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- (57) In particular, the invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result rapidly and which require the minimum degree of skill and involvement from the user. The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace.

CLAIMS

1. An analytical test device incorporating a dry porous carrier to which device a liquid sample suspected of containing an analyte can be applied, the device also incorporating a specific binding reagent attached to a particulate label which labelled specific binding reagent is freely mobile in the porous carrier when in the moist state, and an unlabelled specific binding reagent which is permanently immobilised in a detection zone on the porous carrier, the labelled and unlabelled specific binding reagents being capable of participating in either a

sandwich reaction or a competition reaction in the presence of the analyte, wherein prior to the application to the device of the liquid sample, the particle-labelled specific binding reagent is retained in the dry state in a macroporous body having a pore size not less than 10 times greater than the maximum particle size of the particulate label, through which macroporous body the applied liquid sample must pass en route to the porous carrier material, thus facilitating uptake of the particle-labelled specific binding reagent by the applied liquid sample.

22. A pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member and the macroporous body together acting as a reservoir from which urine is released into the porous carrier, the macroporous body containing a highly-specific anti-hCG antibody bearing a coloured particulate direct label, the labelled antibody having freely mobile within the macroporous body and the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the macroporous body a highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member.

23. A fertile period prediction device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member and the macroporous body together acting as a reservoir from which urine is released into the porous carrier, the macroporous body containing a highly-specific anti-LH antibody bearing a coloured particulate direct label, the labelled antibody being freely mobile within the macroporous body and the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the macroporous body a highly-specific unlabelled anti-LH antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different LH epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member.

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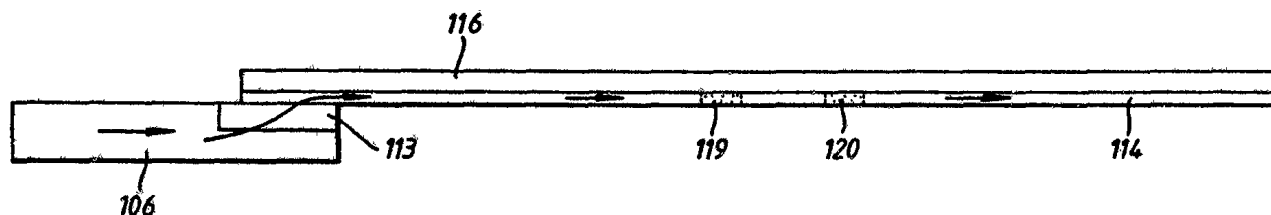
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(54) Title: SOLID-PHASE ANALYTICAL DEVICE**(57) Abstract**

An analytical test device incorporating a dry porous carrier to which a liquid sample, eg. urine, suspected of containing an analyte such as HCG or LH can be applied indirectly, the device also incorporating a labelled specific binding reagent which is freely mobile in the porous carrier when in the moist state, and an unlabelled specific binding reagent which is permanently immobilised in a detection zone on the carrier material, the labelled and unlabelled specific binding reagents being capable of participating in either a sandwich reaction or a competition reaction in the presence of the analyte, in which prior to the application to the device of a liquid sample suspected of containing the analyte, the labelled specific binding reagent is retained in the dry state in a macroporous body, e.g. of plastics material having a pore size of 10 microns or greater, through which the applied liquid sample must pass en route to the porous carrier material, the labelled specific binding reagent being freely soluble or dispersible in any liquid sample which enters the macroporous body.

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Solid-phase analytical device.

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The present invention relates to assays involving specific binding, especially immunoassays.

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In particular, the invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to give an analytical result rapidly and which require the minimum degree of skill and involvement from the user. The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace.

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In the specification of UK patent application GB 2204398A we describe test devices which are readily usable even by an unskilled person and which typically merely require that some portion of the device is contacted with a sample (e.g. urine in the case of a pregnancy or ovulation test) and thereafter no further actions are required by the user before an analytical result can be observed. The analytical result can be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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The use of reagent-impregnated test strips in specific binding assays, such as immunoassays, has previously been proposed. In such procedures a sample is applied to one portion of the test strip and is allowed
5 to permeate through the strip material, usually with the aid of an eluting solvent such as water. In so doing, the sample progresses into or through a detection zone in the test strip wherein a specific binding reagent is immobilised. Analyte present in the sample can
10 participate in a sandwich or a competition reaction within the detection zone, with a labelled reagent which can also be incorporated in the test strip or applied thereto. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB
15 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention provides an analytical test
20 device incorporating a dry porous carrier to which a liquid sample suspected of containing an analyte can be applied indirectly, the device also incorporating a labelled specific binding reagent which is freely mobile in the porous carrier when in the moist state, and an
25 unlabelled specific binding reagent which is permanently immobilised in a detection zone on the carrier material, the labelled and unlabelled specific binding reagents being capable of participating in either a sandwich reaction or a competition reaction in the presence of the
30 analyte, in which prior to the application to the device of a liquid sample suspected of containing the analyte, the labelled specific binding reagent is retained in the dry state in a macroporous body through which the applied liquid sample must pass en route to the porous carrier
35 material, the labelled specific binding reagent being

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freely soluble or dispersible in any liquid sample which enters the macroporous body.

5 The invention also encompasses a macroporous body containing in the dry state a labelled specific binding reagent that is freely soluble or dispersible in an aqueous sample that may be applied to the macroporous body. The invention further encompasses any analytical device that incorporates such a macroporous body together
10 with a test strip or the like into which liquid sample carrying dissolved or dispersed labelled specific binding reagent can flow from the macroporous body. The invention also encompasses the use of such a macroporous body to facilitate the uptake of a labelled specific
15 binding agent by a liquid sample before such a sample is analysed on a test strip or the like.

Preferably, the dry porous carrier material comprises a chromatographic strip, such as a strip of
20 nitrocellulose. If desired, the nitrocellulose can be backed with moisture impermeable material, such as polyester sheet. Using nitrocellulose as the porous carrier material has considerable advantage over more conventional strip materials, such as paper, because
25 nitrocellulose has a natural ability to bind proteins without requiring prior sensitisation. Specific binding reagents, such as immunoglobulins, can be applied directly to nitrocellulose and immobilised thereon. No chemical treatment is required which might interfere with
30 the essential specific binding activity of the reagent. Unused binding sites on the nitrocellulose can thereafter be blocked using simple materials, such as polyvinylalcohol. Moreover, nitrocellulose is readily
35 available in a range of pore sizes and this facilitates the selection of a carrier material to suit particularly requirements such as sample flow rate. Preferably the

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nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns.

