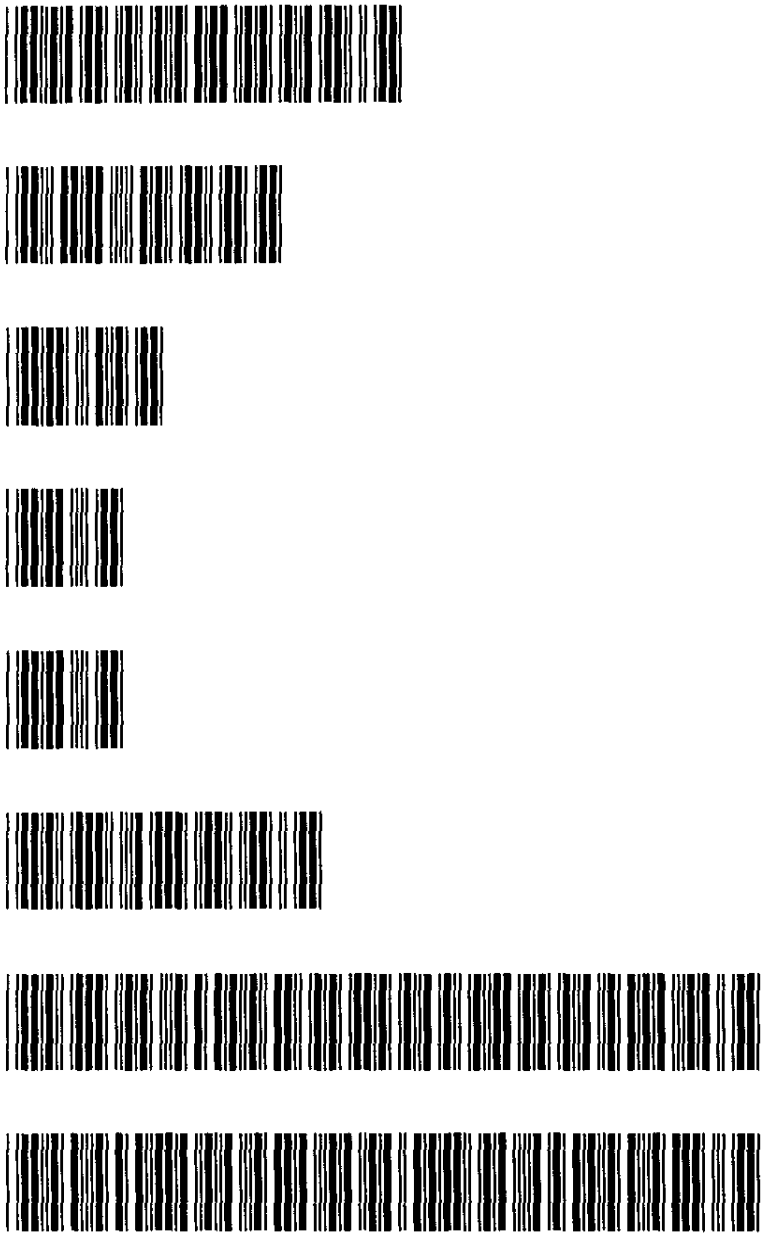

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3:03-CV-00477 ACON LABORATORIES V. INVERNESS MED INC
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AMDCMP.

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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

ACON LABORATORIES, INC.,

Plaintiff,

v.

INVERNESS MEDICAL INNOVATIONS, INC., INVERNESS MEDICAL SWITZERLAND GmbH and UNIPATH DIAGNOSTICS, INC.,

Defendants.

No. 03 CV-477 BTM (AJB)

FIRST AMENDED COMPLAINT FOR DECLARATORY JUDGMENT AND JURY DEMAND

ORIGINAL

FIRST AMENDED COMPLAINT

Plaintiff Acon Laboratories Inc. ("Acon"), by its attorneys, alleges for its first amended complaint against Defendants Inverness Medical Innovations, Inc. ("IMI"), Inverness Medical Switzerland GmbH ("IMS") and Unipath Diagnostics, Inc. ("Unipath") (collectively, "Defendants") the following:

JURISDICTION

1. This is an action for a declaratory judgment pursuant to 28 U.S.C. §§ 2201 and 2202. This Court has jurisdiction over the action pursuant to 28 U.S.C. Sections 1338(a) (action arising under an Act of Congress relating to patents) and 1331 (federal question).

VENUE

2. Venue of this action is properly laid in this district pursuant to 28 U.S.C. § 1391(b), (c), (d) and 28 U.S.C. § 1400(b).

THE PARTIES

3. Plaintiff Acon is a corporation incorporated under the laws of California, with its principal place of business in San Diego.

4. Upon information and belief, Defendant IMI is a corporation incorporated under the laws of Delaware, with its principal place of business in Waltham, Massachusetts.

5. Upon information and belief, Defendant IMS is a corporation organized and existing under the laws of Switzerland, having it principal place of business in Zug, Switzerland.

6. Upon information and belief, Defendant Unipath is a corporation incorporated under the laws of Delaware, with its principal place of business in Waltham, Massachusetts.

FACTUAL BACKGROUND

7. This action seeks a declaratory judgment concerning U.S. Patent No. 6,228,660 (the "'660 patent"), U.S. Patent No. 6,352,862 (the "'862 patent"), U.S. Patent No. 5,656,503 (the "'503 patent"), U.S. Patent No. 6,187,598 (the "'598 patent"), U.S. Patent No. 4,602,040 (the "'040 patent"), and U.S. Patent No. 5,622,871 (the "'871 patent"). Copies of the patents in dispute are attached as Exhibits A through F to this First Amended Complaint.

8. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '660 patent, which is entitled a "Capillary Immunoassay and Device Therefor Comprising Mobilizable particulate Labelled Reagents," and was issued by the United States Patent and Trademark Office on May 8, 2001 to Keith May, *et al.* Defendants IMI and Unipath also claim an interest in the enforcement of the '660 patent.

9. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '862 patent, which is entitled "Analytical Test Device for Immuno Assays and Methods of Using Same," and was issued and was issued by the United States Patent and

Trademark Office on March 5, 2002 to Paul Davis, *et al.* Defendants IMI and Unipath also claim an interest in the enforcement of the '660 patent.

10. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '503 patent, which is entitled "Test Device for Detecting Analytes in Biological Samples," and was issued. and was issued by the United States Patent and Trademark Office on August 12, 1997 to Keith May, *et al.* Defendants IMI and Unipath also claim an interest in the enforcement of the '503 patent.

11. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '598 patent, which is entitled "Capillary Immunoassay and Device Therefor Comprising Mobilizable Particulate Labelled Reagents," and was issued by the United States Patent and Trademark Office on February 13, 2001 to Keith May, *et al.* Defendant Unipath also claims an interest in the enforcement of the '598 patent.

12. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '040 patent, which is entitled "Assays," and was issued by the United States Patent and Trademark Office on February 11, 1997 to Keith May, *et al.* Defendants IMI and Unipath also claim an interest in the enforcement of the '040 patent.

13. Upon information and belief, Defendant IMS claims to be the owner by assignment of the '871 patent, which is entitled "Capillary Immunoassay and Device Therefor Comprising Mobilizable Particulate Labelled Reagents," and was issued by the United States Patent and Trademark Office on April 22, 1997 to Keith May, *et al.* Defendants IMI and Unipath also claim an interest in the enforcement of the '871 patent.

14. Defendants IMS and Unipath commenced an action against Acon in Massachusetts on November 26, 2002, asserting infringement of the '660 and '862 patents (the "Massachusetts Action"). The Massachusetts Action was improperly filed against Acon in a court without jurisdiction over Acon.

15. Defendant IMI sent a letter dated January 31, 2003 to the Perrigo Company ("Perrigo"). In that letter, IMI asserted it believes pregnancy test kits sold by Perrigo may

infringe the '660, '862, '503, '040 and '871 patents. IMI expressly demands that Perrigo "immediately cease and desist in the manufacture, use and/or sale of infringing test kits." Acon is the supplier of Perrigo's pregnancy test kits. Defendant IMI's letter to Perrigo created a reasonable apprehension by Acon that IMI intends to initiate suit against Acon based on the '660, '862, '503, '040 and '871 patents.

16. Defendants IMS and Unipath amended the Massachusetts Action on April 3, 2003, to add allegations of infringement against Acon based on the '503, '598, '040 and '871 patents. IMI has not joined the Massachusetts Action. As amended, the Massachusetts Action remains improperly filed against Acon in a court without jurisdiction over Acon.

17. An actual and justiciable controversy between Acon and Defendants exist regarding the scope, validity and enforceability of the '660, '862, '503, '598, '040 and '871 patents. This controversy is established by the suit filed by Defendants IMS and Unipath against Acon on November 26, 2002, and amended on April 3, 2003. This controversy is further established by Defendant IMI's letter of January 31, 2003 to Acon's customer.

