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(54) **FLUIDIC INDICATOR DEVICE**

FLUIDANZEIGEVORRICHTUNG

DISPOSITIF INDICATEUR FLUIDIQUE

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(56) References cited:
WO-A-2004/083859
WO-A-2006/046054
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Description

Field of the Invention

[0001] The invention relates to a fluidic device for the passage of a liquid. It also relates to an assay device suitable for measurement of the amount and/or presence of an analyte in, or property of, a fluid sample.

Background of the invention

[0002] Simple disposable fluidic devices for the detection of an analyte are known. EP291194 discloses an assay device comprising a lateral flow porous carrier wherein accumulation of a particulate labelled binding reagent in a detection zone provides a visible signal to the user of the presence or absence of analyte in a liquid sample. The signal however requires interpretation by the user. Digital devices have been developed as a consequence wherein on-board optics are able to measure the presence or intensity of the labelled reagent and provide an absolute answer which does not require interpretation. Digital devices however are expensive to produce as they require in addition to the optical components, a power source further processing electronics and a digital display.

[0003] US4963498 discloses a microfluidic device for the measurement of an analyte in, or property of a fluid sample wherein reagents present in the device affect the flow rate of the sample. The device may comprise both a test capillary and a reference or control capillary.

[0004] EP456699 discloses an apparatus for testing the presence of a substance in a liquid comprising a sample application port connected to a number of fluid conduits upstream from respective indicator chambers. According to an example, agglutination reagents present in the fluid conduits interact with the sample in order to change its flow rate, for example, preventing the liquid from reaching an indicator chamber within the time frame of the assay.

[0005] A capillary device for testing for the presence of a substance is also disclosed by WO2004/083859. The device works by causing agglutination of a liquid sample in a test capillary in the presence of an analyte of interest (typically, human chorionic gonadotrophin, hCG), which agglutination prevents the flow of liquid sample in the test capillary but not in a control capillary (which contains no agglutination reagents). The presence or absence of liquid sample at downstream portions of the test and control capillaries is detected by electrodes.

[0006] A problem associated with non-digital assay devices, especially pregnancy-testing devices and/or home-use assay devices, is that they provide an assay result as a signal of variable strength, which can require a degree of interpretation. This leaves the assay result open to misinterpretation, especially where the user or reader of the assay device has a preferred assay result in mind. In the case of some testing devices however, such as a pregnancy-testing device, it is preferred to configure the device such that no interpretation is required and the assay result is provided as one of two alternatives (i.e. pregnant or not pregnant). This may be described as a "binary outcome" device. This provides an unequivocal result which removes the need for interpretation by the user, which is undesirable. This problem has been addressed in the prior art by the provision of assay devices or assay device readers incorporating complicated optical and electronic components to read a variable strength signal and then provide a binary outcome via an electronic (e.g. LCD or LED) display. The present invention provides, in preferred embodiments a simpler method of providing a binary outcome assay device which is far simpler to produce than existing optical/electronic assay devices.

Summary of the Invention

[0007] In a first aspect the invention provides a fluidic assay device for assaying at least one property of a liquid sample, the device being as defined in claim 1 of the claims appended hereto.

[0008] In the event that liquid flowing along the test flow path reaches the junction region before liquid from the reference flow path it is possible, at least in some embodiments, that the flow of liquid along the reference flow path may be prevented. Prevention of the flow of liquid along the reference or test flow path is not necessarily permanent: it is sufficient for the flow of liquid to be prevented within the timescale in which the assay is performed and read.

[0009] The test flow path and/or the reference flow path may comprise or consist of a microfluidic channel, a porous carrier, or a combination of the two. Preferred porous carriers include nitrocellulose and filter paper. The microfluidic channel, if present, is of capillary dimensions such that a typical sample liquid is able to flow along the channel by capillary flow. Preferably the test and/or reference flow paths comprise or consist of channels having at least a portion with a capillary dimension.

[0010] Typical microfluidic channels have an internal cross-sectional dimension of between 0.1 and 500μm, more typically between 1 and 100μm. The microfluidic channels may be formed from synthetic plastics materials such as polycarbonate, epoxy resin etc., glass or metal. The channels may be formed by etching, casting, moulding etc. using conventional techniques.

[0011] Typically, but not necessarily, the property of the liquid sample which is assayed comprises the presence and/or amount of an analyte of interest. The analyte of interest may comprise, for example, a steroid, a hormone, a peptide or polypeptide, a carbohydrate, a lipid, a lipoprotein, a polynucleotide, an enzyme, a blood group marker, a disease marker, a diagnostic or prognostic indicator, a cation, an anion, or a molecular complex such as a virus, bacterium, yeast, fungus, spore or eukaryotic cell. In one preferred embodiment the analyte of interest

comprises hCG. In another embodiment, the analyte is glucose. A property of a liquid sample that may be determined may be for example a coagulation property of blood or plasma such as prothombin time, partial activated thromboplastin time, thrombin time, and activated clotting time.

[0012] The assay device may comprise a control, wherein the control is capable of generating a signal which indicates that sample has been correctly applied to the sample application region and that the assay device is working normally. The control may comprise a control flow path having one or more reagents therein. The reference flow path may also act as a control.

