserted into the test strip in step 904. The conjugate solution may be, for example, a 10μL solution containing target analyte conjugated with an enzyme. As with the sample in step 904, the volume may vary depending on the particulars of the test.

In step 908 a wash buffer solution may be inserted into the test strip via the inlet. The wash buffer helps to prevent contamination of the later substrate solution by the conjugate solution and/or by unbound enzyme-conjugates in the reaction chamber. By reducing the probability of contamination the wash buffer solution may improve the reliability of the results. In some embodiments, the wash buffer solution may have a volume of about 25μL. In optional step 910, additional wash buffer solutions may be used to further reduce the risk of contamination of the substrate solution. The additional wash buffer solutions may have a volume the same as or different from that of the initial wash buffer solution.

In step 912 a substrate solution may be inserted into the test strip via the inlet. The substrate solution and/or volume used may depend on the target analyte and/or enzyme conjugate. In this example process, the substrate solution may have a volume of about 10μL.

In step 914 the user controls the test electrodes in the test chamber to measure the substrate solution. This measurement may be accomplished by using electrochemical transduction to measure the amount of oxidised substrate solution. As discussed above, the test electrodes may be controlled via a reader device, which may then present the results to the user.

Between any two of the above steps the user may allow a certain amount of time for the fluids to propagate through the test strip. Possible wait times between each step are shown in Fig. 9. For example, the wait time between each of steps 904, 906 and 908 may be about 10 minutes, while the wait time between each of steps 908, 910 and 912 may be about 2 minutes. Similarly, the wait time between steps 912 and 914

may be about 10 minutes or 15 minutes. The user may be prompted to proceed to the next step by the reader device, and/or be provided with instructions (separately or by the saliva test system) detailing any such wait time(s).

The volumes and/or incubation times discussed with regard to example process 900 can vary. For example, the short (e.g., 2 minute) wait times may allow each added reagent solution to flow into the test strip channels and/or ensure that the next reagent is added to an empty inlet, thereby reducing or avoiding uncontrolled dilution in the inlet. The longer (e.g., 10 minutes or 15 minutes) incubation times may be set according to the specific antigen-antibody reaction times. Such times may be between about 5 minutes and about 30 minutes. In some embodiments, reactions in the microfluidic channels may reach equilibrium conditions in a shorter period of time than reactions in e.g. in standard plate wells, due to reduced channel dimensions and subsequently shorter diffusion lengths.

Fig. 11 shows a block diagram of an example reader device 1100. The reader device may be any device capable of controlling the test electrodes to perform an electrochemical measurement of the substrate solution, processing of raw test data into analyte concentration and the communication of the analyte concentration to the user, for example via a host computer or mobile phone, and/or by a display on the reader device. Optionally, the reader device may control the test chamber vent to open, for example by actuation. This actuation may be automatic, or it may be controlled by the user through a user control interface 1110, for example a button or a touch screen display. In one embodiment the reader device is a preferably USB-connected multi-channel potentiostat device that may be controlled by software located on a computing device. In this embodiment, the reader device may have a component 1104 configured to communicate with external computing devices, such as a USB port. The reader device may only have one test strip port 1102, however in some embodiments the device may have multiple ports 1102 to enable multiple test strips to be operated simultaneously. For example, a device with 5 ports would enable simultaneous testing with 5 test strips. The reader may be provided with a processor 1106 running software 1112 to guide the user through the multi-step protocol, and/or this functionality may be provided by software on the computing device. The reader device may further be provided with a display screen 1108 for providing information to the user, and/or this functionality may be provided by an external computing device.

The reader device may be configured to further process the test data. For example, the reader device may multiplex measurements by adding signals and/or results obtained from measurement electrodes (e.g., working electrodes) from different test regions of the chamber, and thereby obtain multiple measurements and/or integrate positive and/or negative controls by adequately tailoring the biochemical modification of the surface in the incubation chamber. It will be appreciated that the processing test data (such as multiplexing) may be performed by the reader device itself, or by one or more external computing device(s), such as a mobile phone or a host computer, which may receive test data from the reader device.