18. An actual and justiciable controversy also exists as to the whether Acon infringes the '660, '862, '503, '598, '040 and '871 patents. In the Massachusetts Action, Defendants IMS and Unipath claim Acon infringes the '660, '862, '503, '598, '040 and '871 patents. In its letter to Perrigo, Defendant IMI claims product manufactured by Acon may infringe the '660, '862, '503, '040 and '871 patents. Acon denies these allegations, and contends it does not infringe the '660, '862, '503, '598, '040 and '871 patents or any valid asserted claims thereof.

FIRST CAUSE OF ACTION

(DECLARATORY JUDGMENT OF INVALIDITY AND UNENFORCEABILITY)

19. Acon realleges and incorporates by reference paragraphs 1 through 18 of this Complaint as though fully set forth herein.

20. Each claim of the '660 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

21. Each claim of the '862 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

22. Each claim of the '503 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

23. Each claim of the '598 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

24. Each claim of the '040 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

25. Each claim of the '871 patent is void, invalid, and/or unenforceable under 35 U.S.C. §§ 101, 102, 103 and/or 112.

26. This judicial declaration is necessary and appropriate at this time in order that Acon may ascertain its rights and duties with respect to the '660, '862, '503, '598, '040 and '871 patents.

SECOND CAUSE OF ACTION

(DECLARATORY JUDGMENT OF NON-INFRINGEMENT)

27. Acon realleges and incorporates by reference paragraphs 1 through 26 of this Complaint as though fully set forth herein.

28. Acon does not infringe the '660 patent or any of its claims.

29. Acon does not infringe the '862 patent or any of its claims.

30. Acon does not infringe the '503 patent or any of its claims.

31. Acon does not infringe the '598 patent or any of its claims.

32. Acon does not infringe the '040 patent or any of its claims.

33. Acon does not infringe the '871 patent or any of its claims.

34. This judicial declaration is necessary and appropriate at this time in order that Acon may ascertain its rights and duties with respect to the '660, '862, '503, '598, '040 and '871 patents.

EXHIBIT A

US006228660B1

(12) United States Patent
May et al.

(10) Patent No.: US 6,228,660 B1
(45) Date of Patent: *May 8, 2001

(54) **CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS**

(75) Inventors: **Keith May**, Bedfordshire; **Michael Evans Prior**, Northamptonshire; **Ian Richards**, Bedford, all of (GB)

(73) Assignee: **Conopco Inc.**, New York, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/474,192**

(22) Filed: **Jun. 7, 1995**

Related U.S. Application Data

(62) Continuation of application No. 08/102,313, filed on Jul. 15, 1993, now Pat. No. 5,622,871, which is a continuation of application No. 07/876,449, filed on Apr. 30, 1992, now abandoned, which is a division of application No. 07/795,266, filed on Nov. 19, 1991, now abandoned, which is a continuation of application No. 07/294,146, filed as application No. PCT/GB88/00322 on Apr. 26, 1988, now abandoned.

(30) **Foreign Application Priority Data**

Apr. 27, 1908 (GB) 8709873
Oct. 30, 1988 (GB) 8725457

(51) **Int. Cl.**[7] **G01N 33/533**

(52) **U.S. Cl.** **436/514**; 422/55; 422/56; 422/57; 422/58; 435/7.92; 435/962; 435/969; 435/970; 436/518; 436/525; 436/531; 436/533; 436/534; 436/810; 436/814; 436/817; 436/818

(58) **Field of Search** 422/55, 56, 57, 422/58; 435/7.92, 962, 969, 970; 436/514, 518, 525, 531, 533, 534, 810, 814, 817, 818

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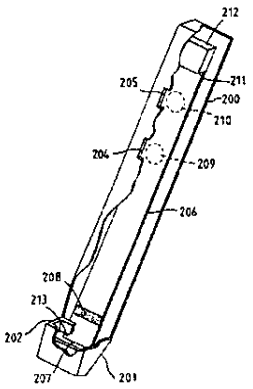
Assistant Examiner—Bao-Thuy L. Nguyen
(74) *Attorney, Agent, or Firm*—Pillsbury Madison & Sutro LLP

(57) **ABSTRACT**

An analytical test device useful for example in pregnancy testing, comprises a hollow casing (**500**) constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier (**510**) which communicates indirectly with the exterior of the casing via a bibulous sample receiving member (**506**) which protrudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilized on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means, such as an aperture (**508**) in the casing, enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

14 Claims, 5 Drawing Sheets



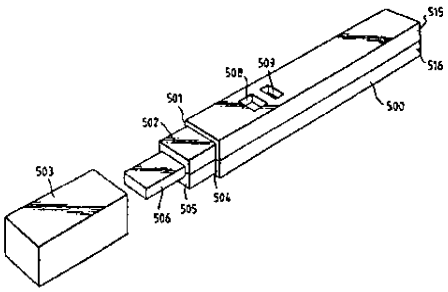


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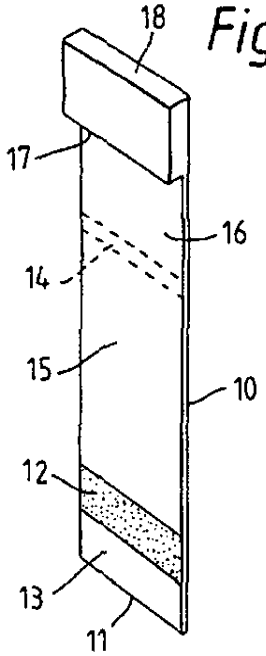
Fig.1.



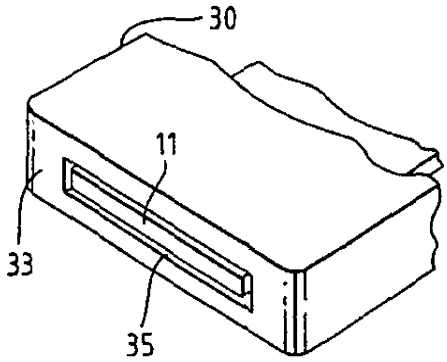
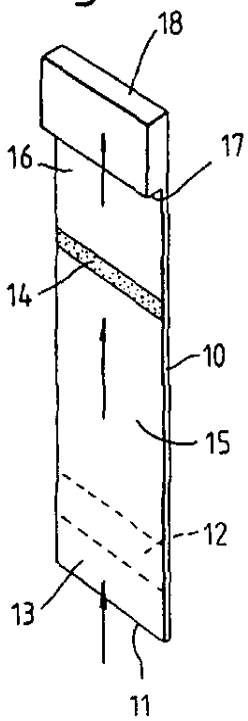


Fig.5.

Fig.2.



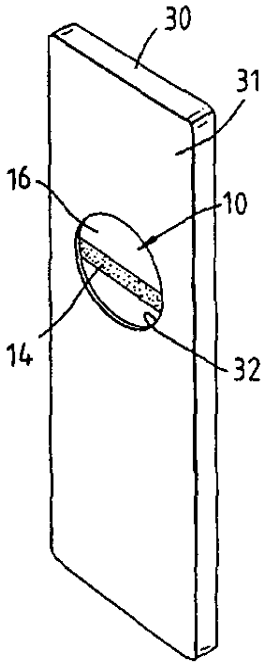


Fig.3.

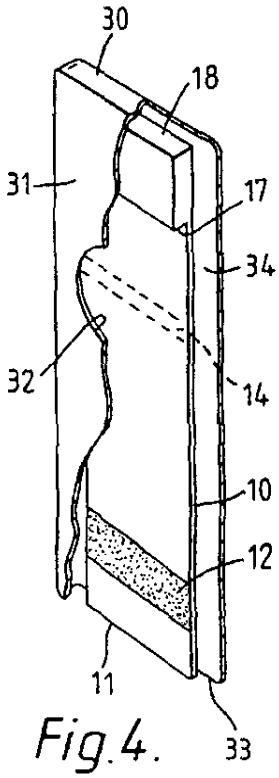
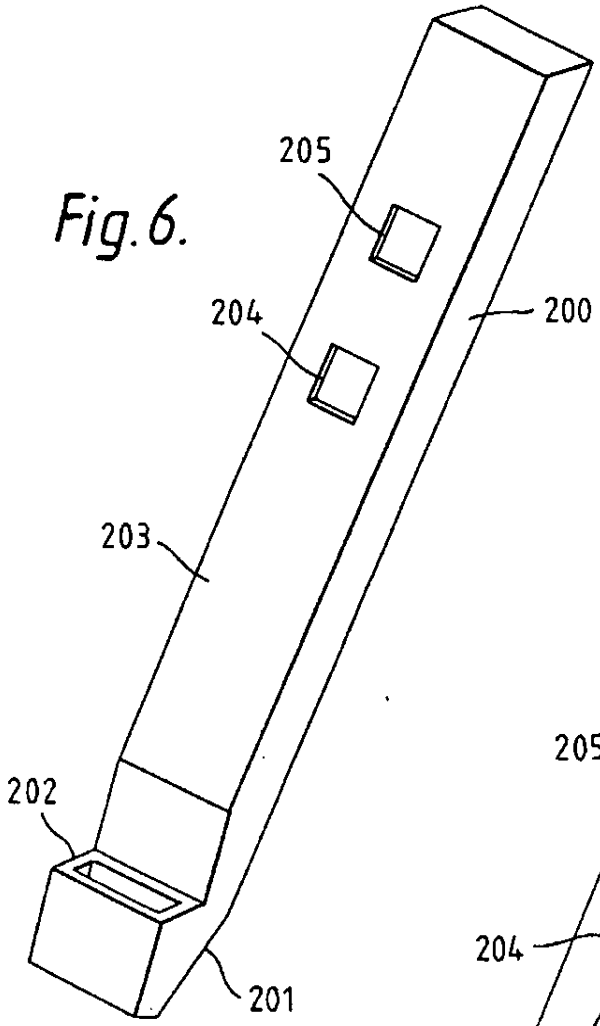


Fig.4.