[0013] Conveniently the control flow path is such that sample liquid applied to the sample application region will flow along the flow path and typically to an indicator region, either upstream or downstream of the junction region and there generate a signal, typically a visible signal.

[0014] The test flow path will generally be substantially similar in character to the reference flow path, but will typically comprise one or more reagents or binding partners which will react with or bind to the analyte of interest. Preferably such reaction or binding event has the effect of altering (typically decreasing) the rate of flow of sample liquid along the test flow path.

[0015] The device of the invention can readily be configured to assay for the presence and/or amount of two or more analytes of interest by providing a two or more test flow paths and, optionally, a corresponding number of reference flow paths.

[0016] In one embodiment, a separate sample application port or input is provided in the sample application region for each test flow path. In another embodiment the sample application region comprises a common sample application port or input, such that sample liquid applied thereto may flow into two or more flow paths (e.g. two or more test flow paths; or at least a test flow path and a reference flow path). Preferably the device comprises a common sample application port or input which supplies sample liquid to all flow paths present in the device, such that a single sample application step is sufficient to initiate the assay.

[0017] The liquid sample may be any suitable liquid, such as water, sewage sample, or an aqueous extract (e.g. an aqueous food or drink sample) or a biological sample e.g. blood, plasma, serum, urine, pus, sweat, saliva, vaginal fluid, or tears. A preferred sample is urine. The liquid sample may be applied to the device 'neat' or may be subjected to a pretreatment step (e.g. including one or more of the following: mixing; agitation; sonication; dilution; incubation; denaturation; or reaction with one or more reagents).

[0018] Performance of the assay conveniently comprises reacting or interacting the sample with one or more substances which have the capacity to affect the rate of flow of liquid sample along the test flow path in order to provide an indication or measure of the presence and/or amount of an analyte in, or other property of, the fluid sample. Preferably at least one of the substances will be provided within the assay device, but additionally or alternatively one or more such substances may be mixed with the sample prior to application of the sample to the assay device. Generally, reaction or interaction of the substance(s) with the sample will tend to alter (i.e. increase or decrease) the rate of progress of sample along the test flow path. The substance(s) may be such as to increase the rate of flow of sample liquid along the test flow path if the sample comprises an analyte of interest above a certain minimum detectable concentration. More preferably however the effects of the substance(s) are such as to impede or decrease the rate of flow of sample liquid along the test flow path if the sample comprises the analyte(s) of interest.

[0019] In a preferred embodiment the device comprises one or more reagents which react with, or binding partners which bind to, the analyte(s) of interest. Convenient binding partners comprise antibodies or antigen-binding fragments thereof (such as Fab, Fv, scFv, domain antibodies and the like), or multimers of antibodies or antigen-binding fragments thereof.

[0020] Other suitable binding partners (depending on the nature of the analyte of interest) may comprise, for example, biotin, streptavidin, complementary polynucleotides (comprising 10 or more, preferably 17 or more, bases of DNA, RNA, PNA, LNA or any combination thereof, optionally including modified or non-naturally occurring bases), and polypeptide receptors or at least portions thereof which retain binding activity for their respective ligand. Receptors include both prokaryotic and eukaryotic polypeptides, numerous examples of which (both full length and truncated) are known.

[0021] The reagents or binding partners may be immobilised on the assay device (i.e. remain attached during performance of the assay) or may be releasably attached (i.e. are released from a support during performance of the assay), or may comprise a combination of immobilised and releasably attached reagents or binding partners. For example, in one embodiment, a releasably attached binding partner is provided on a porous carrier located at an upstream portion of the test flow path. In another embodiment an immobilised binding partner is provided in the test flow path. In yet another embodiment a releasably attached binding partner is provided (on a porous carrier or otherwise) at a relatively upstream portion of the test flow path and an immobilised binding partner is provided at a relatively downstream portion of the test flow path. Methods of releasably attaching or of immobilising antibodies and the like on surfaces are well known to those skilled in the art. Conveniently a binding partner is provided within a capillary channel forming part of the test flow path.

[0022] The binding partner or reagent may advantageously be labelled. Suitable labels include, but are not limited to, an enzyme, a fluorescent dye, a coloured dye and a particle of colloidal gold or other colloidal metal.

[0023] According to an embodiment, the presence of analyte may cause an increase in the flow rate of fluid in the test channel. For example binding of an analyte may cause displacement of a species which is conjugated to a detergent, the presence of which in the fluid channel results in an increase in flow rate of the sample.

[0024] Conveniently the binding partner is particulate or comprises a particulate substance. In one embodiment the binding partner comprises a latex particle or a particle of colloidal gold or other metal. Advantageously the particle comprises a plurality of binding partner molecules, such that a single particle may simultaneously be bound to a plurality of members of the analyte of interest. Preferably the latex particle is loaded or marked with a direct visual label, such as a coloured dye.

[0025] In an embodiment the binding partner or partners are such that an agglutination reaction occurs in the test flow path in the presence of the analyte of interest, which agglutination reaction serves to retard or inhibit the flow of sample liquid along the test flow path. The effect of such retardation or inhibition of flow along the test flow path is that liquid flowing along the reference or control path will reach the junction region first, which in turn blocks the further advance of liquid along the test flow path (as explained below).