5 In a preferred embodiment of the invention, the labelled specific binding reagent comprises a specific binding reagent attached to a particulate label. Such "direct labels", e.g. coloured latex particles, gold
10 sols, non-metallic colloids, and dye sols, are already known per se. They can be used to produce an instant analytical result without the need to add further reagents in order to develop a detectable signal. They are robust and stable and can therefore be used readily in a analytical device which is stored in the dry state.
15 Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes. Preferably, the particulate label is a latex particle, such as a coloured latex particle which can be readily visible to the eye if it becomes bound in the detection
20 zone. If desired, the assay result can be read instrumentally, eg. by colour reflectance. Alternatively, the latex particle can incorporate a fluorescent compound which can respond to applied electromagnetic energy such as ultraviolet light or
25 visible light, to provide an emitted signal that can be measured instrumentally. In a particularly preferred embodiment, the direct label is a coloured latex particle of spherical or near-spherical shape and having a maximum diameter of not greater than about 0.5 micron. An ideal
30 size range for such particles is from about 0.05 to about 0.5 microns.

We have found that use of a macroporous body as the portion of the device wherein the applied liquid sample
35 encounters the particulate label considerably facilitates the ease with which the particulate label is taken up by

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the liquid sample, compared to the situation that usually prevails if the particulate label is incorporated as a pre-dosed reagent on the dry porous carrier strip. To enable the particulate label to migrate freely out of the macroporous body with the liquid sample, the macroporous body preferably has a pore size at least 10 times greater than the maximum particle size of the particulate label. More preferably, the macroporous body comprises plastics material having an average pore size of not less than 10 microns, and ideally about 100 microns, because such larger pore sizes give better release of the labelled reagent. The plastics material should not be protein-binding, or should be easily blockable by means of reagents such as BSA or PVA, to minimise non-specific binding and to facilitate free movement of the labelled reagent after the macroporous body has become moistened with the liquid sample. The plastics material can be pre-treated with surface active agent or solvent, if necessary, to render it more hydrophilic and to promote rapid uptake of the liquid sample. Alternatively, if desired, a surface active agent can be incorporated in the solution containing the labelled reagent when this is applied to the macroporous material during manufacture of the device.

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The labelled reagent is preferably incorporated in the macroporous material in bulk, eg. large sheet, form before it is subdivided into individual bodies for use in a testing device of the invention.

30

After a solution containing the labelled reagent has been allowed to saturate the macroporous material, the macroporous material should be dried, eg. by vacuum or air-drying, or preferably by freeze-drying. Optionally, the solution can also contain a surface active agent, such as a detergent, and/or a glazing material, such as a

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sugar, e.g. sucrose. The presence of the glazing material appears to enhance release of the labelled reagent and promotes stability of delicate specific binding reagents such as antibodies.

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By incorporating the labelled reagent in a separate macroporous body, rather than pre-dosed onto the carrier material that also incorporates the detection zone, the following advantages can be obtained:

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Enhanced sensitivity of the test, because a substantial quantity of the liquid sample is able to take up the labelled reagent before migrating through the carrier material to the detection zone, enhancing potential reaction time without significantly increasing overall test time. Also, the liquid which permeates the carrier is of a more uniform and consistent composition. Whereas the test devices as described in our earlier patent application GB 2204398A are primarily, although not exclusively, suited to qualitative assays, those of the present invention are especially suitable for quantitative assays as well as for qualitative assays.

Enhanced perceived performance of the test. For example, when the device incorporates a carrier strip and the detection zone comprises a line of immobilised reagent, and the label is a visible direct label, a positive result shows up more clearly, with much reduced temporary background caused by the visible labelled reagent being progressively conveyed past the detection zone.

Ease of manufacture, because the incorporation of the labelled reagent in the separate macroporous body avoids the need to apply the labelled reagent in a

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special zone in the carrier, which may need careful pre-treatment, as described in our GB 2204398A.

If the assay device is intended to identify more
5 than one analyte in a single sample, the macroporous body
can incorporate several labelled specific binding
reagents each carrying a different label, eg. having
different colours or fluorescent properties. This will
facilitate the manufacture of a multiple analyte testing
10 device.

Ideally, the macroporous body is in direct
moisture-conductive contact with the porous material, and
the detection zone on the porous carrier material is
15 spaced away from the region of contact between the porous
carrier material and the macroporous body. In such an
embodiment, the quantity of liquid sample required to
saturate the macroporous body is preferably not less than
the quantity of liquid sample capable of being absorbed
20 by the mass of porous carrier material linking the
macroporous body and the detection zone. In other words,
the liquid capacity of the macroporous body is at least
equal to the liquid capacity of the working portion of
the porous carrier.

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The invention also provides an analytical method in
which a device as set forth above is contacted with an
aqueous liquid sample suspected of containing the
analyte, such that the sample permeates by capillary
30 action via the macroporous body through the porous solid
carrier into the detection zone and the labelled reagent
migrates therewith to the detection zone, the presence of
analyte in the sample being determined by observing the
extent (if any) to which the labelled reagent becomes
35 bound in the detection zone.

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In one embodiment of the invention, the labelled reagent is a specific binding partner for the analyte. The labelled reagent, the analyte (if present) and the immobilised unlabelled specific binding reagent cooperate
5 together in a "sandwich" reaction. This results in the labelled reagent being bound in the detection zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

10

In another embodiment of the invention, the labelled reagent is either the analyte itself which has been conjugated with a label, or is an analyte analogue, ie a chemical entity having the identical specific binding
15 characteristics as the analyte, and which similarly has been conjugated with a label. In the latter case, it is preferable that the properties of the analyte analogue which influence its solubility or dispersibility in an aqueous liquid sample and its ability to migrate through
20 the moist porous solid phase material should be identical to those of the analyte itself, or at least very closely similar. In this second embodiment, the labelled analyte or analyte analogue will migrate through the porous carrier into the detection zone and bind with the
25 immobilised reagent. Any analyte present in the sample will compete with the labelled reagent in this binding reaction. Such competition will result in a reduction in the amount of labelled reagent binding in the detection zone, and a consequent decrease in the intensity of the
30 signal observed in the detection zone in comparison with the signal that is observed in the absence of analyte in the sample.