Such a process may be implemented using test strip embodiments as described in the following. Such embodiments may allow multiple reagent solutions to be added to a test strip in a simple way and/or

without the need for any active pumping mechanism. In this regard, one way to achieve passive pumping of fluids is to utilize capillary pressure. Zimmerman et al (LabChip, 2007, 7, 119–125, Capillary pumps for autonomous capillary systems) defines the capillary pressure Pc of a liquid–air meniscus in a microchannel as:

$$P_c = -\gamma\left(\frac{\cos\alpha_b + \cos\alpha_t}{a} + \frac{\cos\alpha_l + \cos\alpha_r}{b}\right)$$

where γ is the surface tension of the liquid, $\alpha_{b,t,l,r}$ are the contact angles of the liquid on the bottom, top, left, and right wall, respectively, and a and b are the depth and width of the microchannel, respectively. Microfluidic components typically have sub-millimeter dimensions and thus may allow for precise control and manipulation of fluids via capillary action. References to microfluidic channels and/or chambers throughout the description generally refer to channels and/or chambers of dimensions at which the mass transport of fluids is primarily governed by capillary pressure. References to capillary pressure herein generally relate to capillary pressure of a saliva-air interface, and may be approximated based on the above equation for capillary pressure Pc based on an assumption that the relevant structure, e.g., channel or chamber, can be approximated as having a substantially rectangular cross-section.

The above definition of a liquid-air capillary pressure Pc may generally be applied to references to capillary pressure throughout the present disclosure, for example in relation to the inlet retention valve having a greatest capillary pressure in the test strip, which may in turn specifically relate to capillary pressure of a saliva-air interface. In embodiments, liquid in the channel may be pulled from each end by the capillary pressure (or combined surface tension) at the liquid-air interface. Liquid may flow into the capillary pump when there is liquid in the inlet since the pull from the inlet may be less than the pull from the capillary pump. (In addition to this capillary pressure difference, liquid in the inlet with height greater than the depth of the channels may generally exert a hydrostatic pressure). Once the inlet is empty and the upstream liquid-air interface is located in the retention valve then the pull there is generally greater than the capillary pump pull so it becomes pinned there. Inlet and test chamber retention valves jointly may have the greatest capillary pressure in a test strip embodiment.

Fig. 1 shows a known saliva test strip 100, such as the test strip device of GB2593199 A1. Test strip 100 has an inlet 102, a microfluidic reaction chamber 106, a capillary pump 110 and a test chamber 116. The reaction chamber 106 is used for incubation or reaction of solution(s) received from the inlet 102, such as saliva, with bioreceptor molecules. Test chamber 116 is provided in a side microfluidic circuit that branches off from the main fluidic channel between reaction chamber 106 and capillary pump 110. The inclusion of an additional side branch in the test strip 100 results in additional dilution and losses of the sample by possible nonspecific adsorption on the device walls, and as therefore reduces the accuracy of the results obtained from any tests.

The test chamber 116 includes a vent channel 120 that connects the test chamber 116 to a vent hole 118. The vent hole 118 is initially sealed to reduce flow into the test chamber 116. The user of test strip 100 is therefore required to open the vent hole 118 after a specified amount of time to initiate flow into the test chamber 116 from the reaction chamber 106 after the substrate has incubated with the bioreceptors in the reaction chamber 106. The vent hole 118 is opened by, for example, piercing or removing a cover or film. The requirement for the user to open the vent hole 118 increases the complexity of the process to the user, and provides an additional source for measurement error, as different users (or even the same user over multiple tests) may open the vent hole 118 at different times even when attempting to follow the same set of instructions. Moreover, the presence of an open hole (e.g. the opened vent hole 118) from which test fluids could escape (e.g. leak) from test strip 100 into the environment represents a potential safety hazard.

Fig. 2A shows an example saliva test strip 200. The test strip 200 comprises an inlet that includes an inlet port 202 and a microfluidic retention valve 204 immediately adjacent to inlet port 202. The inlet retention valve 204 may at least temporarily maintain a position of fluid within retention valve 204 when inlet 202 is at least substantially (e.g., fully) otherwise empty of input solution(s). Advantageously, the retention valve may pin the fluid in position once the inlet is effectively empty. The retention valve 204 may help to reduce or avoid bubble formation in a microfluidic channel 208 leading toward the combined incubation and test chamber 206, even upon multiple serial additions of solutions. Inlet 202 is connected to the chamber 206 via inlet retention valve 204 and the microfluidic channel 208. Test strip 200 further comprises a capillary pump 210 that is connected to chamber 206 by a further microfluidic channel. Capillary pump 210 may comprise vent hole(s) 212. Preferably, the capillary pump 210 has a capillary pressure greater than the rest of microfluidic circuit of test strip 200 except for any microfluidic retention valve(s), such as retention valve 204.