[0026] In a further embodiment, the test flow path may comprise a reagent such as thromboplastin, or one or more of the various clotting factors, for the determination of a coagulation property of blood or plasma.

[0027] According to a further embodiment, the reagent may be Concanavalin A which is able to react with glucose to cause an increase in viscosity in the fluid sample. The test flow path may comprise a solvent swellable polymer gel which swells in the presence of a particular solvent to cause an increase in viscosity. An example of such is a dextran polymer when the analyte to be detected is water.

[0028] The assay device of the present invention can be thought of as using a "race" between the liquid flowing along the test flow path and that flowing along the reference flow path - the first liquid to reach the junction region will win the "race" and block further advance of liquid along the other flow path.

[0029] The way of forming the block is to provide a number (one or more) of vents downstream of the junction region. Displacement of the gas (typically air) filling the microfluidic channel of the test and flow paths, via these vents, is necessary to allow liquid to advance along the flow paths. However, once liquid from one of the flow paths has reached the junction it prevents the venting of gas from the other flow path, forming a gas block (typically an air block), preventing liquid advancing along the blocked flow path. This arrangement is extremely simple, requires no moving parts, and is easy to manufacture.

[0030] One or both of the test and reference flow paths may additionally comprise partial barriers to flow, such as constrictions, filters, weirs or the like, which encourage the formation of more total barriers or obstructions in the presence of e.g. an agglutination reaction. Typically such a partial barrier or obstruction is provided in the one or more test flow paths but not in the reference flow path.

[0031] The device conveniently comprises at least one indicator region. In one embodiment there is an indicator region located downstream of the junction region. In one embodiment there is an indicator region located upstream of the junction region. In one embodiment there is an indicator region in or on the test flow path and an indicator region in or on the reference flow path, both indicator regions being located between the sample application region and the junction region.

[0032] The indicator region comprises a display which displays information about the assay result to a person using the assay device. Typically the assay result is displayed, at least in part, by a colour change.

[0033] There are a great many ways by which a colour change, visible in the indicator region or regions of the device, could be effected.

[0034] In one example, there is an indicator region downstream of the junction region. In a simple embodiment, dyes of different colours are provided in the respective test and control flow paths, such that the presence of a dye of a particular colour in the indicator region reveals by which route (the test or control flow path) liquid first reached the indicator region. Alternatively, two different enzymes (e.g. horseradish peroxidase and glucose oxidase) could be provided in the indicator region, and a respective substrate for one of the enzymes could be provided in the flow paths which, reacts, in the presence of the relevant enzyme catalyst, to produce a coloured product. The colour of the product reveals which substrate was introduced into the indicator region (and hence by which flow path liquid first arrived there). In general terms, the indicator region (if located downstream of the junction region) may comprise components of two different signal-generating means which generate detectably different signals, with one or more further components of each signal-generating means being mobilisably disposed upstream, the further component of one signal-generating means being disposed in the test flow path, and the further component of the other signal-generating means being disposed in the reference flow path, the further component being required to contact the other component in the indicator region in order to generate a signal. Which of the two signal-generating means is activated depends on which of the further components reaches the indicator region first, which in turn depends on the relative rates of flow of liquid along the test and reference flow paths.

[0035] In one embodiment, the indicator region comprises a pH-sensitive indicator, and the test and reference flow paths each comprise a different pH-affecting agent e.g. one comprises a buffer at relatively acidic pH and one comprises a buffer at relatively alkaline pH. The flow path by which liquid first reaches the indicator region will therefore determine the pH in the indicator region and hence the colour of the indicator.

[0036] Embodiments of this general type, with a downstream indicator region, have the advantage that it is not necessary to impede or retard the flow of liquid along the test flow path by a large amount in order for the liquid flowing along the reference flow path to reach the indicator region first - a time differential of as little as 1 or 2 seconds will suffice.

[0037] In other embodiments an indicator region is provided, upstream of the junction region, in each of the reference and the test flow paths. In one embodiment, flow of liquid along the reference flow path to a certain point acts to block flow of liquid along the test flow path before the liquid reaches the indicator region on the test flow path, such that a certain assay result is displayed in the indicator region. In some embodiments it may be advantageous to provide an indicator substance, such as a dye, upstream of the indicator region, such that a visible change can be seen if/when liquid reaches the indicator region of the test and/or reference+ flow paths.

[0038] In some embodiments, the indicator region comprises a microfluidic channel, such as a capillary, which is visible to a user (e.g. through a window or aperture in an otherwise opaque housing). In one embodiment the indicator region comprises two channels or capillaries, one forming part of the test flow path and one forming part of the reference flow path. In one embodiment, the microfluidic channels or capillaries in the indicator region became filled with a coloured liquid during performance of the assay. The colour of the liquid may itself indicate the result of the assay. Alternatively, the coloured liquid may simply serve to alter the visibility of the channel or capillary. For example, a clear plastics or glass capillary against a clear or white background may not be readily apparent. Introduction of a coloured liquid into such a channel or capillary will increase contrast and render the channel or capillary readily visible. Alternatively, if the channel or capillary is initially of high contrast with its background (e.g. a white capillary against a red background), then introduction of a coloured liquid into the channel or capillary which is of the same colour as the background will reduce the contrast and render the capillary or channel difficult to observe. These all represent different methods of conveying or displaying a visible signal concerning the outcome of the assay.