In a further alternative embodiment, an analyte or
35 analyte analogue is immobilised in the detection zone, and the labelled reagent is specific for the analyte. If

an analyte-containing sample is applied to the device, competition between the immobilised and free analyte reduced the extent to which the labelled reagent may become bound in the detection zone.

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In a further embodiment of the present invention, the porous carrier is linked via the macro-porous body to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into
10 the porous carrier. Preferably, the porous carrier and the macroporous body are contained within a moisture-impermeable casing or housing and the porous receiving member extends out of the housing and can act as a means for permitting a liquid sample to enter the
15 housing and reach the porous carrier. The housing should be provided with means, e.g. appropriately placed apertures, which enable the detection zone of the porous solid phase carrier material (carrying the immobilised unlabelled specific binding reagent) to be observable
20 from outside the housing so that the result of the assay can be observed. If desired, the housing may also be provided with further means which enable a further zone of the porous solid phase carrier material to be observed from outside the housing and which further zone
25 incorporates one or more control reagents which enable an indication to be given as to whether the assay procedure has been completed. Preferably the housing is provided with a removable cap or shroud which can protect the protruding porous receiving member during storage before
30 use. If desired, the cap or shroud can be replaced over the protruding porous receiving member, after sample application, while the assay procedure is being performed.

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An important embodiment of the invention is a pregnancy testing device comprising a hollow elongated

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casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member and the macroporous body together acting as a reservoir from which urine is released into the porous carrier, the macroporous body containing a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the macroporous body and the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the macroporous body an highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member. A fertile period prediction device, essentially as just defined except that the analyte is LH, is an important alternative.

Such devices can be provided as kits suitable for home use, comprising a plurality (e.g. two) of devices individually wrapped in moisture impervious wrapping and packaged together with appropriate instructions to the user.

The porous sample receiving member can be made from any bibulous, porous or fibrous material capable of

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absorbing liquid rapidly. The porosity of the material can be unidirectional (ie with pores or fibres running wholly or predominantly parallel to an axis of the member) or multidirectional (omnidirectional, so that the member has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight), polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene can be used.

It can be advantageous to pre-treat the member with a surface-active agent during manufacture, as this can reduce any inherent hydrophobicity in the member and therefore enhance its ability to take up and deliver a moist sample rapidly and efficiently. Porous sample receiving members can also be made from paper or other cellulosic materials, such as nitro-cellulose. Materials that are now used in the nibs of so-called fibre tipped pens are particularly suitable and such materials can be shaped or extruded in a variety of lengths and cross-sections appropriate in the context of the invention. Preferably the material comprising the porous receiving member should be chosen such that the porous member can be saturated with aqueous liquid within a matter of seconds. Preferably the material remains robust when moist, and for this reason paper and similar materials are less preferred in any embodiment wherein the porous receiving member protrudes from a housing. The liquid must thereafter permeate freely from the porous sample receiving member into the macroporous body.

30

If present, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the control zone can be loaded with an antibody that will bind to the labelled reagent, e.g. an "anti-mouse" antibody if the labelled reagent is an antibody that has been derived using a

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murine hybridoma, to confirm that the sample has permeated the test strip. Alternatively, the control zone can contain an anhydrous reagent that, when moistened, produces a colour change or colour formation, e.g. anhydrous copper sulphate which will turn blue when moistened by an aqueous sample. As a further alternative, a control zone could contain immobilised analyte which will react with excess labelled reagent from the first zone. As the purpose of the control zone is to indicate to the user that the test has been completed, the control zone should be located downstream from the detection zone in which the desired test result is recorded. A positive control indicator therefore tells the user that the sample has permeated the required distance through the test device.

The label can be any entity the presence of which can be readily detected. Preferably the label is a direct label, ie. an entity which, in its natural state, is readily visible either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g. UV light to promote fluorescence. For example, minute coloured particles, such as dye sols, metallic sols (e.g. gold), and coloured latex particles, are very suitable. Of these options, coloured latex particles are most preferred. Concentration of the label into a small zone or volume should give rise to a readily detectable signal, e.g. a strongly-coloured area. This can be evaluated by eye, or by instruments if desired.

Indirect labels, such as enzymes, e.g. alkaline phosphatase and horse radish peroxidase, can be used but these usually require the addition of one or more developing reagents such as substrates before a visible signal can be detected. Hence these are less preferred. Such additional reagents can be incorporated in the

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porous solid phase material or in the macroporous body, or in the sample receiving member if present, such that they dissolve or disperse in the aqueous liquid sample. Alternatively, the developing reagents can be added to
5 the sample before contact with the porous material or the porous material can be exposed to the developing reagents after the binding reaction has taken place.

Coupling of the label to the specific binding
10 reagent can be by covalent bonding, if desired, or by hydrophobic bonding. Such techniques are commonplace in the art, and form no part of the present invention. In the preferred embodiment, where the label is a direct label such as a coloured latex particle, hydrophobic
15 bonding is preferred.

In all embodiments of the invention, it is essential that the labelled reagent migrates with the liquid sample as this progresses to the detection zone. Preferably,
20 the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous carrier material in order that this may occur and that any excess labelled reagent which does not participate in any binding reaction in the detection zone is flushed away
25 from the detection zone by this continuing flow. If desired, an absorbant "sink" can be provided at the distal end of the carrier material. The absorbent sink may comprise, for example, Whatman 3MM chromatography paper, and should provide sufficient absorptive capacity
30 to allow any unbound conjugate to wash out of the detection zone. As an alternative to such a sink it can be sufficient to have a length of porous solid phase material which extends beyond the detection zone.

35 The presence or intensity of the signal from the label which becomes bound in the detection zone can

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provide a qualitative or quantitative measurement of analyte in the sample. A plurality of detection zones arranged in series on the porous solid phase material, through which the aqueous liquid sample can pass progressively, can also be used to provide a quantitative measurement of the analyte, or can be loaded individually with different specific binding agents to provide a multi-analyte test.