In contrast to test strip 100 of Fig. 1, test strip 200 does not comprise a dedicated test chamber. Instead, chamber 206 may be used for both the incubation or reaction of solution(s) such as saliva with bioreceptor molecules and the testing and/or measurement of the incubated solution. As such, the combined incubation and test (CIT) chamber 206 may be used for determining hormone and/or other analyte levels in a sample by performing a measuring or sensing test on a solution such as a substrate solution. For example, as discussed in more detail with reference to Fig. 10 above, a measurement of a level of oxidization of a substrate solution (e.g., a measure of an amount of oxidised TMB, which may be indicative of concentration of hormone or other analytes) may be used to determine a corresponding analyte level in a test sample. (Embodiments may not measure hormones directly). The CIT chamber 206 may further allow electrochemical measurement of such a solution by exposing the solution to test electrode(s) 216 at one or more test regions 214. The CIT chamber 206 may be pre-functionalised by dispensing bioreceptor molecules along one or more of its surfaces to assist with the incubation or reaction of solution(s). The bioreceptor molecules may comprise, for example, antibodies to bind with a target hormone analyte in a saliva sample.

Beneficially, by incubating and testing the sample in a single chamber 206, the length of the fluidic circuit traversed by the sample before measurements are taken is reduced. Test strip 200 may therefore improve the accuracy of test results by e.g. reducing the measurement error from dilution and losses by possible nonspecific adsorption on the device walls.

In addition, as the flow of the solutions through the fluidic circuit is controlled entirely by passive means (e.g. capillary pressure from capillary pump 210), the user is not required to interact with the device after supplying the samples and solutions required for a particular test. Instead, the timing of the measurements may be controlled entirely by e.g. software, such as software on an accompanying reader device. Test strip 200 therefore may reduce the potential for user error and thereby increases the reliability and/or replicability of the test results.

The test strip 200 may comprise a stack formed of a plurality of layers, such as the example layers shown in Figs. 2B-E. Examples of such layers are described below as first to fourth layers, however any one or more of those layers may not be present, and/or additional layer(s) may be provided above, below or in-between those layers.

A first layer 224 shown in Fig. 2E may be an electrode film, for example a Au/PET film, for example a preferably sputtered gold film with a thickness of, e.g., about (i.e., exactly or approximately) 20-1000nm on a PET film. In one embodiment a thickness of about 50nm is used, and/or the Au/PET film has a square resistance of 5 Ω/□. The gold may be patterned for example by laser ablation to form an electrical circuit for use in an electrochemical measurement. Alternatively or additionally, the electrodes may be screen printed electrodes or part of a circuit formed by lithographic processes.

A second layer 222 shown in Fig. 2D may be a laminate layer such as a 2-ply laminate stack of a double-sided adhesive film (such as PET with inert, acrylic, pressure-sensitive and/or medical-grade adhesive on each side) with a preferably single-side adhesive film (such as PET with preferably hydrophilic and/or pressure-sensitive adhesive). In another embodiment, laminate layer may comprise a single preferably double-sided adhesive film, where the adhesive on at least one side may be hydrophilic. The laminate stack may have a thickness of between about 5 μm and about 300 μm. More specifically, an example thickness may be approximately 200 μm, e.g., 183 μm. The second layer may comprise formations, cuttings and/or cavities corresponding to, for example, one or more of the inlet 202, channel 208 and CIT chamber 206.

A third layer 220 shown in Fig. 2C may comprise a microfluidic cartridge such as a PMMA layer. The PMMA layer may have a thickness of greater than about 0.5 mm. For example, a preferred embodiment may have a thickness of at least 2mm. The microfluidic channels, including for example the inlet and/or CIT chamber, may be formed by laser ablation, injection moulding and/or hot embossing. Additional or alternative polymers may include cyclic olefin polymer (COP), cyclic olefin copolymer (COC), polycarbonate (PC) and/or polystyrene (PS). The depths of the channels in this layer may be less than or about 300μm. However, a total thickness of at least 2mm may be preferred to provide a sufficient inlet

volume such that dispensed reagent volumes may be confined. Alternatively, a thinner PMMA cartridge may have a wider inlet area to compensate for the lost volume. The depths of the channels along with the channel width and length may define the volume capacity of the test strip, and the reagent volumes may be set accordingly. (Thickness of the cartridge may be driven by volumes such as capacity of the test strip in total, balance between reaction and test chamber volumes, and/or maximum reagent volume to be added to the test strip. In embodiments, the channels themselves may be less than about 0.3mm deep so that the strip could then be about 0.5mm thick to robustly accommodate these. However, for a preferred volume, e.g., up to 40uL, of reagent volume may be added to the inlet, the inlet capacity is preferably suitable to hold this without overflowing. A thinner PMMA strip of thickness, say, about 0.5mm and a separate inlet apparatus may increase the capacity there).

A fourth layer may comprise a test strip label and may be a printed and/or die-cut PVC (vinyl) or PET (polyester terephthalate) label. The test strip label may include test strip information and/or branding for the test strip. The label may screen the CIT chamber 206 from exposure to light. This may be advantageous, as light exposure can oxidise a TMB substrate and reduce accuracy of any measurement.