[0039] In some embodiments, the indicator region may comprise one or more channels or capillaries which form one or more words or symbols (such as "PREGNANT", or a plus or minus symbol). In one particular embodiment, in which an assay device in accordance with the invention is provided as a pregnancy test device, one flow path comprises an indicator region in which a channel or capillary forms the word "NOT", and another flow path comprises an indicator region in which a channel or capillary forms the word "PREGNANT". Typically the word "NOT" is formed in the test flow path and the word "PREGNANT" is formed in the reference flow path. If a sample is applied the device which does not contain any hCG (i.e. the subject is not pregnant), liquid is free to flow along both the test and reference flow paths. A coloured label e.g. a dye, is transported along both flow paths, making the words "NOT" and "PREGNANT" appear as a message in a display. If a sample comprising hCG is applied to the device, agglutination reagents (e.g. particles of latex coated with anti-hCG antibodies) present in the test flow path reduce the rate of flow so much that liquid in the reference flow path reaches the junction before the liquid in the test flow path can reach the indicator region. This effectively blocks the test flow path, so that the word "NOT" does not become visible and instead the display gives the message "PREGNANT".

[0040] In some embodiments it may be preferred to bias the assay device, so as to configure the device such that liquid flowing along the reference flow path will, in the absence of analyte of interest in the sample, reach the junction region slightly before the liquid flowing along the test flow path. This feature applies particularly, but not exclusively, to those embodiments in which an indicator region is provided downstream of the junction region, and in which, for example, the test and reference flow paths are provided with a respective indicator or label. If the times taken for the liquid sample to reach the junction region via the reference flow path and the test flow path were identical, it is at least conceivable that liquid from both flow paths would reach the junction region exactly simultaneously and hence become mixed in the indicator region, which would fail to provide a clear assay result. This can be avoided by making the reference flow path shorter and/or by making the rate of flow along the reference flow path more rapid (e.g. by using a thinner bore capillary).

[0041] In a second aspect the invention provides a method of testing for the presence of an analyte of interest in a liquid sample, the method comprising the step of applying the liquid sample to the sample application region of a device in accordance with the first aspect of the invention; and noting or recording the assay result displayed by the device.

[0042] For the avoidance of doubt it is hereby expressly stated that any features described herein as "preferred", "advantageous", "desirable", "convenient", "typical" or the like may be present in the invention in isolation or in combination with any other feature so described, unless the context dictates otherwise.

[0043] The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, in which

Figures 1 and 2 show schematic representations of different embodiments of an assay device in accordance with the present invention.

EXAMPLES

Example 1

[0044] **Figure 1** shows a device according to the in-

vention. The device has a sample application region 2 fluidically connected to test flow path 4 and a reference flow path 6, which both comprise a capillary channel. A filter 8 may optionally be provided in one or both of the flow paths. The flow paths converge downstream at a junction region 10 leading to a common channel 12. An indicator region 14 may be provided downstream from the junction region 10.

[0045] Liquid sample applied to the device via a sample application port in the sample application region 2 is able to flow respectively along the test and reference flow paths 4, 6 and towards the junction region 10. One or more vents are provided in the common channel 12 and the indicator region 14 to allow air to be displaced from the device by the advance of liquid along the capillaries. However, once one of the fluid fronts has reached the junction region 10, it blocks off the other flow path from the vents, preventing further advance of the liquid along the other flow path. Thus the device only allows for the arrival in the indicator region 14 of fluid flowing along the flow path whose fluid front first reaches the junction region 10. An indication means may be provided in the fluid channels to enable an observer to determine which fluid in the respective channel arrived first. For example dyes of different colours may be provided in each channel such that the fluid sample is able to interact with the dye to produce liquid of a particular colour. Thus the presence of a particular coloured dye in the indicator region would enable a user to determine which fluid reached the fluid gate first.

Preparation of the assay device according to Fig 1.

[0046] A base layer was prepared from agarose coated 200μm polyester (GelBond, BMA). The appropriate microfluidic features were cut out of a 75μm thick heat sealing adhesive PE layers using a GraftTeC cutter and the two layers laminated together. Finally a third layer was laminated to the intermediate layer to provide microfluidic channels of 75μm.

Example 2

[0047] An alternative embodiment of an assay device in accordance with the invention is illustrated in Figure 2. Components functionally equivalent to those of the embodiment illustrated in Figure 1 are denoted by common reference numerals.

[0048] As in the previous example, the assay device comprises a sample application port in a common sample application region 2, from which liquid sample can flow into a capillary forming part of the test flow path 4 and a separate capillary forming part of the reference flow path 6. Alternatively each flow path may be provided with a unique, separate sample application region. Those skilled in the art will appreciate that the assay device described in the present examples may be provided with further test flow paths to test for the presence of further analytes of interest. The or each further test flow path can, if desired, be provided with a corresponding reference flow path.

[0049] In the embodiment depicted in Figure 2, each flow path comprises a filter element 8 and an indicator region 14, upstream of a junction region 10.