Fig.6.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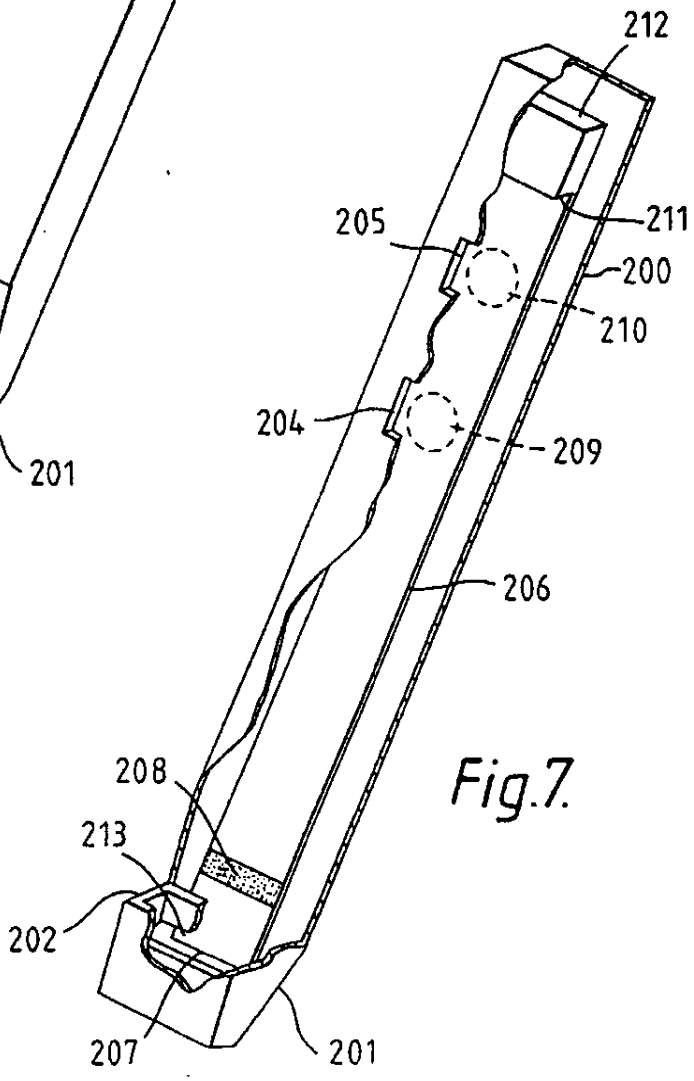
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Fig.7.

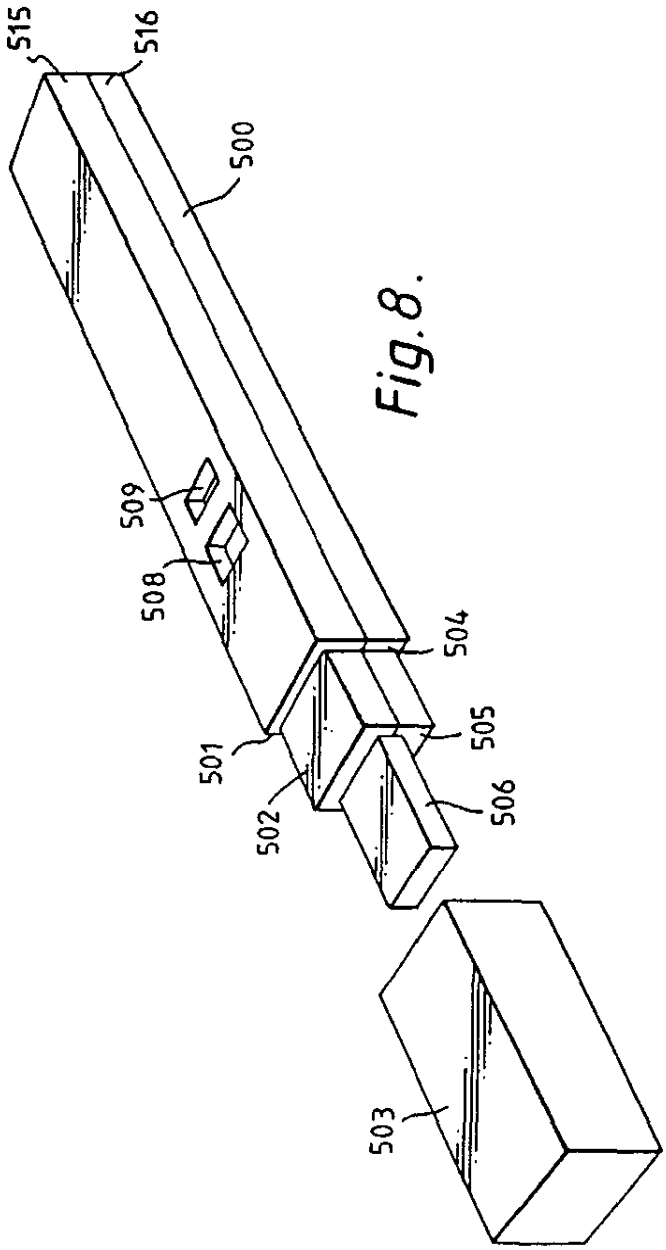
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Fig. 8.

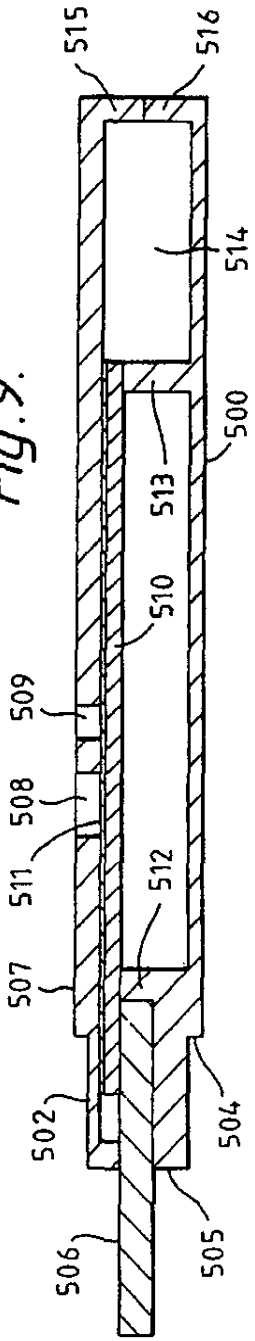
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Fig. 9.

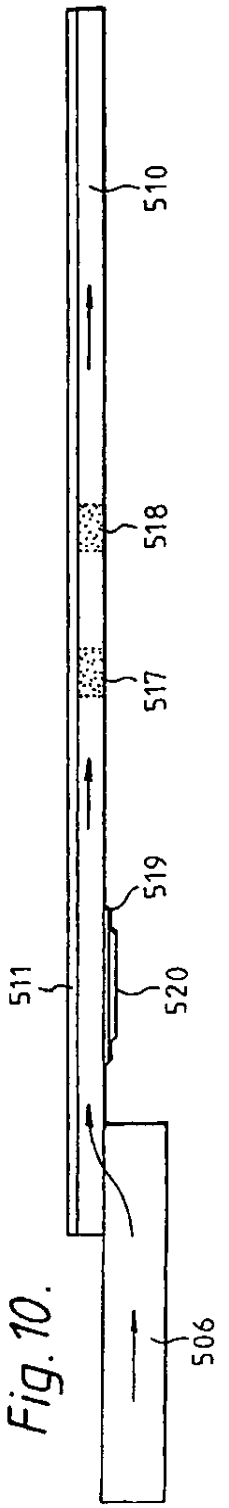

Fig. 10.