Fig. 3 shows a comparison of the electrode structure 302 of a test strip 100 of Fig. 1 and an example electrode structure 304 of test strip 200 of Fig. 2. As described above, the measurement electrodes of test strip 100 are configured to allow electrochemical measurements of a solution in a dedicated measurement chamber that forms part of an additional side branch of the fluidic circuit. The measurement electrodes of test strip 200, meanwhile, are configured to allow electrochemical measurements of a solution or solutions in a multi-purpose chamber that may also be utilized for e.g. incubating or enabling reactions of the solutions.

Fig. 4 shows a comparison of the measurement chamber 116 of test strip 100 of Fig. 1 and an example CIT chamber 206 of test strip 200 of Fig. 2. In test strip 200, the CIT chamber 206 may be formed in a polymer layer of the test strip (e.g. the third (PMMA) layer as described above). The CIT chamber 206 may be formed, for example, by injection molding techniques. Formation by this or similar methods may enable increased control over the volume and shape of the CIT chamber 206. In contrast, in test strip 100 the size of the test chamber 116 is defined by a deformable layer, such as the second (laminate) layer described above. This may result in a greater variability in the volume of the test chamber 116 as compared to CIT chamber 206, and thus a reduced accuracy of the measurement results.

Figs. 5 and 6 show diagrams of example adhesive layers 500 and 600. The adhesive layers may correspond to the second (laminate) layers described above. In both cases, one or more surfaces of the adhesive layers 500, 600 may be or comprise a hydrophilic surface. As such, adhesive layers 500, 600 may alternatively be referred to alternatively as hydrophilic and/or laminate layers. As shown in Fig. 5, the adhesive layer 500 may comprise cuttings, cavities or formations corresponding to an inlet 502, retention valve 504, channel 506 and CIT chamber 508. In some cases, it has been found that the potential energy barrier between the channel and the CIT chamber of the test strip 200 may introduce a variable delay into

the time for the fluid to flow into the CIT chamber from the inlet. In some example devices, this delay may vary between as much as 30 and 90 seconds. The variability of this delay may reduce the reliability of the measurement results by e.g. altering the wait time between the user steps shown in Fig. 9.

By cutting a channel cavity 506 into the adhesive layer 500 corresponding to the channel between the inlet and the CIT chamber, the potential barrier may be reduced as the fluid in the channel is no longer in contact with a hydrophobic surface. This can be shown by considering the extended Bernoulli equation along a central fluid line:

$$\frac{{v_2}^2 - {v_1}^2}{2} + g(Z_2 - Z_1) + \frac{P_2 - P_1}{\rho} = -w_F - w_S$$

Where v is the fluid velocity, ρ is the fluid density, P is the pressure, and gZ is the potential energy of the fluid. Subscripts 1 and 2 correspond to the fluid in the channel and the CIT chamber respectively. The presence of channel 506 cut into the adhesive layer may result in a reduction or, preferably, a removal of the potential energy term $g(z_2 - z_1)$, and therefore may further result in a corresponding reduction in the kinetic energy required by the fluid to enter the CIT chamber.

Therefore, by providing a channel 506 cut into the adhesive layer 500 the variability of the delay, and in some cases the delay itself, may be reduced. In some implementations, the variability and/or the delay itself may be reduced to about or under 1 second. Delays of this level may have only minor or negligible effects on the measurement results.

It will be appreciated that the inlet, retention valve, channel and chamber may also comprise corresponding formations in other layers of the test strip, for example in a polymer (e.g. third) layer. In some implementations, the cuttings, cavities and/or formations in the adhesive layer may have different dimensions compared to the corresponding formations in one or more other layers. For example, the CIT chamber cavity 508 in the adhesive layer 500 may be larger than the corresponding CIT chamber formed in e.g. the polymer layer. In these implementations, when part of a test strip the adhesive layer may therefore comprise a recess 602 about the CIT chamber. This is shown in Fig. 6A-D, which show a test strip comprising an example adhesive layers 600 and an example polymer layer 606. When formed into such a test strip, the recess 602 may be provided along and under at least part of a sidewall of the CIT chamber of the polymer layer 606. It will be understood that the term “under” is used herein in a non-limiting manner, and in embodiments which provide an adhesive layer “above” the polymer layer 606 recess 602 may be instead provided along and above at least part of a sidewall of the CIT chamber of the polymer layer 606.

As discussed above, one or more surfaces of the adhesive layer 600 may be a hydrophilic surface. In some test strips, the presence of the hydrophilic walls of the exposed cut adhesive layer 600 around the CIT chamber may result in fluid flowing along the walls of the CIT chamber due to the interaction of the fluid with a hydrophilic surface of the exposed adhesive layer 600. Potentially, in some test strips, a large enough proportion of the fluid may flow along the edge of the CIT chamber that the interaction between

the fluid solution and some, most or even all of the antibodies disposed in the CIT chamber is reduced. This may result in a reduction in the accuracy of any measurements, particularly in e.g. ELISA arrays.