[0050] The filer element 8 comprises one or more binding partners for the analyte of interest, in this instance hCG. In the presence of the analyte of interest the binding partner, particles coated with anti-hCG monoclonal antibody, mediates an agglutination reaction.

[0051] Each flow path is also provided with a coloured dye which is mobilised by contact, and migrates, with the liquid sample.

[0052] The indicator region 14 of each flow path comprises a capillary channel forming the word "NOT" in the test flow path 4 and the word "PREGNANT" in the reference flow path. These capillaries are formed from clear synthetic plastics material and are against a low contrast background (e.g. white or clear synthetic plastics material). Accordingly, prior to performance of the assay, the capillaries are not highly visible.

[0053] However, once the assay is initiated, the dye located in the flow paths upstream of the indicator region is mobilised by the advancing liquid sample. If the sample does not contain hCG, liquid is free to flow along both flow paths. The dye-containing liquid thus fills both capillaries, displaying the assay result "NOT PREGNANT". Vents may be provided at several points along the reference flow path to encourage the flow of liquid therealong. In particular these vents may be provided to assist the liquid in filling the indicator region of the reference flow path. Preferably there are no such vents in the test flow path, air being vented from the test flow path capillary 4 only via one or more vents downstream of the junction region 10, in the common channel 12, such that if liquid flowing along the reference flow path 6 reaches the junction region 10 before the liquid front flowing along the test flow path 4, air can no longer be displaced from the test flow path capillary and further advance of the liquid along that channel is prevented.

[0054] The rate of flow of liquid along the test and reference flow paths, and/or the length of the respective flow paths, is adjusted such that, in the absence of hCG, liquid flows along both flow paths 4, 6 and fills the respective indicator regions. Typically, in the absence of hCG in the sample, the liquid flowing along the reference flow path will reach the junction region 10 either simultaneously with the liquid flowing along the test flow path or just 1 or 2 seconds in advance thereof.

[0055] If however the applied sample comprises hCG, agglutination will take place in the test flow path 4 which substantially retards the advance of liquid along the test flow path capillary towards the indicator region. This allows liquid flowing along the reference flow path to "win the race" to the junction region easily. The liquid flowing along the reference flow path reaches the junction region 10 before the liquid flowing along the test flow path 4

reaches the indicator region. In this instance, the word "NOT" does not become filled with dye and remains indistinct, whilst the word "PREGNANT" becomes highly visible and thus displays the assay result.

Example 3

[0056] In order to provide a practical demonstration of the feasibility of the invention 15μm polystyrene beads (Polysciences) were coated with aminodextran 500,000 RMM, then with NHS-LCLC-Biotin to prepare biotinylated latex beads (NHS = N-hydroxy succinimidyl, LCLC = "long chain", i.e. a 12 carbon spacer). Into 50 μl of a 200 μg/ml solution of BSA (to block non-specific binding sites), was added 50 μl of a 5% solution of the 15 μm biotin particles, mixed on a vortex. To the biotinylated particle solution in BSA, 5μl of streptavidin 1μm magnetic particle in solution were added solution, while mixing on a vortex to prepare a test fluid. Immediately after preparation, the fluid was added to a microfluidic device as described below.

[0057] A reference fluid consisting of BSA buffer was also prepared.

[0058] A microfluidic device was prepared having a sample application port provided upstream from a fluid channel of dimensions, 5mm wide by 3cm long by 100μm in height. Provided at a distance of 2cm along the fluid channel was a filter zone of 5mm in length comprising channels running parallel to the fluid channel having a 30μm gap.

[0059] Two such devices were prepared and a test solution and reference solution were added respectively to both and the time taken for the fluid front to reach the end of the fluid channel was measured. In this particular example, the test solution took 60s to reach the end of the channel. In contrast, the reference fluid took just 10s.

[0060] The delay in flow of the test fluid was due to the agglutinated particles becoming stuck in the filter zone. In the case of the reference fluid, no agglutination took place and therefore the fluid is able to flow unimpeded.

Claims

1. A fluidic assay device for assaying at least one property of a liquid sample, the device comprising:

(i) a liquid sample application region (2);
(ii) at least one test flow path (4) in liquid flow communication with the sample application region;
(iii) a reference flow path (6) in liquid flow communication with the sample application region;
(iv) a junction region (10), at which the test flow path and the reference flow path contact one another, the junction region comprising an outlet, conduit, chamber or other portion (12) which permits the onward flow of liquid; and
(v) one or more vents provided downstream of the junction region, whereby displacement of air or other fluid through the vents is necessary to allow liquid to advance along the test and reference flow paths;

the device being adapted such that when liquid flowing along one of the test flow path or the reference flow path reaches the junction region first, it creates an air lock or other gas lock, upstream of the vents, which blocks further advance of the flow of liquid along the other of the test flow path or the reference flow path; and wherein the test and/or reference flow paths comprise a microfluidic channel, a porous carrier, or a combination of both; and wherein the microfluidic channel, if present, comprises at least a portion which is of capillary dimension.

2. An assay device according to claim 1, wherein the test and/or reference flow paths comprise one or more of the following: a filter (8); an incubation region; a chamber; a flow restriction, and a label or indicator.