10 The immobilised reagent in the detection zone is preferably a highly specific antibody, and more preferably a monoclonal antibody. In the embodiment of the invention involving the sandwich reaction, the labelled reagent is also preferably a highly specific
15 antibody, and more preferably a monoclonal antibody.

Preferably the porous carrier material is in the form of a strip or sheet to which during manufacture of the device, one or more reagents can be applied in
20 spacially distinct zones. During use, the liquid sample is allowed to permeate through the sheet or strip from one side or end to another.

If desired, a device according to the invention can
25 incorporate two or more discrete bodies of porous solid phase carrier material, e.g. separate strips or sheets, each carrying immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device
30 initiates sample flow in the discrete bodies simultaneously. The separate analytical results that can be determined in this way can be used as control results, or if different reagents are used on the different carriers, the simultaneous determination of a plurality
35 of analytes in a single sample can be made. Alternatively, multiple samples can be applied

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individually to an array of carriers and analysed simultaneously.

5 The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that proteinaceous reagents, such as an antibody, in the detection zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of an
10 antibody in the second zone needs to be performed by chemical coupling using, for example, CNBr, carbonyldiimidazole, or tresyl chloride.

Following the application of the specific binding
15 reagent to the detection zone, the remainder of the porous solid phase material should be treated to block any remaining binding sites elsewhere. Blocking can be achieved by treatment with protein (e.g. bovine serum albumin or milk protein), or with polyvinylalcohol or
20 ethanolamine, or any combination of these agents, for example. Between these process steps the porous solid phase carrier material should be dried.

Preferably the porous solid phase material is
25 nitrocellulose sheet having a pore size of at least about 1 micron, even more preferably of greater than about 5 microns, and yet more preferably about 8-12 microns. Very suitable nitrocellulose sheet having a nominal pore size of up to approximately 12 microns, is available
30 commercially from Schleicher and Schuell GmbH.

Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strength. This can be manufactured easily by forming a
35 thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose

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when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose
5 can be tightly sandwiched between two supporting sheets of solid material, e.g. plastics sheets.

It is preferable that the flow rate of an aqueous sample through the porous solid phase material should be
10 such that in the untreated material, aqueous liquid migrates at a rate of 1cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the macroporous body
15 and the detection zone, and the flow rate characteristics of the porous carrier material, can be selected to allow adequate reaction times during which the necessary specific binding can occur. Further control over these parameters can be achieved by the incorporation of
20 viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the detection zone is impregnated throughout the thickness of the
25 carrier in the detection zone (e.g. throughout the thickness of the sheet or strip if the carrier is in this form). Such impregnation can enhance the extent to which the immobilised reagent can capture any analyte or labelled reagent, present in the migrating sample.

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Reagents can be applied to the porous carrier material in a variety of ways. Various "printing" techniques have previously been proposed for application of liquid reagents to carriers, e.g. micro-syringes, pens
35 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the

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present context. To facilitate manufacture, the carrier (e.g. sheet) can be treated with the reagents and then subdivided into smaller portions (e.g. small narrow strips each embodying the required reagent-containing zones) to provide a plurality of identical carrier units.

An assay based on the above principles can be used to determine a wide variety of analytes by choice of appropriate specific binding reagents. The analytes can be, for example, proteins, haptens, immunoglobulins, hormones, polynucleotides, steroids, drugs, infectious disease agents (e.g. of bacterial or viral origin) such as Streptococcus, Neisseria and Chlamydia. Sandwich assays, for example, may be performed for analytes such as hCG, LH, and infectious disease agents, whereas competition assays, for example, may be carried out for analytes such as E-3-G and P-3-G.

The determination of the presence (if any) of more than one analyte in sample can have significant clinical utility. For example, the ratio of the levels of apolipoproteins A₁ and B can be indicative of susceptibility to coronary heart disease. Similarly, the ratio of the levels of glycated haemoglobin (HbA) to unglycated (HbAo) or total (Hb) haemoglobin can aid in the management of diabetes. Additionally it is possible to configure tests to measure two steroids simultaneously, e.g E-3-G and P-3-G.

The determination of the presence of more than two (ie multiple) analytes in any sample may have significant clinical utility. For example, the detection of the presence of various different serotypes of one bacterium, or the detection of the presence of soluble serological markers in humans may be useful. By way of example, a multiple analyte test for the detection of the

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presence of different serotypes of Streptococcus can be prepared for groups A, B, C and D. A cocktail of monoclonal antibodies, each specific for various pathologically important group serotypes, or a polyclonal antiserum raised against a particular Streptococcal group, can be dispensed onto a porous carrier strip as a line extending the width of the strip of approximately 1mm zone length. Multiple lines be dispensed in spatially discrete zones, each zone containing immunochemically reactive component(s) capable of binding the analyte of interest. Following the application of the multiple zones, via a suitable application procedure (eg ink-jet printing, metered pump and pen, airbrush), the remainder of the porous material should be treated with a reagent (eg bovine serum albumin, polyvinylalcohol, ethanolamine) to block any remaining binding sites elsewhere.

By way of example only, some preferred embodiments of the invention will now be described in detail with reference to the accompanying drawings.

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Embodiment 1

Figure 1 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and Figure 2 represents a cross-sectional side elevation of the device shown in Figure 1.

Referring to Figure 1, the device comprises a housing or casing 100 of elongate rectangular form having at one end 101 a portion 102 of reduced cross-sectional area. A cap 103 can be fitted onto portion 102 and can abut against the shoulder 104 at end 101 of the housing. Cap 103 is shown separated from housing 100. Extending beyond end 105 of portion 102 is a porous sample collector 106. When cap 103 is fitted onto portion 102 of the housing, it covers porous sample collector 106. Upper face 107 of housing 100 incorporates two apertures 108 and 109. The housing is constructed of an upper half 110 and a lower half 111.