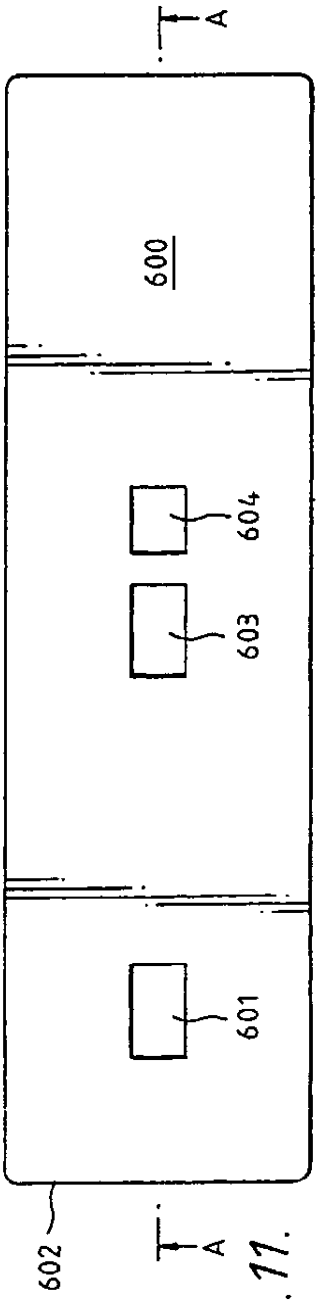

Fig. 11.

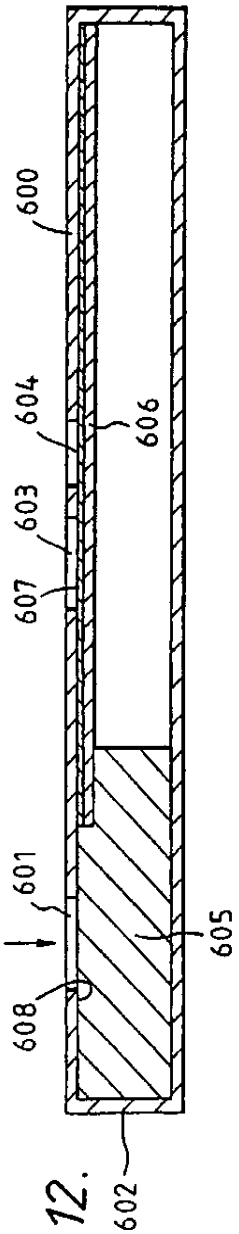

Fig. 12.

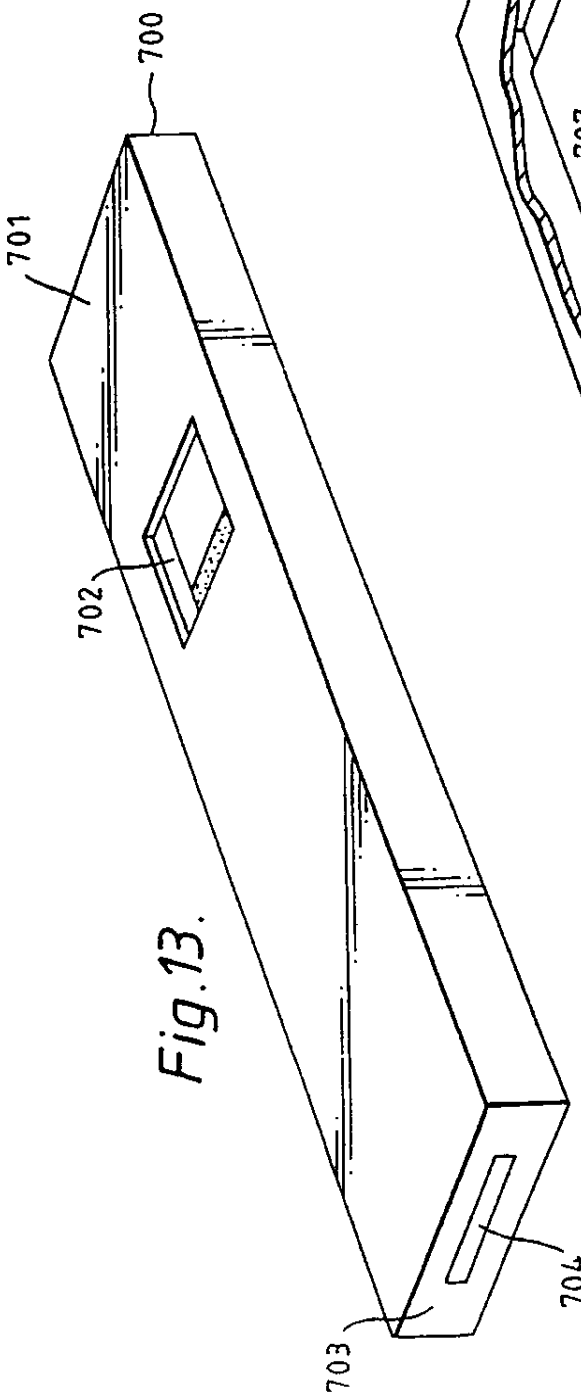

Fig. 13.

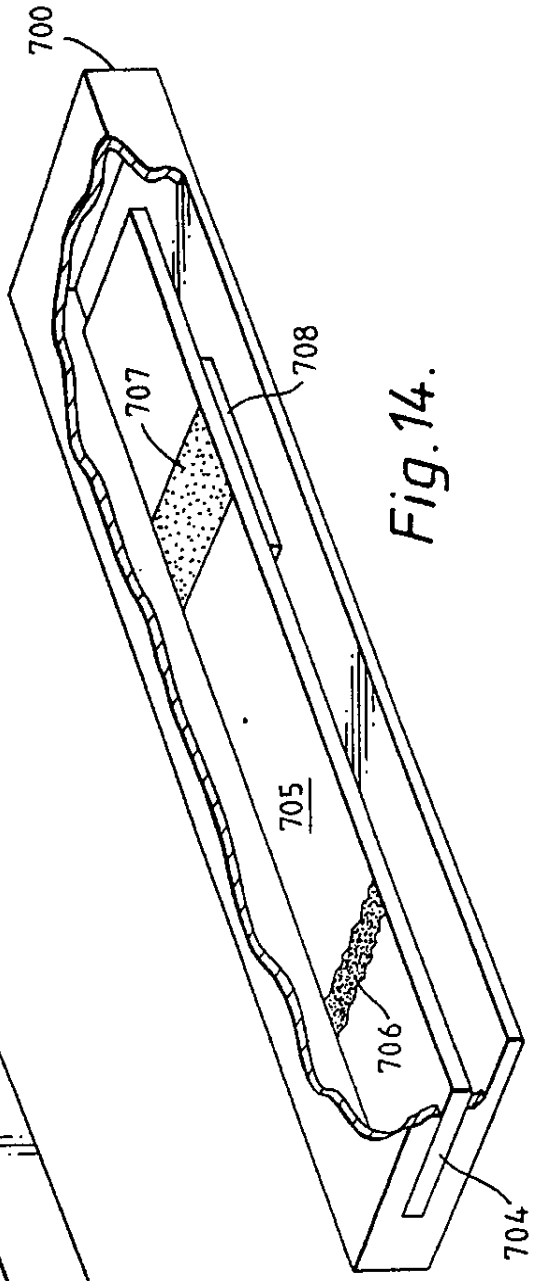

Fig. 14.

CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS

This is a continuation of application Ser. No. 08/102,313, filed Jul. 15, 1993, now U.S. Pat. No. 5,622,871, which was a continuation of 07/876,449 filled Apr. 30, 1992, now abandoned; which was a division of application Ser. No. 07/795,266, filed Nov. 19, 1991, now abandoned; which was a continuation of application Ser. No. 07/294,146, filed Feb. 27, 1989, now abandoned, which was a U.S. national phase of PCT/GB88/00322, filed Apr. 26, 1988. Priority is claimed in this case from each of the above-listed applications.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to analytical devices which are suitable for use in the home, clinic or doctor's surgery and which are intended to given an analytical result which is rapid and which requires the minimum degree of skill and involvement from the user.

2. Description of the Related Art

The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream 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. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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 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 for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is in the moist state.

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

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 together in a "sandwich" reaction. This results in the labelled reagent being ground in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

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 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 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 solid phase material into the second zone and bind with the immobilized 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 second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it 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 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 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.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols 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. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. 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 size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable 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 material to be observed from outside the housing and which further zone incorporates 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 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. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but this is not preferred.

An important embodiment of the invention is 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 and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone 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 two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and 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 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 multi-directional (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 flouride, 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 porous solid phase material.

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 antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a 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 form 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 second 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 an 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 an enzymes, e.g. alkaline phosphatase and horseradish 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 porous solid phase material 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 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 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 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, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent form the first zone which does not participate in any binding reaction in the second zone is flushed away 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 3 MM chromatography paper, and should provide sufficient absorptive capacity to allow any unbound conjugage 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.

The presence or intensity of the signal from the label which becomes bound in the second zone can 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.

The immobilised specific binding reagent in the second 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 antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and 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 incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the 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 antibody 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 ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of

sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is 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 commercially from Schleicher and Schuell GmbH.

Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strenth. This can be manufactured easily by forming a thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose 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 such that in the untreated material, aqueous liquid migrates at a rate of 1 cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the zones, 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, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated through the thickness of the carrier in the second 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 present in the migrating sample.