3. An assay device according to claim 2, wherein the label or indicator is mobilisable upon contact with the liquid sample.

4. An assay device according to claim 2 or 3, wherein the label or indicator is selected from the group consisting of: an enzyme; a fluorescent dye, a coloured dye; and a particle of colloidal gold or other metal.

5. An assay device according to any one of the preceding claims, wherein the assay device comprises a reagent which reacts with, or a binding partner which binds to, an analyte of interest present in the sample.

6. An assay device according to claim 5, wherein the reagent or binding partner is located in the test flow path.

7. An assay device according to claim 6, wherein reaction of the reagent with the analyte, or binding of the binding partner to the analyte, decreases the rate of flow of the liquid sample along the test flow path.

8. An assay device according to claim 5 or 6, wherein the binding partner comprises an antibody, an antigen-binding fragment of an antibody, or a multimer of an antibody or an antigen-binding fragment thereof.

9. An assay device according to any one of claims 5, 6 or 8, wherein the reagent or binding partner is particulate or associated with a particle.

10. An assay device according to any one of claims 5-8,

wherein the reagent or binding partner causes agglutination in the presence of the analyte of interest, sufficient to impede or retard the flow of sample liquid along the test flow path.

11. An assay device according to any one of the preceding claims, further comprising an indicator region (14) which indicates the result of the assay, said indicator region being either upstream or downstream of the junction region.

12. An assay device according to claim 11, wherein an indicator region is provided in both the test and the reference flow paths.

13. An assay device according to any one of the preceding claims, which provides a binary outcome for the assay result, yet is a non-digital assay device.

14. An assay device according to any one of the preceding claims, which is a pregnancy-testing device and the analyte of interest comprises hCG.

15. A method of detecting the presence and/or amount of an analyte of interest in a liquid sample, the method comprising the steps of: applying the liquid sample to the sample application region of an assay device in accordance with any one of the preceding claims; and noting or recording the assay result.

Patentansprüche

1. Fluidtestvorrichtung zum Testen mindestens einer Eigenschaft einer Flüssigkeitsprobe, wobei die Vorrichtung Folgendes umfasst:

(i) einen Flüssigkeitsproben-Applikationsbereich (2);
(ii) mindestens einen Testflussweg (4) in flüssiger Flusskommunikation mit dem Probenapplikationsbereich;
(iii) einen Referenzflussweg (6) in flüssiger Flusskommunikation mit dem Probenapplikationsbereich;
(iv) einen Vereinigungsbereich (10), bei welchem der Testflussweg und der Referenzflussweg einander kontaktieren, wobei der Vereinigungsbereich einen Auslass, eine Rohrleitung, eine Kammer oder einen anderen Abschnitt (12) umfasst, welcher den weiteren Fluss von Flüssigkeit erlaubt; und
(v) eine oder mehrere Belüftungen, welche dem Vereinigungsbereich nachgeschaltet bereitgestellt sind, wobei ein Verdrängen von Luft oder anderer Flüssigkeit durch die Belüftungen nötig ist, um zu gestatten, dass sich Flüssigkeit entlang des Test- und des Referenzflusswegs fortbewegt;

wobei die Vorrichtung derartig eingerichtet ist, dass, wenn Flüssigkeit, welche entweder entlang des Testflusswegs oder entlang des Referenzflusswegs fließt, den Vereinigungsbereich zuerst erreicht, sie eine Luftschleuse oder eine andere Gasschleuse, den Belüftungen vorgeschaltet, erzeugt, die eine weitere Fortbewegung des Flusses von Flüssigkeit entlang des jeweils anderen des Testflusswegs oder des Referenzflusswegs blockiert; und wobei der Test- und/oder der Referenzflussweg einen mikrofluidischen Kanal, einen porösen Träger oder eine Kombination aus beidem umfassen; und wobei der mikrofluidische Kanal, falls vorhanden, mindestens einen Abschnitt umfasst, welcher eine Kapillarabmessung aufweist.

2. Testvorrichtung nach Anspruch 1, wobei der Test- und/oder der Referenzflussweg mindestens eines von Folgendem umfassen: einen Filter (8); einen Inkubierungsbereich; eine Kammer; eine Flussbegrenzung und ein Kennzeichen oder eine Anzeige.

3. Testvorrichtung nach Anspruch 2, wobei das Kennzeichen oder die Anzeige bei Kontakt mit der Flüssigkeitsprobe mobilisierbar ist.

4. Testvorrichtung nach Anspruch 2 oder 3, wobei das Kennzeichen oder die Anzeige aus der Gruppe ausgewählt ist, welche aus Folgendem besteht: einem Enzym; einem Fluoreszenzfarbstoff, einem gefärbten Farbstoff; und einem Partikel kolloidales Gold oder anderes Metall.

5. Testvorrichtung nach einem der vorhergehenden Ansprüche, wobei die Testvorrichtung ein Reagenz, welches mit einem interessierenden Analyten, welcher in der Probe vorhanden ist, reagiert, oder einen Verbindungspartner umfasst, welcher sich damit verbindet.