Referring to Figure 2, it can be seen that housing 100 is of hollow construction. Porous sample collector 106 extends into housing 100. The inner end 112 of sample collector 106 is recessed to accommodate a macroporous body 113 of plastics material. Aqueous liquid sample applied to collector 106 can pass freely into macroporous body 113, rapidly saturating it. In turn, macroporous body 113 is in liquid permeable contact with a strip of porous carrier material 114. The housing is constructed of an upper half 110 and a lower half 111 and strip 114 overlap to ensure that there is adequate contact between these two components and that a liquid sample applied to sample collector 106 can permeate via macroporous body 113 and into strip 114. Strip 114 extends further into housing 100. To help ensure that no

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liquid sample reaches Strip 114 without first passing through macroporous body 113, a gap 115 can be left in the housing 100 by arranging for the strip 114 to overlap macroporous body 113 only partially. Strip 114 is

5 "backed" by a supporting strip 116 formed of transparent moisture-impermeable plastics material. Strip 114 extends beyond apertures 108 and 109. Means are provided within housing 100 by webbs 117 and 118 to hold strip 114 firmly in place. In this respect, the internal

10 constructional details of the housing are not a significant aspect of the invention as long as the strip is held firmly in place within the housing, sample collector 106 is firmly retained in the housing, and adequate fluid permeable contact is maintained between

15 sample collector 106, macroporous body 113 and strip 114. The transparent backing strip 116 lies between strip 114 and apertures 108 and 109 and can act as a seal against ingress of moisture from outside the housing 100 via these apertures. If desired, the residual space 119

20 within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip 114 in the dry state during storage. The reagent-containing detection zone in strip 114 is not depicted in Figure 2, but the zone containing the

25 immobilised unlabelled reagent will lie in the region exposed through aperture 108 in order that when the device has been used in an assay, the result can be observed through aperture 108. Aperture 109 provides means through which a control zone containing further

30 reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap 103 is removed from the holder and sample collector 106 is exposed to a

35 liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing sample

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collector 106 to the liquid sample for a time sufficient to ensure that the collector 106 is saturated with the sample, the cap 103 can be replaced and the device placed aside by the user for an appropriate period time (e.g. two or three minutes) while the sample permeates test strip 114 to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures 108 and 109 and can ascertain whether the assay has been completed by observing the control zone through aperture 109, and can ascertain the result of the assay by observing the second zone through aperture 108.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing 100 being moulded in two parts (e.g. upper and lower halves 110 and 111) which can be securely fastened together (e.g. by ultrasonic welding) after the sample collector, macroporous body and test strip have been placed within one of the halves and then sandwiched between the two halves. The act of forming this sandwich construction can be used to "crimp" the sample collector macroporous body and test strip together to ensure adequate contact between them. Cap 103 can be moulded as a separate complete item. If desired, apertures 108 and 109 can be provided with transparent inserts which may insure greater security against ingress of extraneous moisture from outside the housing. By providing a tight fit between the end 105 of housing 100 and the protruding sample collector 106, the application of sample to the protruding member will not result in sample entering the device directly and by-passing collector 106. Collector 106 therefore provides the sole route of access for the sample to the strip within the housing, and can deliver sample to the strip in a controlled manner. The device

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as a whole therefore combines the functions of sampler and analyser.

By using the test strip materials and reagents as
5 herein described, a device in accordance with Figures 1
and 2 can be produced which is eminently suitable for use
as a pregnancy test kit or fertile period test kit for
use in the home or clinic. The user merely needs to
apply a urine sample to the exposed porous member and
10 then (after optionally replacing the cap) can observe the
test result through aperture 108 within a matter of a few
minutes.

Although described with particular reference to
15 pregnancy tests and fertile period tests, it will be
appreciated that the device, as just described, can be
used to determine the presence of a very wide variety of
analytes if appropriate reagents are incorporated in the
test strip. It will be further appreciated that aperture
20 109 is redundant and may be omitted if the test strip
does not contain any control means. Further, the general
shape of the housing and cap, both in terms of their
length, cross-section and other physical features, can be
the subject of considerable variation without departing
25 from the spirit of the invention.

Figure 3 of the accompanying drawings shows an
enlarged view of the sample collector, macroporous body
and test strip in the device illustrated in Figures 1 and
30 2.

The bibulous sample collector 106 is linked to the
macroporous body 113 and test strip 114, backed by the
transparent plastics sheet 116, such that liquid can flow
35 in the direction shown by the arrows from the sample
collector through the macroporous body and into the

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porous strip. Test zone 120 incorporates the immobilised specific binding reagent, and control zone 121 contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip.

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An aqueous sample deposited in collector 106 can flow into macroporous body 113 and take up labelled reagent therein. The sample can permeate from macroporous body 113 along the length of strip 114 and in so doing will carry the labelled reagent along the strip and through zone 120.

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If the desired, eg. for ease of manufacture, the collector 106 need not be recessed to accommodate the macroporous body 113. Instead, these components can simply be placed in an overlapping arrangement, together with the porous strip 114, and pressed together during assembly of the complete device. This will in practice provide a physical arrangement in which the liquid path will be essentially as depicted in Figure 3.

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Embodiment 2

Figures 4 and 5 illustrate another embodiment of the invention, which is seen in plan view in Figure 4 and in cross-section in Figure 5, the cross-section being an elevation on the line A seen in Figure 4.

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Referring to Figure 4, the test device comprises a flat rectangular casing 400 incorporating a centrally disposed rectangular aperture 401, adjacent the left hand end 402, and two further apertures 403 and 404 near the mid point of the device and arranged such that apertures 401, 403 and 404 lie on the central longitudinal axis of the device corresponding to line A. Although all three

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apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in Figure 5, the device is hollow and incorporates within it a macroporous sample receiving member 405 adjacent end 402 of casing 400 and lying directly beneath aperture 401. Sample receiving member 405 is in liquid-conductive contact with one end of a test strip 406 backed by a transparent plastics sheet 407 also contained within casing 400, and which extends to the extreme other end of the casing. The transparent backing sheet 407 is in firm contact with the upper inner surface 408 of casing 400, and provides a seal against apertures 403 and 404 to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip 406 incorporates a test zone and a control zone placed appropriately in relation to apertures 403 and 404, in a manner analagous to that described in Embodiment 1. The macroporous sample receiving member incorporates a labelled reagent which is readily soluble or dispensable in an applied liquid sample.

In operation, an aqueous sample can be applied through aperture 401, e.g. by means of a syringe, to saturate porous receiving member 405 which contains labelled reagent which can be taken up by the sample. Thereafter, the aqueous sample can permeate the test strip and, after an appropriate time, the test result can be observed through apertures 403 and 404.