Beneficially, the recess 602 in the adhesive layer 600 may reduce the interaction between the fluid and the hydrophilic surface(s) of the cut adhesive layer 600, thereby reducing the risk of fluid bypassing some, most or all of the bioreceptor molecules in the CIT chamber by flowing along the edge of the CIT chamber. This may in turn increase the interaction between the fluid and the antibodies. In some implementations, the recess 602 may be at least 50μm, for example 65μm. The recess 602 distance may additionally be less than the thickness of the adhesive layer 600, for example the recess distance may be less than 200μm. This may reduce the risk of a trapped fluid pocket forming in the recess 602, and/or reduce the risk of fluid bypassing the antibodies in the CIT chamber (for example, as a result of fluid flowing and/or crawling along the recess rather than the through the CIT chamber).

In some implementations, the test strip may comprise tapered or narrowed end regions 604 at one or both of the inlet end 608 and the outlet end of the CIT chamber. The recess 602 in the adhesive layer 600 about the CIT chamber may be less than 50μm at one or both of the tapered end regions 604 of the CIT chamber. For example, the adhesive layer 600 at the entrance 608 to the CIT chamber (i.e. the end of the chamber for receiving fluids from the inlet) may have no recess 602, or may have a variable recess 602 that increases to e.g. 50μm or more at the end of the tapered region 604. Beneficially, the reduced recess 602 in the tapered region(s) 604 may assist in increasing the flow rate of the fluid into and/or out of the CIT chamber. The reduced recess size in these regions 604 may therefore further assist in overcoming any remaining potential barrier between the channel and the CIT chamber.

A cross section of a portion of an example test strip 700 is shown in Fig. 7. The example test strip 700 comprises a polymer layer 702 and a laminate layer 704. As discussed above, the laminate layer may comprise, for example one or more adhesive layers and one or more hydrophilic surfaces. A channel 706 and CIT chamber 710 are formed in the polymer layer 702. Additionally, corresponding cuts and/or formations may be provided in the laminate layer 704, for example channel formation 708 and CIT chamber formation 714. The CIT chamber formation 714 may additionally comprise recesses 712 around at least part of the CIT chamber 710. The recesses 712 may have a size D that is, for example, between 50μm and 200μm, such as 65μm or 100μm. As discussed in further detail above, channel formation 708 may assist in reducing a variable delay in the time taken for fluid to flow from the channel 706 to the CIT chamber 710. Additionally or alternatively, the recesses 712 may assist in increasing the interaction between the fluid (e.g. solutions and/or a sample) and biorecepters (such as antibodies) disposed on the polymer layer 702, for example in the CIT chamber 710.

Fig. 8 shows a further example of an electrode structure suitable for use with a test strip 800 according to the present invention. The electrodes 802a-e correspond to three test regions 804, 806 and 808 in the CIT chamber. It will be understood that fewer or additional test regions may also be provided. The provision of multiple test regions 804, 806, 808 may enable the test strip to be used to obtain multiple measurements and/or to integrate positive and/or negative controls by adequately tailoring the biochemical modification

of the surface in the CIT chamber. The multiple measurements may then be multiplexed by e.g. a reader device or an external computing device. The test regions 804, 806, 808 may be separated to isolate the sample(s) and/or solution(s) of each test region 804, 806, 808 during the incubation and measurement periods. The distance L between the samples may be selected based on the expected diffusion time during the duration τ of the incubation and/or measurement period using the equation:

$$L = \sqrt{D\tau}$$

where D is the diffusion coefficients of the fluid(s). It will be understood that the preferred separation distances between the test regions 804, 806, 808 will vary depending on the solutions and required incubation times. For example, some tests may utilise incubation periods of 5 minutes, 10 minutes, 15 minutes or more. As a specific example provided for illustrative purposes, a test strip may be configured to perform an assay with an incubation period of 15 minutes using a solution with a diffusion coefficient D of approximately 10^{-9} m^2/s. Such a test strip would preferably have a separation distance L between the test regions 804, 806, 808 of at least 0.9mm. Further advantageously, the separation distance between the test regions 804, 806, 808 may be doubled to reduce the mixing of the solutions from the test regions 804, 806, 808 during the incubation period. In other words, in the above example a separation distance of 2L, or 1.8mm, may be used. It will be understood that the above incubation periods and diffusion coefficient values are merely provided as illustrative examples, and should not be construed as in any way limiting the scope of the invention.