6. Testvorrichtung nach Anspruch 5, wobei das Reagenz oder der Verbindungspartner auf dem Testflussweg lokalisiert ist.

7. Testvorrichtung nach Anspruch 6, wobei eine Reaktion des Reagenzes mit dem Analyten oder eine Verbindung des Verbindungspartners mit dem Analyten die Fließgeschwindigkeit der Flüssigkeitsprobe entlang des Testflusswegs vermindert.

8. Testvorrichtung nach Anspruch 5 oder 6, wobei der Verbindungspartner einen Antikörper, ein Antigen-Verbindungsfragment eines Antikörpers oder ein Multimer eines Antikörpers oder eines Antigen-Verbindungsfragments davon umfasst.

9. Testvorrichtung nach einem der Ansprüche 5, 6 oder 8, wobei das Reagenz oder der Verbindungspartner partikelförmig oder einem Partikel zugeordnet ist.

10. Testvorrichtung nach einem der Ansprüche 5 bis 8, wobei das Reagenz oder der Verbindungspartner eine Agglutination in der Gegenwart des interessierenden Analyten bewirkt, welche ausreichend ist, um den Fluss der Probenflüssigkeit entlang des Testflusswegs zu behindern oder zu hemmen.

11. Testvorrichtung nach einem der vorhergehenden Ansprüche, weiterhin umfassend einen Anzeigebereich (14), welcher das Ergebnis des Tests anzeigt, wobei der Anzeigebereich dem Vereinigungsbereich entweder vorgeschaltet oder nachgeschaltet ist.

12. Testvorrichtung nach Anspruch 11, wobei ein Anzeigebereich sowohl in dem Test- als auch in dem Referenzflussweg bereitgestellt wird.

13. Testvorrichtung nach einem der vorhergehenden Ansprüche, welche ein binäres Resultat für das Testergebnis bereitstellt, jedoch eine nicht-digitale Testvorrichtung ist.

14. Testvorrichtung nach einem der vorhergehenden Ansprüche, welche eine Schwangerschaftstestvorrichtung ist, und wobei der interessierende Analyt hCG umfasst.

15. Verfahren zum Detektieren der Gegenwart und/oder der Menge eines interessierenden Analyten in einer Flüssigkeitsprobe, wobei das Verfahren die folgenden Schritte umfasst: Applizieren der Flüssigkeitsprobe auf den Probenapplikationsbereich einer Testvorrichtung nach einem der vorhergehenden Ansprüche; und Vermerken oder Aufzeichnen des Testergebnisses.

Revendications

1. Dispositif d'analyse fluidique destiné à analyser au moins une propriété d'un échantillon liquide, le dispositif comportant :

(i) une région (2) d'application d'échantillon liquide ;
(ii) au moins un passage (4) d'écoulement d'essai en communication d'écoulement de liquide avec la région d'application d'échantillon ;
(iii) un passage (6) d'écoulement de référence en communication d'écoulement de liquide avec la région d'application d'échantillon ;
(iv) une région (10) de jonction, au niveau de laquelle le passage d'écoulement d'essai et le passage d'écoulement de référence entrent en contact, la région de jonction comportant une sortie, un conduit, une chambre ou une autre partie (12) qui permet au liquide de continuer à s'écouler ; et
(v) un ou plusieurs évents pratiqués en aval de la région de jonction, un déplacement d'air ou d'un autre fluide à travers les évents étant ainsi nécessaire pour permettre à du liquide d'avancer le long des passages de test et d'écoulement de référence ;

le dispositif étant conçu de telle façon que, lorsqu'un liquide circulant le long de l'un des passages d'écoulement d'essai et d'écoulement de référence atteint la région de jonction en premier, il bloque toute progression supplémentaire de l'écoulement de liquide le long de l'autre des passages d'écoulement d'essai et d'écoulement de référence ; et les passages d'écoulement d'essai et /ou de référence comportant un canal microfluidique, un support poreux, ou une combinaison des deux ; et le canal microfluidique, s'il est présent, comporte au moins une partie qui est de dimension capillaire.

2. Dispositif d'analyse selon la revendication 1, les passages d'écoulement d'essai et /ou de référence comportant un ou plusieurs des éléments suivants ; un filtre (8) ; une région d'incubation ; une chambre ; un étranglement, et une étiquette ou un indicateur.

3. Dispositif d'analyse selon la revendication 2, l'étiquette ou l'indicateur pouvant être mobilisé suite à un contact avec l'échantillon liquide.

4. Dispositif d'analyse selon la revendication 2 ou 3, l'étiquette ou l'indicateur étant choisi dans le group constitué : d'une enzyme, une teinture fluorescente, une teinture colorée, et une particule d'ou colloïdal ou d'un autre métal.

5. Dispositif d'analyse selon l'une quelconque des revendications précédentes, le dispositif d'analyse comportant un réactif qui réagit avec, ou un partenaire de liaison qui se lie à, un analyte d'intérêt présent dans l'échantillon.

6. Dispositif d'analyse selon la revendication 5, le réactif ou le partenaire de liaison étant situé dans le passage d'écoulement d'essai.

7. Dispositif d'analyse selon la revendication 6, la réaction du réactif avec l'analyte, ou la liaison du partenaire de liaison à l'analyte, diminue le débit d'écoulement de l'échantillon liquide le long de le passage d'écoulement d'essai.