The reagents can be applied to the 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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **2** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **3** is a perspective view of a device utilizing a porous strip of the type illustrated in FIGS. **1** and **2**;

FIG. **4** is a perspective view, partially broken away, revealing a porous strip within the device of FIG. **3**;

FIG. **5** is an enlarged view of one end of the device of FIG. **3**;

FIG. **6** is a perspective view of another test device according to the invention;

FIG. **7** is a perspective view, similar to FIG. **6**, but partially cut away to reveal the porous test strip contained within the body of the device;

FIG. **8** is an exploded perspective view of an assay device in accordance with the present invention;

FIG. **9** is a cross-sectional side elevation of the device shown in FIG. **8**;

FIG. **10** is an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**;

FIG. **11** is a plan view of another embodiment of the invention;

FIG. **12** is a schematic cross-sectional view taken along line **12**—**12** in FIG. **11**;

FIG. **13** is a perspective view of yet another embodiment of the invention; and

FIG. **14** is a partially cut away view of the device of FIG. **13**.

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

EMBODIMENT 1

FIGS. **1** and **2** represent a typical strip of porous solid phase material for use in any assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to FIG. **1**, the assay test strip **10** is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end **11** of strip **10** is a narrow band or zone **12** extending across the entire width of the strip. A small region **13** of strip **10** lies vertically below zone **12**. Above zone **12** is a second zone **14** lying a discrete distance up strip **10** and similarly extending the entire width of the strip. The region **15** of strip **10** between zones **12** and **14** can be of any height as long as the two zones are separate. A further region **16** of the strip extends above zone **14**, and at the top **17** of the strip is a porous pad **18** firmly linked to strip **10** such that pad **18** can act as a "sink" for any liquid sample which may be rising by capillary action through strip **10**.

Zone **12** is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone **14**, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

FIG. **2** illustrates what happens when the assay strip is used in an analytical procedure. The lower end **11** of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad **18**. In so doing, the sample traverses zone **12** and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone **14**, the labelled antibody can bind to any analyte present in the sample. On reaching zone **14**, any analyte molecule should become bound to the second antibody, so immobilising the labelled "sandwhich" so produced. If a significant concentration of the analyte to be determined is present in the liquid sample, in a short period of time a distinct accumulation of the visible label should occur in zone **14**.

As an example of an analysis to which this embodiment can be applied, the analyte can be hCG, the reagents in zones **12** and **14** can be monoclonal antibodies to hCG which can participate in a "sandwich" reaction with hCG, and the label can be a particulate dye, a gold sol or coloured latex particles.

Although described above in relation to a "sandwhich" reaction, it will be readily apparent to the skilled reader that this can be modified to a "competition" reaction format if desired, the labelled reagent in zone **12** being the analyte or an analogue of the analyte.

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 (estrone-3-glucuronide) and P-3-G (progesterone-3-glucuronide).

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 A_1 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. By way of example, a dual analyte test for apolipoproteins A_1 and B may be prepared by depositing, as two spacially distinct zones, antibody specific for apolipoprotein A_1 throughout a first zone and depositing a second antibody specific for apolipoprotein B, throughout the second zone of a porous carrier matrix. Following the application of both antibodies to each of their respective zones via a suitable application procedure (e.g. ink-jet printing, metered pump and pen, or airbrush), the remainder of the porous material should be treated with a reagent, e.g. bovine serum albumin, polyvinyl alcohol, or ethanolamine, to block any remaining binding sites elsewhere. A third and fourth reagent, bearing a label, may then be dispensed onto the dry carrier in one or more zones near to one end of the strip, the strip being allowed to dry between applications of the two reagents to the same zone. Reagent **3** and Reagent **4** may comprise conjugates of anti-apolipoprotein A_1 antibody and anti-apolipoprotein B antibody respectively. Both of these conjugates will become mobile in and on the carrier when in the moist state. Reagents **3** and **4** can migrate with the solvent flow when an aqueous sample is applied to the first end of the carrier strip. While migrating towards the two zones further along the strip, reagent **3** may bind any apolipoprotein A_1 present in the sample and reagent **4** may bind any apolipoprotein B present in the sample. On reaching the first second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein A_1 molecules should become bound to the second antibody, immobilising the labelled 'sandwich' so produced. No labelled apoliprotein B molecules will bind to this first zone. On reaching the second second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein B molecules should become bound to the second antibody (solid-phase antibody), immobilising the labelled 'sandwich' so produced. No labelled apolipoprotein A_1 molecules will bind to the second zone. An accumulation of each of the direct label may occur at both or either zones to a lesser or greater extent resulting in a visible signal at either or both of the solid phase antibody zones. Excess unbound conjugate (of both reagent **3** and reagent **4**) can pass freely over the two antibody zones and will be washed into the distal end of the strip.

The development of a quantifiable colour in both of the second-antibody zones may be assessed with an appropriate form of instrumentation, yielding a ratio of colour density between the two sites.

The determination of the presence of more than two (ie multiple) analytes in any example 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 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 1 mm 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. Conjugates of label, e.g. a dye sol, and each immunochemically-reactive component specific for each bacterial group may then be dispensed either onto a single zone at the bottom end of the strip, proximal to the sample application zone, or as a series of separate zones.

FIGS. **3**, **4** and **5** of the accompanying drawings depict a complete device utilising a porous strip as just described above. FIG. **3** represents the complete device viewed from the front, FIG. **4** shows the same device partially cut away to reveal the details of the strip inside, and FIG. **5** shows the underside of the device.

Referring to FIG. **3**, the device comprises a flat rectangular body **30** the front face **31** of which is perforated by a circular hole or window **32** which reveals the porous test strip **10** within the body. The region of the test strip **10** visible through the window **32** incorporates a narrow horizontal zone **14**.

Referring to FIG. **4**, the device comprises a dry rectangular test strip **10** made from porous material which extends from the bottom end **33** of the body **30** within the body between the front **31** and back **34** of the body. Near the bottom end **11** of the strip **10** is a horizontal zone **12** bearing a labelled specific binding reagent for an analyte, the binding reagent being mobile in the test strip in the moist state. Further up the test strip is the narrow horizontal zone **14** which is visible through the window **32**. At the top **17** of the test strip **10** is a porous 'sink' **18** which can absorb any liquid sample that has permeated upwards through the strip.

Referring to FIG. **5**, the bottom edge **35** of the body **30** incorporates a lateral aperture in which the bottom end **11** of the strip lies.

In operation, the bottom end **33** of the body **30** is immersed in a liquid sample (eg urine) so that the liquid sample can be absorbed by the bottom end **11** of the test strip

20 and rise by capillary action to the top 17 of the test strip and into the sink 18. In so doing, the liquid sample progresses via zone 12 to zone 14. Specific binding reactions as described above occur, and the test result is visible to the user through the window 32.

EMBODIMENT 2

FIGS. 6 and 7 of the accompanying drawings illustrate another test device according to the invention. FIG. 6 illustrates the complete device viewed from the front, and FIG. 7 depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to FIG. 6, the device comprises an elongate body 200 terminating at its lower end 201 in a small integral receptacle 202 which can hold a predetermined volume of a liquid sample, eg urine. The front face 203 of the body 200 incorporates two square small square apertures or windows 204 and 205 located one above the other.

Referring to FIG. 7, the elongate portion of the body 200 is hollow and incorporates a test strip 206 running almost the full height of the body. This test strip is of similar construction to those described under Embodiment 1, and incorporates near its lower end 207 a horizontal zone 208 bearing a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones 209 and 210 adjacent to the windows 204 and 205 and visible therethrough. The strip terminates at its top end 211 in a porous sink 212. At the bottom end 201 of the device, the receptacle 202 communicates with the hollow body via a lateral aperture 213.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacle 202. From the receptacle 202 the liquid sample rises by capillary action through the test strip 206 and conveys and labelled reagent from zone 208 to the two circular zones 209 and 210. A series of specific binding reactions as described in relation to Embodiment 1 above occur. In this embodiment the second circular zone 210 can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone 209. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone 210 can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone 208 by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone 209 by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone 208, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

EMBODIMENT 3

FIG. 8 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and FIG. 9 represents a cross-sectional side elevation of the device shown in FIG. 8.

Referring to FIG. 8, the device comprises a housing or casing 500 of elongate rectangular form having at one end 501 a portion 502 of reduced cross-sectional area. A cap 503 can be fitted onto portion 502 and can abut against the shoulder 504 at end 501 of the housing. Cap 503 is shown separated from housing 500. Extending beyond end 505 of portion 502 is a porous member 506. When cap 503 is fitted onto portion 502 of the housing, it covers porous member 506. Upper face 507 of housing 500 incorporates two apertures 508 and 509.