By separating the test regions 804, 806, 808 by a suitable distance (e.g. L or 2L), the interaction between the test electrodes 802 of each test region 804, 806, 808 and reagents produced in different test regions 804, 806, 808 may be reduced. This is because the incubation period may not provide a sufficient time for diffusion between the test regions 804, 806, 808. Additionally or alternatively, the CIT chamber may be modified to comprise narrower and/or shallower sections (e.g. reduced width and/or depth sections) between the test regions 804, 806, 808. The narrower and/or shallower regions may slow the diffusion of molecules in these sections, and therefore may slow the diffusion of molecules between test regions 804, 806, 808. The inclusion of narrower and/or shallower regions may therefore allow the separation distance L between the test regions 804, 806, 808 to be reduced. It will be appreciated that the narrower and/or shallower regions may be provided between only some but not all test regions (e.g. between test regions 804 and 806 only, or between test regions 806 and 808 only), or they may be provided between each of the test regions.

Fig. 12 shows a block diagram of an example sample collection device 1200. The sample collection device may comprise an inlet 1202 for receiving a sample, a storage chamber 1206 for storing a received sample and an outlet 1206 for inserting the sample into a test strip. The collector device may additionally or alternatively be used to insert other fluids, such as a substrate solution or wash buffer, into the test strip. In some embodiments, the inlet 1202 may also be the outlet 1206. Storage chamber 1206 may be designed to hold a suitable volume of a sample for use in a test strip.

Fig. 13 shows a block diagram of an example test system 1300 comprising a test strip 1302, a collection device 1304 and a reader device 1306. The test strip 1302 may be, for example, test strip 200 of Fig. 2. Similarly, collection device 1304 and reader device 1306 may be e.g. collection device 1200 of Fig. 12 and reader device 1100 of Fig. 11 respectively.

Figs. 14 shows a further schematic diagram of suitable electrode structures for use with test strips according to the present invention. The electrode structure of Fig. 14a corresponds to the electrode structure of test strip 800 of Fig. 8. Figs. 14b and c show schematic diagrams of alternative methods of providing multiple test regions. In Figs. 14b and c, multiple test chambers 1402 are provided, each test chamber 1402 corresponding to a test region 1404. The flow of the fluids into and around the test chambers 1402 may be controlled in a similar manner to the flow of fluids in and around the test chamber of test strip 100 of Fig. 1 (for example, via opening of the corresponding vent holes 1406 of each of the test chambers 1402).

Fig. 15 outlines an example operational flow of the above-mentioned process 900 through example test strip 1500. We describe below each of steps a) – f), any one or more of which may be optional.

In step a), a sample (shown in blue) such as saliva is added into inlet 1502. The sample flows through the test strip and into capillary pump 1508 via the CIT chamber 1506 and the connecting microfluidic channels, such as inlet channel 1504. As capillary pump 1508 fills with fluids, vent holes 1510 may allow air in capillary pump to be displaced or escape. While in the CIT chamber 1506, target analytes (such as hormones) in the sample may bind to any bioreceptor molecules (such as antibodies) that were disposed in the CIT chamber 1506 during the test strip manufacturing process. The target analytes may bind to the bioreceptors through biorecognition. As the sample flows into the test strip, inlet 1502 preferably becomes empty, however an inlet retention valve may be provided to preferably retain a portion of the sample.

In step b), a conjugate solution (shown in green) may be inserted into inlet 1502. The conjugate solution may be, for example, a solution formed of the target analyte conjugated with an enzyme. The solution may flow into the test strip in the same way as the sample, displacing the sample through the test strip and into capillary pump 1508. As with the sample, inlet 1502 preferably becomes empty as the conjugate solution flows into the test strip, however the inlet retention valve may again retain a portion of the conjugate solution. As the conjugate has displaced the sample, the sample may no longer be retained by the inlet retention valve. The conjugate may bind with at least some of the remaining unbound bioreceptor molecules. At this stage many (preferably most or all) of the bioreceptor molecules may be bound to either the target analyte or the conjugate.

In step c), a wash buffer solution (shown in red) may be placed in inlet 1502. The wash buffer solution may displace the conjugate solution through the test strip and into the capillary pump 1508. Advantageously, the wash buffer solution may reduce the number of unbound conjugate molecules in the CIT chamber 1506. This may prevent the removed conjugates from potentially reacting with later solutions and reducing the reliability of test results. Once again, inlet 1502 preferably becomes empty as the wash buffer flows

into the test strip, however the inlet retention valve may retain a portion of the wash buffer solution. As the wash buffer has displaced the conjugate solution, the conjugate may then no longer be retained by the inlet retention valve.