Example

A sheet (1.4mm thick) of commercially-available, detergent pre-treated, macroporous polyethylene having a pore size of about 100 microns was saturated with an

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aqueous suspension of blue-coloured latex particles (prepared as described in GB 2204398A) of particle size about 0.4 microns. The latex particles carried an anti-beta LH monoclonal antibody. The solution also
5 contained 3% BSA and 4% sugar. The sheet was then freeze-dried and cut into portions each 6 x 12mm, having a liquid capacity of about 50 μ L. These were incorporated in test devices as described above under embodiment 1, with the test strip comprising backed nitrocellulose with
10 an anti-alpha LH monoclonal antibody immobilised in the test zone. The liquid capacity of the "working length" of the test strip between the macroporous body and the detection zone was about 40 μ L.

15 When a LH-containing urine sample was applied to the device, a positive result showed up as a very clear blue line, with negligible background blue colour being visible in the detection window while the assay was being run.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An analytical test device incorporating a dry porous carrier to which device a liquid sample suspected of containing an analyte can be applied, the device also incorporating a specific binding reagent attached to a particulate label which labelled specific binding reagent is freely mobile in the porous carrier when in the moist state, and an unlabelled specific binding reagent which is permanently immobilised in a detection zone on the porous carrier, the labelled and unlabelled specific binding reagents being capable of participating in either a sandwich reaction or a competition reaction in the presence of the analyte, wherein prior to the application to the device of the liquid sample, the particle-labelled specific binding reagent is retained in the dry state in a macroporous body having a pore size not less than 10 times greater than the maximum particle size of the particulate label, through which macroporous body the applied liquid sample must pass en route to the porous carrier material, thus facilitating uptake of the particle-labelled specific binding reagent by the applied liquid sample.

2. An analytical test device according to claim 1, wherein the macroporous body has an average pore size of not less than 10 microns.

3. An analytical test device according to claim 1 or claim 2, wherein the macroporous body has an average pore size of about 100 microns.

4. An analytical test device according to any one of claims 1 to 3, wherein the macroporous body comprises plastics material.



5. An analytical device according to claim 4, wherein the plastics material has been pre-treated to render it more hydrophilic.

5 6. An analytical device according to claim 4 or claim 5, wherein the plastics material is polyethylene.

7. An analytical test device according to any one of the preceding claims, wherein the particulate label is
10 selected from the group consisting of metallic (e.g. gold) sols, non-metallic sols, dye sols, and latex particles having a maximum diameter of not greater than about 0.5 micron.

15 8. An analytical test device according to claim 7, wherein the particulate label is latex.

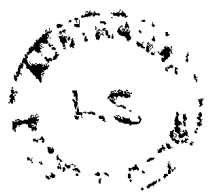
9. An analytical test device according to claim 8, wherein the latex is coloured.
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10. An analytical test device according to claim 8, wherein the latex is fluorescent.

11. An analytical test device according to any one of the preceding claims, wherein the dry porous carrier
25 material comprises a chromatographic strip.

12. An analytical test device according to any one of the preceding claims, wherein the porous carrier material
30 is nitrocellulose.

13. An analytical test device according to claim 1, wherein the nitrocellulose has a pore size of at least 1 micron.



14. An analytical test device according to any one of the preceding claims, wherein the macroporous body is in direct moisture-conductive contact with the porous carrier material, and the detection zone on the porous carrier material is spaced away from the region of contact of the porous carrier material with the macroporous body.

15. An analytical test device according to claim 14, wherein the quantity of liquid sample required to saturate the macroporous body is not less than the quantity of liquid sample capable of being absorbed by the mass of porous carrier material linking the macroporous body and the detection zone.

16. An analytical test device according to any one of the preceding claims, wherein the macroporous body and porous carrier are contained within a casing or housing constructed of moisture-impermeable material and having a sample entry port communicating with the macroporous body, the casing or housing also incorporating means to enable the detection zone to be observable from outside the casing or housing.

17. An analytical device according to any one of claims 1 to 15, wherein the porous carrier is linked via the macroporous body to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous carrier.

18. An analytical device according to claim 17, wherein the porous carrier and the macroporous body are contained within a casing or housing constructed of moisture-impermeable material and the porous receiving member extends out of the casing or housing and can act as



a means for permitting a liquid sample to enter the housing and reach the porous carrier, the casing or housing being provided with means which enable the detection zone of the porous carrier to be observable from outside the casing or housing so that the result of the assay can be observed.

19. An analytical device according to any one of claims 16 to 18, wherein the casing or housing is provided with means which enable a further zone of the porous carrier to be observed from outside the housing and which further zone incorporates one or more control reagents which enable an indication to be given as to whether the assay procedure has been completed.

20. An analytical device according to claim 18, provided with a removable cap or shroud which can protect the protruding porous receiving member during storage before use.

21. An analytical device according to any one of the preceding claims, wherein the liquid sample is aqueous.

22. A pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member and the macroporous body together acting as a reservoir from which urine is released into the porous carrier, the macroporous body containing a highly-specific anti-hCG antibody bearing a coloured particulate



direct label, the labelled antibody having freely mobile within the macroporous body and the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the macroporous body a highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member.

23. A fertile period prediction device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing, the porous nitrocellulose carrier and the sample receiving member being linked via a macroporous body such that any sample reaching the porous carrier must first pass through the macroporous body, the sample receiving member and the macroporous body together acting as a reservoir from which urine is released into the porous carrier, the macroporous body containing a highly-specific anti-LH antibody bearing a coloured particulate direct label, the labelled antibody being freely mobile within the macroporous body and the porous carrier when in the moist state, and in a detection zone on the carrier spatially distant from the macroporous body a highly-specific unlabelled anti-LH antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different LH



epitopes, the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member.

24. An analytical device according to claim 1, substantially as hereinbefore described with reference to Figures 1 and 2 of the accompanying drawings.

25. An analytical device according to claim 1, substantially as hereinbefore described with reference to Figures 4 and 5 of the accompanying drawings.

DATED THIS 9TH DAY OF SEPTEMBER 1993

UNILEVER PLC

By its Patent Attorneys:

GRIFFITH HACK & CO

Fellows Institute of Patent
Attorneys of Australia.



Fig. 1.