8. Dispositif d'analyse selon la revendication 5 ou 6, le

partenaire de liaison comportant un anticorps, un fragment fixateur d'antigène d'un anticorps, ou un multimère d'un anticorps ou d'un fragment fixateur d'antigène de celui-ci.

9. Dispositif d'analyse selon l'une quelconque des revendications 5, 6 ou 8, le réactif ou le partenaire de liaison étant sous forme particulaire ou associé à une particule.

10. Dispositif d'analyse selon l'une quelconque des revendications 5-8, le réactif ou le partenaire de liaison provoquant, en présence de l'analyte d'intérêt, une agglutination suffisante pour gêner ou ralentir l'écoulement d'échantillon liquide le long du passage d'écoulement d'essai.

11. Dispositif d'analyse selon l'une quelconque des revendications précédentes, comportant en outre une région indicatrice (14) qui indique le résultat de l'analyse, ladite région indicatrice se trouvant soit en amont, soit en aval de la région de jonction.

12. Dispositif d'analyse selon la revendication 11, une région indicatrice étant aménagée dans les deux passages d'écoulement de test et de référence.

13. Dispositif d'analyse selon l'une quelconque des revendications précédentes, qui présente une issue binaire du résultat de l'analyse, mais est un dispositif d'analyse non numérique.

14. Dispositif d'analyse selon l'une quelconque des revendications précédentes, qui constitue un dispositif de test de grossesse et où l'analyte d'intérêt comporte de l'hCG.

15. Procédé de détection de la présence et / ou de la quantité d'un analyte d'intérêt dans un échantillon liquide, le procédé comportant les étapes consistant à appliquer l'échantillon liquide à la région d'application d'échantillon d'un dispositif d'analyse selon l'une quelconque des revendications précédentes ; et à noter ou à enregistrer le résultat de l'analyse.

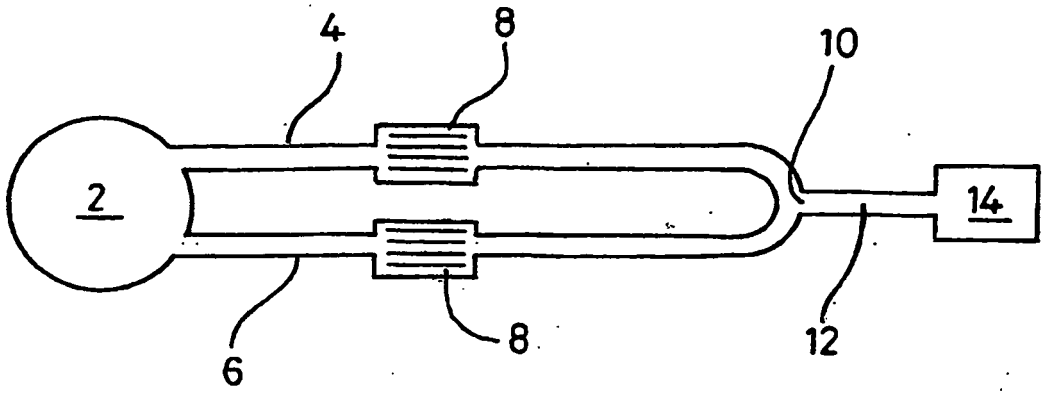

Fig. 1

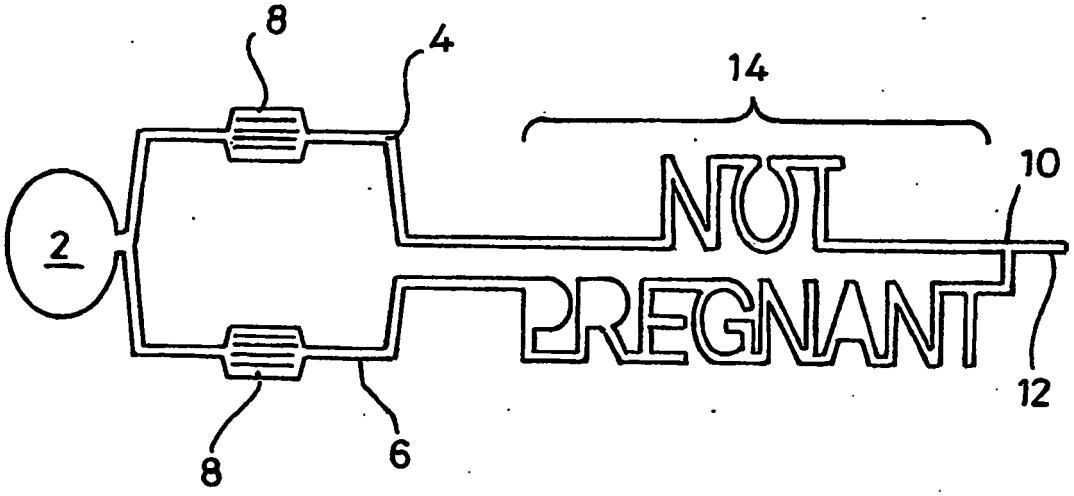

Fig. 2

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- EP 291194 A **[0002]**
- US 4963498 A **[0003]**
- EP 456699 A **[0004]**
- WO 2004083859 A **[0005]**