In step d), one or more additional wash buffer solutions (also shown in red) are added to inlet 1502. These additional buffers may displace the previous solutions as discussed in the previous steps. Each additional wash buffer inserted into the test strip may further reduce the number of unbound conjugate molecules in the CIT chamber 1506, further improving the accuracy and/or reliability of the test.

In step e), a substrate solution (shown in dark blue) is introduced into inlet 1502. The substrate solution may flow through the test strip, displacing the previous solutions such as the wash buffer into capillary pump 1508. Substrate solution in the CIT chamber 1506 may incubate by reacting with the bound enzyme-conjugate molecules. Such reaction may comprise oxidisation of the substrate solution. The incubating/incubated substrate solution is shown in steps e) and f) in purple. The total volume of the fluids introduced to the test strip by this stage should preferably not exceed the total volume of the microfluidic circuit, in order to reduce leakage from vents 1510. Preferably, the inlet retention valve may retain a portion of the substrate solution as discussed for other solutions above, to ensure that there is remaining solution in inlet 1502 during the substrate incubation. When inlet 1502 is not empty, this may increase the hydrostatic pressure in the test strip 1500. The increased hydrostatic pressure may in turn lead to parasitic movement of the substrate relative to the test electrodes. This movement may lead to a change in the location of the test electrodes relative to the intended test areas in the CIT chamber 1506, thereby reducing the accuracy of the measurement results. In addition, the movement may also lead to a change in the portion of the substrate in close contact with the immobilised enzyme conjugate. This may result in a greater volume of the substrate solution (e.g. TMB) reacting with the enzyme less intensely compared to a static volume of the substrate solution. The portion to be measured by the electrodes would therefore be less oxidised and ultimately introduce additional error and reduce accuracy.

In step f), after a preferably predetermined incubation period (for example 10 or 15 minutes) the user or reader device may initiate testing of the incubated substrate solution (i.e. the purple solution). A stationary or slow flowing solution will generally produce more accurate results than a faster flowing solution. A portion of the substrate solution is preferably retained by inlet retention valve, such that the hydrostatic pressure assists in reducing the flow speed of the solution in the CIT chamber 1506. The test electrodes may be controlled (for example, by means of a reader device or other computing device such as a general purpose computer or mobile computing device) to perform an electrochemical transduction. Generally, for a given incubation period, the greater the proportion of bound enzyme-conjugates the more reacted substrate solution will be produced. As the analytes in the conjugate solution and the sample compete for binding spots, in general, an increase in the amount of reacted substrate produced in a given period of time may correlate with a decrease in the levels of the target analyte in the saliva. Therefore, by testing the incubated solution using the test strip of an embodiment, it may be possible to determine a level of the target analyte in the sample.

Although the disclosure has been described in terms of preferred embodiments as set forth above, it should be understood that these embodiments are illustrative only and that the claims are not limited to those embodiments. Those skilled in the art will be able to make modifications and alternatives in view of the disclosure which are contemplated as falling within the scope of the appended claims. Each feature disclosed or illustrated in the present specification may be incorporated in any embodiments, whether alone or in any appropriate combination with any other feature disclosed or illustrated herein.

CLAIMS:

1. An integrated fluid sample test strip comprising:

a first layer;

an inlet for receiving a series of solutions, said solutions comprising at least a fluid sample and a substrate solution;

a test chamber for incubating and performing biosensing tests on one or more solutions received from the inlet, the test chamber formed in the first layer and configured to receive the solutions from the inlet, the test chamber functionalized with one or more bioreceptors for binding to a target analyte, the test chamber comprising a plurality of test electrodes to perform at least part of a biosensing test of the substrate solution; and

a second layer comprising a cavity that is adjacent to the test chamber, wherein the cavity is recessed along and under at least part of a sidewall of the test chamber, the second layer comprising a hydrophilic surface adjacent to the first layer.

2. The test strip of claim 1, comprising a channel for guiding a solution from the inlet to the test chamber, wherein the channel is formed in a hydrophilic surface of the second layer, and optionally wherein the channel is formed in the first layer and the hydrophilic surface of the second layer.

3. The test strip of claim 1 or 2, wherein the second layer is recessed relative to the sidewall of the test chamber by between about 50µm and about 200µm, and further preferably wherein the second layer is recessed relative to the sidewall of the test chamber by about 65µm.

4. The test strip of any preceding claim, wherein the second layer is recessed relative to the sidewall of the test chamber by less than a thickness of the second layer.

5. The test strip of any preceding claim, wherein the test chamber comprises a tapered end for receiving solutions from the inlet.

6. The test strip of claim 5, wherein the second layer is recessed along the at least part of the sidewall of the test chamber relative to the sidewall of the test chamber by a first distance, and wherein the second layer is recessed along at least part of a sidewall of the tapered end of the test chamber relative to the sidewall of the tapered end of the test chamber by a second distance, and wherein the second distance is less than the first distance.