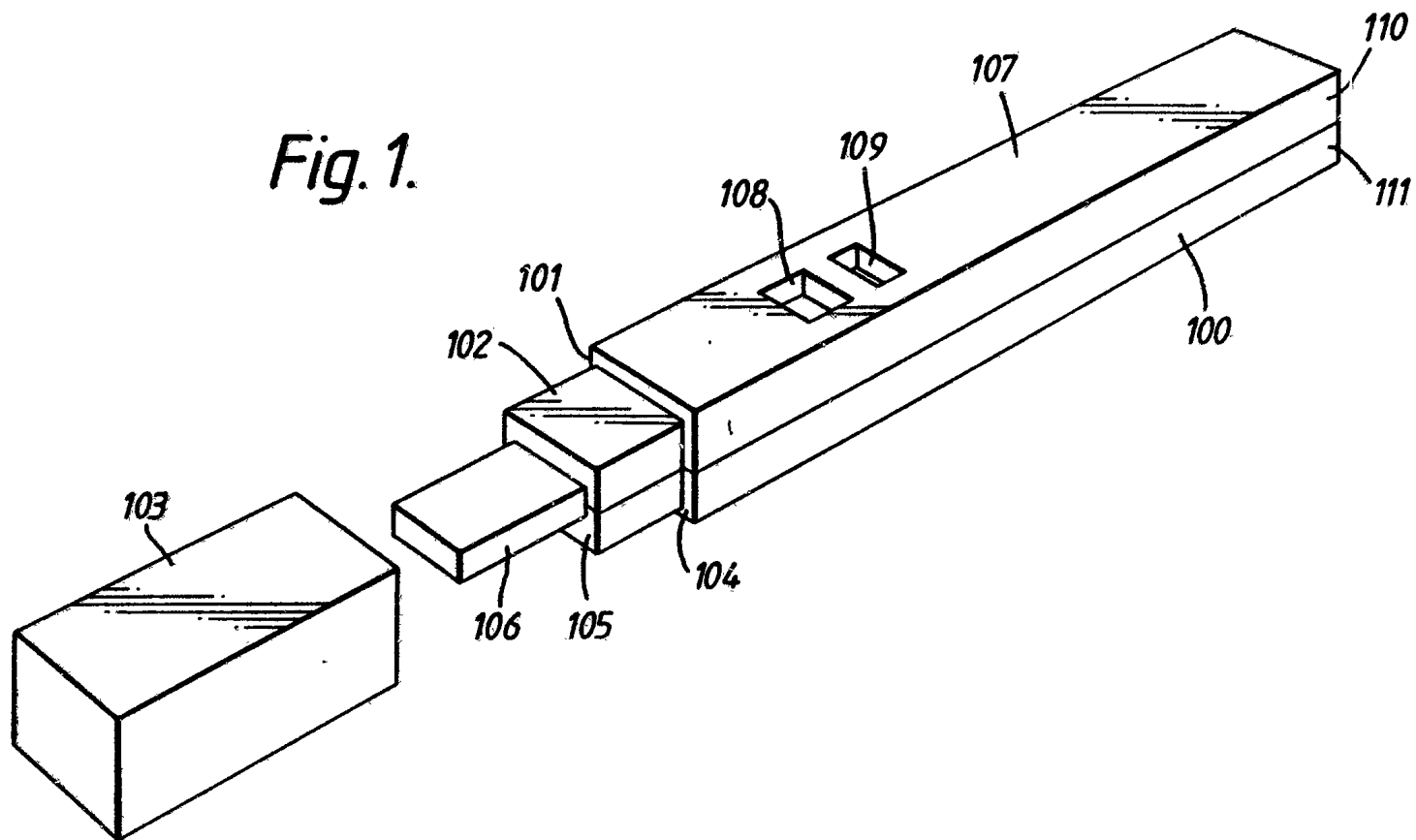


Fig. 2.

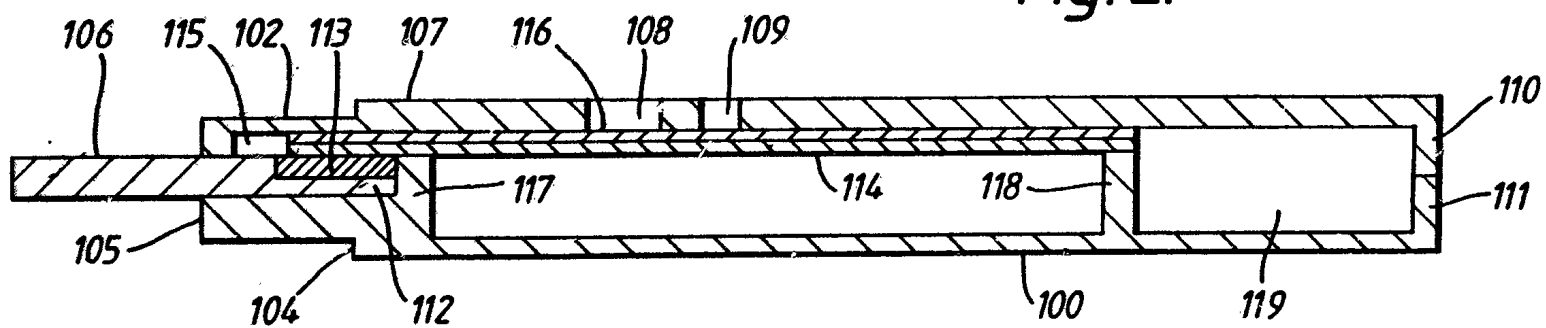


Fig. 3.

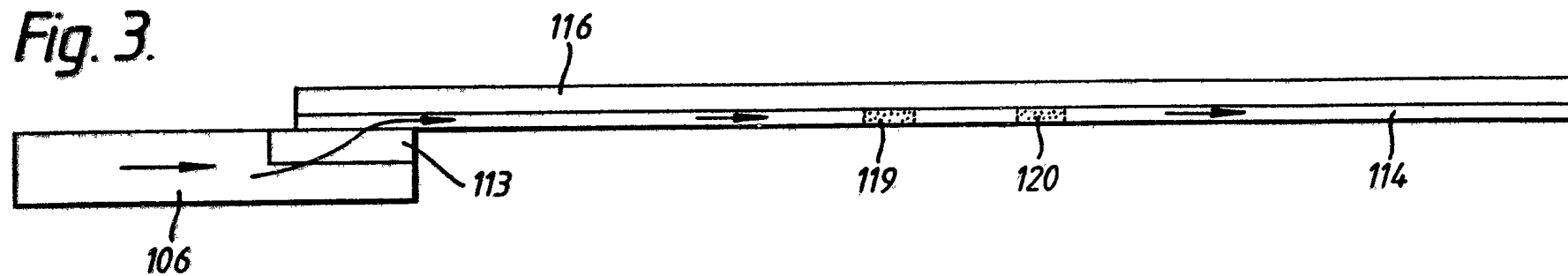


Fig. 4.