Referring to FIG. 9, it can be seen that housing 500 is of hollow construction. Porous member 506 extends into housing 500 and contacts a strip of porous carrier material 510. Porous member 506 and strip 510 overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member 506 can permeate member 506 and progress into strip 510. Strip 510 extends further into housing 500. Strip 510 is "backed" by a supporting strip 511 formed of transparent moisture-impermeable plastics material. Strip 510 extends beyond apertures 508 and 509. Means are provided within housing 500 by webbs 512 and 513 to hold strip 510 firmly in place. In this respect, the internal 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, and porous member 506 is firmly retained in the housing and adequate fluid permeable contact is maintained between member 506 and strip 510. The transparent backing strip 511 lies between strip 510 and apertures 508 and 509 and can act as a seal against ingress of moisture from outside the housing 500 via these apertures. If desired, the residual space 514 within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip 510 in the dry state during storage. The reagent-containing zones in strip 510 are not depicted in FIG. 8, but the first zone containing the labelled regent which is mobile when the strip is moistened will lie in the region between the porous member 506 and aperture 508. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 508 in order that when the device has been used in an assay, the result can be observed through aperture 508. Aperture 509 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap 503 is removed from the holder and member 506 is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member 506 to the liquid sample for a time sufficient to ensure that member 506 is saturated with the sample, the cap 503 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 510 to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures 508 and 509 and can ascertain whether the assay has been completed by observing the control zone through aperture 509, and can ascertain the result of the assay by observing the second zone through aperture 508.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing 500 being moulded in two parts (e.g. upper and lower halves 515 and 516) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member 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 porous

member and test strip together to ensure adequate contact between them. Cap **503** can be moulded as a separate complete item. If desired, apertures **508** and **509** 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 **505** of housing **500** and the protruding porous member **506**, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member **506**. Member **506** 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 as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with FIGS. **8** and **9** 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 then (after optionally replacing the cap) can observe the test result through aperture **508** within a matter of a few minutes.

Although described with particular reference to 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 **509** 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 from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member **506**.

FIG. **10** of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**.

The porous receiving member **506** is linked to the porous test strip **510**, backed by the transparent plastics sheet **511**, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone **517** incorporates the immobilised specific binding reagent, and control zone **518** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip **511** and adjacent the porous receiving member **506**, carries a glaze **519** on which is deposited a layer **520** of labelled specific binding reagent. The thickness of these two layers as depicted in FIG. **10** is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member **506** can flow therefrom along the length of strip **510** and in so doing, will dissolve glaze **519** and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone **517**.

Embodiment 4

FIGS. **11** and **12** illustrate another embodiment of the invention, which is seen in plan view in FIG. **11** and in cross-section in FIG. **12**, the cross-section being an elevation on the line **12—12** seen in FIG. **11**.

Referring to FIG. **11**, the test device comprises a flat rectangular casing **600** incorporating a centrally disposed rectangular aperture **601**, adjacent the left hand end **602**, and two further apertures **603** and **604** near the mid point of the device and arranged such that apertures **601**, **603** and **604** lie on the central longitudinal axis of the device corresponding to line **12—12**. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **12**, the device is hollow and incorporates within it a porous sample receiving member adjacent end **602** of casing **600** and lying directly beneath aperture **601**. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip **606** backed by a transparent plastics sheet **607** is also contained within casing **600**, and extends from the porous receiving member **602**, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet **607** is in firm contact with the upper inner surface **608** of casing **600**, and provides a seal against apertures **603** and **604** to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip **606** will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures **603** and **604**, in a manner analagous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture **601**, e.g. by means of a syringe, to saturate porous receiving member **605**. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures **603** and **604**.

Embodiment 5

A yet further embodiment of the invention is illustrated in FIGS. **13** and **14** of the accompanying drawings. FIG. **13** shows a device comprising a rectangular casing **700** having in its upper surface **701** a rectangular aperture **702**. One end wall **703** of the device **703** incorporates an aperture **704** through which a porous test element communicates with the exterior of the device. Aperture **702** is situated in surface **701** at a point relatively remote from the end **703** containing the aperture **704**.

FIG. **14** shows a partially cut-away view of the device in FIG. **13**. The hollow device incorporates a porous test strip **705**, running almost the entire length of casing **700** from aperture **704**. Test strip **705** incorporates a first zone **706** containing a labelled specific binding reagent and a further zone **707**, remote from aperture **704**, incorporating an immobilised specific reagent. Zone **706** lies directly beneath aperture **702** is therefore observable from outside casing. Beneath strip **705** and adjacent zone **707**, is a crushable element **708** containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone **707**, if labelled reagent from **706** has become bound in zone **707** following use of the device. Release of the reagents from member **708** can be effected by applying pressure to the outside of the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end **703** of casing **700** into

a vessel containing the sample. The liquid sample will then permeate the length of test strip **705**, taking labelled reagent from zone **706** and passing through zone **707** where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the sample has permeated the test strip, reagent can be released from the crushable member **708** and the result of the test observed through aperture **702**.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45 mm (minutes)
Schleicher + Schuell nitrocellulose (unbacked)	3μ	3.40
	5μ	3.30
	8μ	3.00
	12μ	2.20
polyester-backed	8μ (nominal)	3.40
Whatman Nitrocellulose	5	19.20
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20 nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22μ filtered 0.1M K_2CO_3, and 20 mls is added to a clean glass beaker. 200 μl of anti-alpha hCG antibody, prepared in 2 mM borax buffer pH9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2 mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000 g, 30 minutes, and 4° C., with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20 mM Tris, 150 mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specification No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 liter of distilled water by mixing on a magnetic stirrer for 2–3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500 g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000 g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500 mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100 mls distilled water.

The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657 nm for Foron Blue, and 690 nm for Resolin Blue. The absorbance at lambda-max, for 1 cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoassays are available commercially. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the preformed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chloroform which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5 ml (12.5 mg solids) of suspension is diluted with 1 ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1 ml borate buffer, mixed with 300 μg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16–20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000 rpm, the supernatant is discarded and the pellet resuspended in 1.5 mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5 mg/ml BSA in phosphate buffered

saline pH7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5 mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4° C. until used.

(A) Anti-hCG—Dye Sol Preparation

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction mixture is prepared which contains 100 μl antibody solution, 2 mls dye sol, 2 mls 0.1M phosphate buffer pH 5.8 and 15.9 mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4 mls of 150 mg/ml BSA in 5 mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000 g for 10 minutes, and the pellet resuspended in 10 mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH—Dye Sol Preparation

Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8μ nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30° C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5 cm in length and 1 cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8μ nitrocellulose, 25 cm in length and 20 cm in width with zones of immobilised antibody at 5 cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6 cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5 cm in length and 1 cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used as a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% TRITON X305 (Trademark—obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1 mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of

estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA—Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of E-3-G (2 nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10 nM) in dry DMF were equilibrated separately at 4° C. Using pre-cooled glassware E-3-G in DMF (1.25 ml) and TnB in DMF (0.25 ml) were added to a pre-cooled 5 ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10 nM) was prepared and an aliquot (0.25 ml) was cooled to 4° C. and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4° C. and a solution of BSA (1 mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5 ml) and stirred on a magnetic stirrer for 4 hours at 4° C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 SEPHADEX G-25 column, transferred to an amber glass storage bottle and stored at 4° C.

Preparation of BSA-E-3-G Dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850 rpm (1500 g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10 μg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5 mM, pH 7.4) to yield a final BSA concentration of 25 mg/ml. The reaction mixture was centrifuged at 4850 rpm (3000 g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH 5.8) buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA—E-3G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to all combinations and subcombinations of the features of this description and the accompanying drawings.

What is claimed is:

1. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample and requiring solely the application thereto of said liquid biological sample to enable a test to be performed and a test result to be provided, said device comprising:

a hollow casing constructed of moisture impervious solid material;

a test strip, comprising a dry porous carrier disposed in said casing, said dry porous carrier comprising a test result zone and a control zone; and

a labeled reagent capable of specifically binding with said analyte to form a first complex of said labeled reagent and said analyte, said labeled reagent being positioned within said casing as part of said dry porous carrier prior to use, said labeled reagent being a particulate direct label,

said casing comprising a sample application aperture through which liquid biological sample can be applied directly or indirectly to said carrier, said labeled reagent being located upstream from said test result zone prior to use and released into mobile form by application of said liquid biological sample,

wherein said carrier comprises, immobilized in said test result zone, means for binding said first complex, and wherein migration of said applied liquid biological sample through said dry porous carrier conveys by capillarity said first complex to said test result zone of said dry porous carrier, whereat said binding means binds said first complex thereby to form a second complex and to thereby indicate the presence of said analyte in said liquid biological sample,

said casing further comprising a test result observation aperture remote from said sample application aperture, said test result zone at least being visible through said test result observation aperture, said sample application aperture and said test result observation aperture being spaced apart and disposed so that sample liquid can be applied to said sample application aperture to initiate said test but said test strip is substantially shielded from accidental initial application of sample liquid directly to a portion of said test strip downstream from said location of said labeled reagent in the dry state,

wherein said control zone comprises a material for indicating that said liquid biological sample has been conveyed thereto by capillarity along said carrier irrespective of a presence or absence of said analyte in said liquid biological sample.

2. The analytical test device of claim 1, further comprising a control zone observation aperture, said control zone being visible through said control zone observation aperture; said control zone observation aperture being defined separately from said test result observation aperture.