7. The test strip of claim 5, wherein the second layer is not recessed along a sidewall of the tapered end of the test chamber.

8. An integrated fluid sample test strip comprising:

a first layer;

a second layer comprising a hydrophilic surface adjacent to the first layer;

an inlet for receiving a series of solutions, said solutions comprising at least a fluid sample and a substrate solution;

a test chamber for incubating and performing biosensing tests on one or more solutions received from the inlet, the test chamber, the test chamber configured to receive the solutions from the inlet, the test chamber functionalized with one or more bioreceptors for binding to a target analyte, the test chamber comprising a plurality of test electrodes to perform at least part of a biosensing test of the substrate solution; and

a channel for guiding a solution from the inlet to the test chamber, wherein the channel is formed in at least the hydrophilic surface of the second layer.

9. The test strip of any preceding claim, wherein the test chamber comprises a plurality of test regions, each test region comprising one or more of the plurality of test electrodes.

10. The test strip of claim 9, wherein the chamber comprises reduced width sections between at least two of the test regions.

11. The test strip of claim 9 or 10, wherein two or more of the plurality of test regions are separated by a distance of at least about 0.9 mm, and preferably wherein two or more of the plurality of test regions are separated by a distance of at least about 1.8 mm.

12. The test strip of any preceding claim, comprising a retention valve for temporarily retaining each said solution in the inlet to thereby reduce air flow through the retention valve.

13. The test strip of any preceding claim, comprising a capillary pump to receive from the test chamber at least one of the solutions including at least the fluid sample, the capillary pump comprising at least one vent hole to allow any air to escape from the capillary pump and thereby reduce pressure in the capillary pump.

14. The test strip of any preceding claim, wherein the capillary pump comprises at least one capillary channel defined by an array of micropillars.

15. The test strip of claim 14, wherein at least one said micropillar comprises a substantially diamond-shaped cross section, and/or wherein the capillary pump comprises a bypass channel along at least part of a perimeter of the capillary pump, wherein a smallest cross-sectional width of the bypass channel is greater than a smallest separation of between adjacent said micropillars.

16. The test strip of any one of claims 13 to 15, wherein a smallest separation between adjacent said micropillars is less than a smallest width of a solution flow path from the reaction chamber to the capillary pump.

17. The test strip of any one of claims 13 to 16, wherein the capillary pump has an inlet comprising a constriction.

18. The test strip of any preceding claim, wherein:

- a channel for guiding solution from the test chamber to the capillary pump is formed in at least the first layer;
- the inlet is formed at in least the first layer;
- the capillary pump is formed in at least the first layer;
- the first layer comprises a polymer layer; and/or
- the second layer comprises an adhesive layer.

19. The test strip of any preceding claim, wherein the fluid sample comprises saliva, blood, blood serum, blood plasma, urine, nasal fluid or solutions thereof.

20. The test strip of any preceding claim, configured to measure levels of the analyte, wherein the analyte is a hormone.

21. The test strip of any preceding claim, configured to perform an ELISA or ELONA test.

22. A fluid sample test system comprising the fluid sample test strip of any preceding claim and at least one of:

- a fluid sample collector device for collecting the fluid sample and inputting the fluid sample into the inlet; and
- a reader device for controlling at least one of the test electrodes to perform the at least part of the biosensing test, and to output a result of the biosensing test.

23. The fluid test system of claim 22, wherein the reader device is configured to multiplex signals from the plurality of test regions.

24. Use of the test strip of any one of claims 1 to 21 or the test system of claims 22 or 23, to perform an ELISA or ELONA test.

25. The use according to claim 24, comprising:

(i) receiving the fluid sample in the inlet; and/or

(ii) receiving the substrate solution in the inlet; and

optionally comprising:

(iii) receiving a solution comprising an enzyme-conjugate in the inlet; and/or

(iv) receiving one or more wash-buffer solutions in the inlet.

Intellectual Property Office

Application No:	GB2204764.1	**Examiner:**	Miss Elizabeth Price
Claims searched:	1-7 and in part 9-25	**Date of search:**	3 October 2022

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
A	-	WO 2022/036346 A1 (UNIV COLORADO STATE RES FOUND)
A	-	WO 2018/152296 A1 (NEW JERSEY INST TECHNOLOGY)
A	-	WO 2018/187535 A1 (TOKITAE LLC)
A	-	US 2019/0118178 A1 (LEE et al.)
A	-	WO 2014/035167 A1 (NANOBIOSYS INC)
A	-	GB 2593199 A (MINT DIAGNOSTICS LTD)

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

B01L; G01N

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC

Intellectual Property Office

International Classification:

Subclass	Subgroup	Valid From
None		