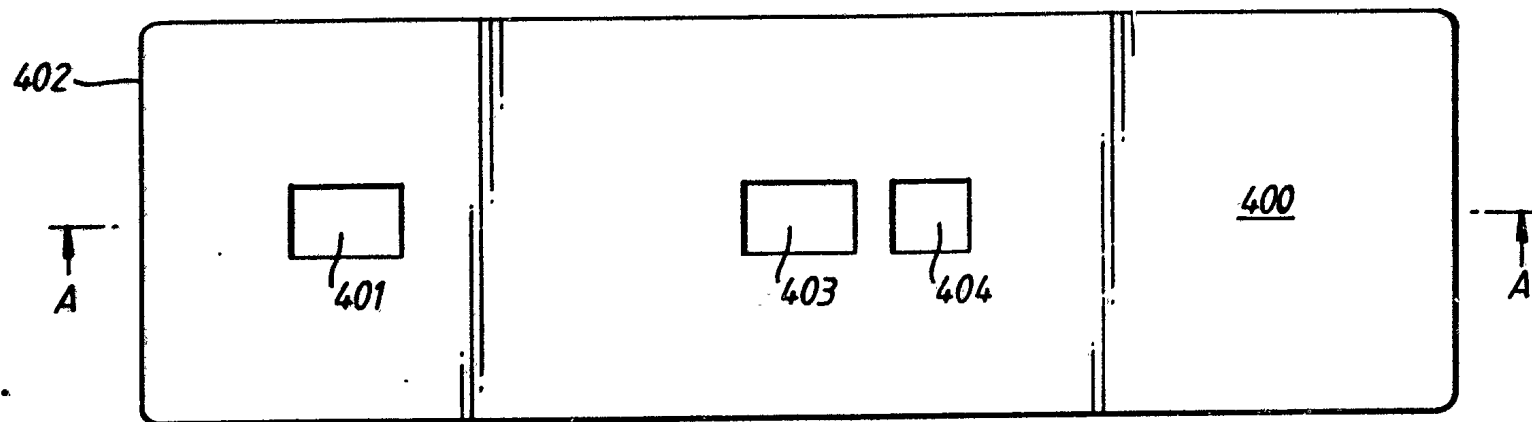
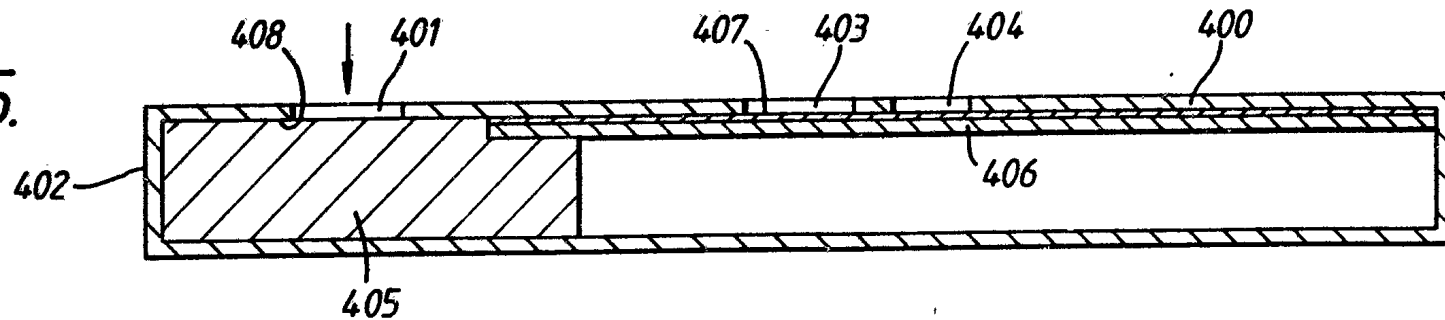


Fig. 5.

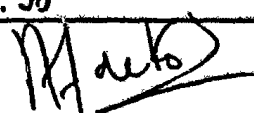


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INTERNATIONAL SEARCH REPORT

PCT/GB 90/00254

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 G01N33/558 ; G01N33/543 ; G01N33/58 ; G01N33/76		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	G01N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,291194 (UNILEVER NV) 17 November 1988 see page 2, lines 54 - 55 see page 4, lines 15 - 22 see page 5, lines 23 - 24 see page 5, lines 43 - 50; figures 1-14	1, 8, 23-25
A	see page 1, line 24 - page 2, line 54 see page 10, line 1 - page 14, line 36 & GB,A,2204398 (cited in the application)	
X	WO,A,8604683 (BOEHRINGER BIOCHEMIA ROBIN) 14 August 1986 see the whole document	1-3, 20-25
¹⁰ Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "T" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
06 JUNE 1990	16.07.90	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	DE KOK A.J. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
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X	EP,A,277723 (UNILEVER PLC) 10 August 1988 see page 5, lines 1 - 10; claims 1-3; figures 1-3 ---	1, 23-25
X	EP,A,299359 (ABBOTT LABORATORIES) 18 January 1989 see column 4, line 42 - column 5, line 20 see column 4, lines 19 - 29 see column 5, line 49 - column 9, line 5; figures 1-3 ---	1, 8-10 23-25
X	EP,A,238012 (FUJI PHOTO FILM CO., LTD) 23 September 1987 see the whole document ---	1, 23-25
X	EP,A,284232 (BECTON, DICKINSON AND COMPANY) 28 September 1988 see the whole document ---	1-14, 23-25
X	EP,A,279097 (GENESIS LABS INC) 24 August 1988 see the whole document ---	1, 23-25
P,X	WO,A,8906799 (BOEHRINGER MANNHEIM CORPORATION) 27 July 1989 see pages 9 - 14 see pages 17 - 19; figures 1-7 ---	1, 2, 15-17 23-25

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

PCT/GB 90/00254
SA 34693

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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