3. The analytical test device of claim 1, wherein said control zone comprises means for binding labeled reagent conveyed thereto by said liquid biological sample, irrespective of a presence or absence of said analyte.

4. The analytical test device of claim 1, wherein said carrier communicates indirectly with an exterior of the casing via a sample receiving member.

5. The analytical test device of claim 4, wherein the sample receiving member protrudes through the sample application aperture.

6. The analytical test device of claim 1, wherein said casing has first and second longitudinal ends, said sample

application aperture being disposed adjacent said first longitudinal end and further comprising a displaceable shroud disposed on said first end for shielding said liquid biological sample application aperture.

7. The analytical test device of claim 1, wherein said analyte is human chorionic gonadotrophin (hCG).

8. The analytical test device of claim 1, wherein said analyte is luteinizing hormone (LH).

9. The analytical test device of claim 1 wherein said label is a gold sol.

10. The analytical test device of claim 1, wherein said label is colored latex particles.

11. A pregnancy testing device for detecting human chorionic gonadotrophin (hCG) suspected of being present in a urine sample and requiring solely the application thereto of said urine sample to enable a test to be performed and a test result to be provided, said device comprising:

a hollow casing constructed of moisture impervious solid material;

a test strip, comprising a dry porous carrier disposed in said casing, said dry porous carrier having a detection zone and a control zone; and

labeled reagent comprising an anti-hCG antibody bearing a particulate direct label capable of specifically binding with said hCG to form a first complex of said labeled anti-hCG antibody and said hCG, said labeled hCG antibody reagent being at least one of disposed on and contained in said test strip in a dry state prior to use,

said casing comprising a sample application aperture through which urine can be applied directly or indirectly to said carrier, said labeled hCG antibody being located upstream from said detection zone prior to use and released into mobile form by application of said urine sample,

wherein said carrier comprises, immobilized in said detection zone, means for binding said first complex, and wherein migration of said applied urine sample through said dry porous carrier conveys by capillarity said labeled hCG antibody and said hCG to said detection zone of said dry porous carrier, whereat said binding means binds said first complex thereby to form a second complex, thereby to indicate the presence of said hCG in said urine sample,

said casing comprising a test result observation aperture remote from said sample application aperture, said detection zone at least being visible through said test result observation aperture, said sample application aperture and said test result observation aperture being spaced apart and disposed so that urine can be applied to said sample application aperture to initiate said test but said test strip is substantially shielded from accidental initial application of urine directly to a portion of said test strip downstream from said location of said labeled hCG antibody in the dry state,

wherein said control zone comprises a material for indicating that said urine sample has been conveyed thereto by capillarity along said carrier irrespective of a presence or absence of hCG in said urine sample.

12. The pregnancy testing device of claim 11, wherein said control zone is downstream from said detection zone.

13. The pregnancy testing device of claim 11 wherein said label is a gold sol.

14. The pregnancy testing device of claim 11 wherein said label is colored latex particles.

* * * * *

EXHIBIT B

US006352862B1

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Davis et al.

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(45) Date of Patent: *Mar. 5, 2002

(54) ANALYTICAL TEST DEVICE FOR IMUNO ASSAYS AND METHODS OF USING SAME

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(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(58) **Field of Search** 435/7.92–7.95, 435/805, 806, 810, 970, 6; 436/518, 525, 528, 530, 541, 810, 814, 818; 422/101, 55

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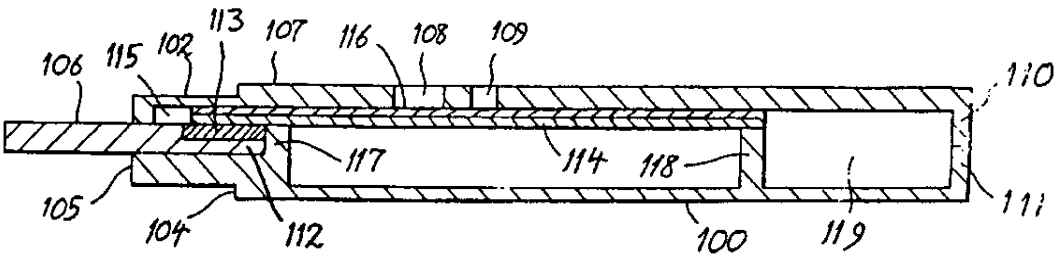
(57) **ABSTRACT**

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, eg. 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.

21 Claims, 2 Drawing Sheets



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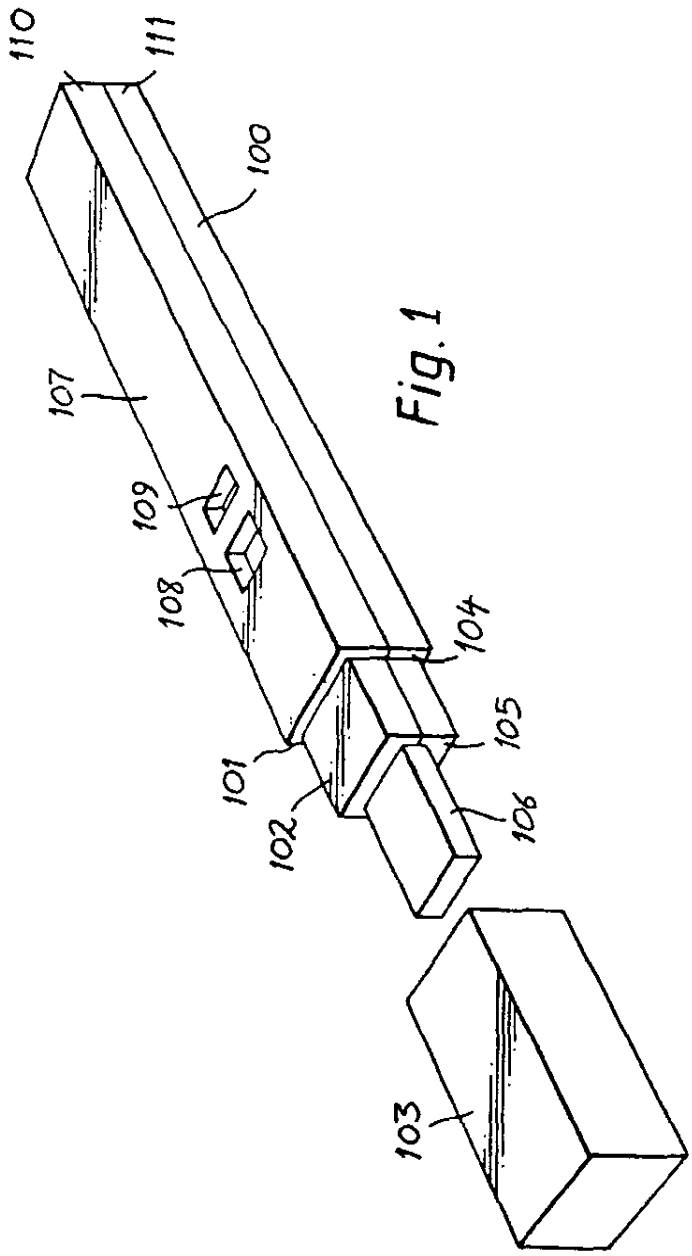
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Fig. 1

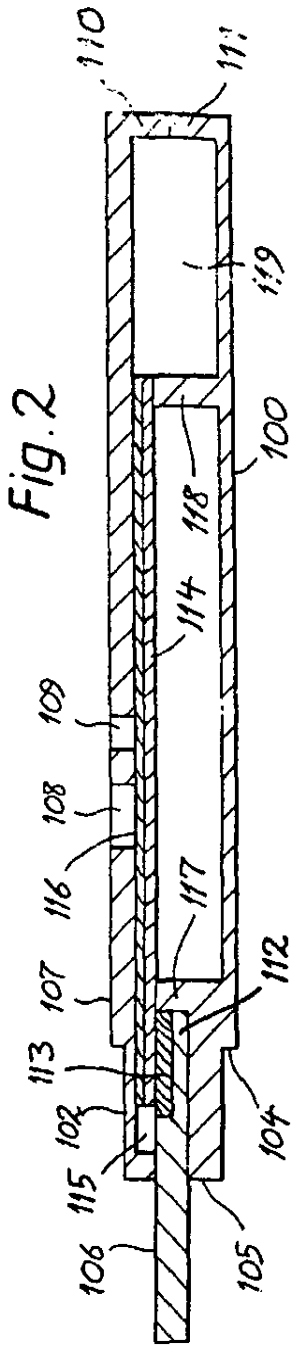
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Fig. 2

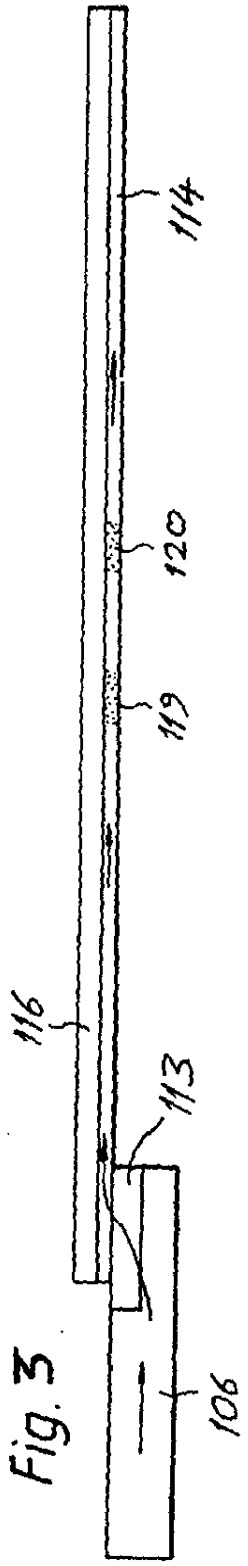

Fig. 3

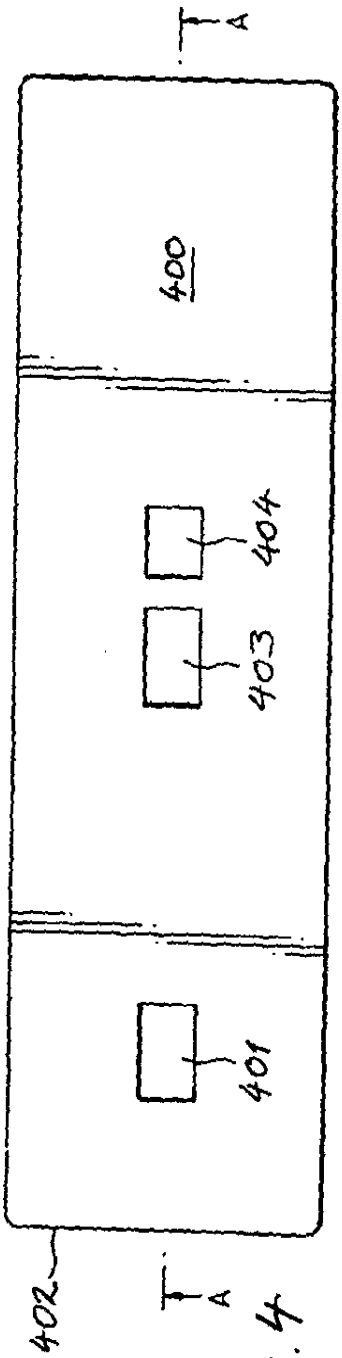

Fig. 4

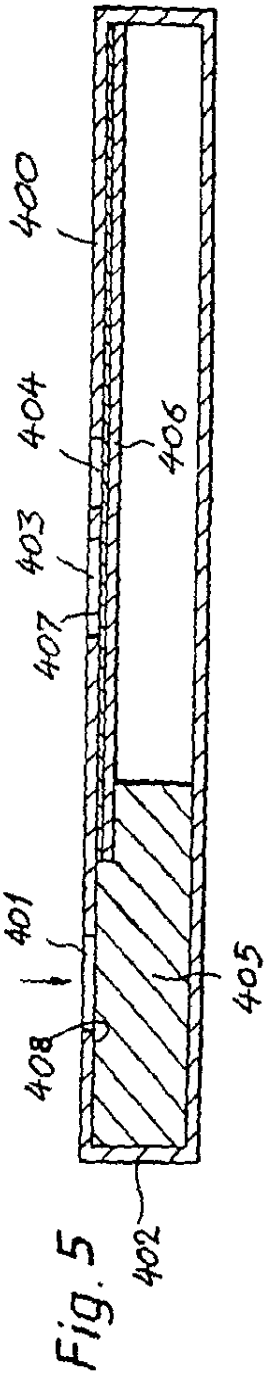

Fig. 5

ANALYTICAL TEST DEVICE FOR IMUNO ASSAYS AND METHODS OF USING SAME

This is a continuation of application Ser. No. 8/488,460, filed on Jun. 7, 1995, which was abandoned upon the filing hereof; a cont. of application Ser. No. 08/400,500 filed Mar. 8, 1995, abandoned; which is a cont. of application Ser. No. 08/183,263 filed Jan. 19, 1994, now abandoned; which was a cont. of application Ser. No. 07/585,066 filed May 19, 1992, now abandoned.

FIELD OF THE INVENTION

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

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.

BACKGROUND PRIOR ART TO THE INVENTION

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.

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 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 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 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

SUMMARY OF THE INVENTION

The present invention provides an analytical test 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 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 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.

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 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 binding agent by a liquid sample before such a sample is analysed on a test strip or the like.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** represents an isometric view of an assay device in accordance with the invention;

FIG. **2** represents a cross-sectional side elevation of the device shown in FIG. **1**.

FIG. **3** shows an enlarged view of the sample collector, macroporous body and test strip in the device illustrated in FIGS. **1** and **2**.

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

Preferably, the dry porous carrier material comprises a chromatographic strip, such as a strip of 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 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 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 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 nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns.

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 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. 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 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 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 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 encounters the particulate label considerably facilitates the ease with which the particulate label is taken up by 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.

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.

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 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.

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:

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 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 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 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 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 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.

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 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 bound in the detection zone.

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 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.

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 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 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 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 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 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.

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 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 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 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 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 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.

An important embodiment of the invention is 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 "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 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 multi-directional (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.

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 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 horseradish 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 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 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 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 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, 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 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 to allow any unbound conjugage 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.

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

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 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 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 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 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

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 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 biding 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 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 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 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 thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose 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 such that in the untreated material, aqueous liquid migrates at a rate of 1 cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the macroporous body 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 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 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.

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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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 mbodying 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 (human chorionic gonadotrophin), LH (luteinizing hormone), and infectious disease agents, whereas competition assays, for example, may be carried out for analytes such as E-3-G (estrone-3-glucoronide) and P-3-G (pregnanediol-3-glucoronide).

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 A_1

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 sereotypes 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 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 lmm 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.

Referring to FIG. **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 FIG. **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 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 "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 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 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** 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 FIG. **2**, but the zone containing the 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 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 liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing sample 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 as a whole therefore combines the functions of sampler and analyzer.

By using the test strip materials and reagents as herein described, a device in accordance with FIGS. **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 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 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 **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 from the spirit of the invention.

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 in the direction shown by the arrows from the sample collector through the macroporous body and into the porous strip. Test zone **120** incorporates the immobilized specific binding reagent, and control zone **121** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip.

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**.

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 FIG. **3**.

Embodiment 2

Referring to FIG. **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 apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **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 analogous 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 (1.4 mm thick) of commercially-available, detergent pre-treated, macroporous polyethylene having a pore size of about 100 microns was saturated with an 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 contained 3% BSA and 4% sugar. The sheet was then freeze-dried and cut into portions each 6×12 mm, having a liquid capacity of about 50 μL. These were incorporated in test devices as described above under embodiment 1, with the test strip comprising backed nitrocellulose with an anti-alpha LH monoclonal antibody immobilized 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 μL.

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.

What is claimed is:

1. An analytical test device suitable for analyzing a liquid sample suspected of containing an analyte, said device comprising the following separate components in sequential fluid communication: (1) a liquid sample application member; (2) a macroporous body positioned to receive liquid sample from said sample application member, said macroporous body including a mobilizable labeled specific binding reagent for binding to analyte in said sample; and (3) a dry porous carrier strip downstream of said macroporous body, said carrier strip including a detection zone comprising an unlabeled immobilized specific binding reagent for binding to said analyte, said mobilizable labeled reagent being freely soluble or dispersible in liquid sample applied to the liquid application member and free to move therewith through the pores of said macroporous body whereby it is transported by said liquid sample from said macroporous body to said detection zone, said macroporous body and carrier strip comprising separate and different materials which overlap at their adjacent ends to provide effective contact between these components to ensure that liquid sample applied to said application member can permeate sequentially through the macroporous body and the carrier strip.

2. The analytical test device according to claim **1**, wherein the dry porous carrier strip comprises a chromatographic strip.

3. The analytical test device according to claim **2**, wherein the labelled specific binding reagent comprises a specific binding reagent attached to a particulate label.

4. The analytical test device according to claim **3**, wherein the particulate label is latex.

5. The analytical test device according to claim **4**, wherein the latex comprises particles having a maximum dimension of not greater than about 0.5 micron.

6. The analytical test device according to claim **4**, wherein the latex is coloured.

7. The analytical test device according to claim **4**, wherein the latex is fluorescent.

8. The analytical test device according to claim **1**, wherein the macroporous body comprises plastics material.

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

10. The analytical test device according to claim **3**, wherein the macroporous body has a pore size not less than 10 times greater than the maximum particle size of the particulate label.

11. The analytical test device according to claim **1**, wherein the porous dry carrier is nitrocellulose.

12. The analytical test device according to claim 11, wherein the nitrocellulose has a pore size of greater than about 1 micron.

13. The analytical test device according to claim 1, wherein the macroporous body is capable of absorbing at least as much of the liquid sample as can be absorbed by the dry porous carrier linking the macroporous body and the detection zone.

14. The analytical test device according to claim 1 wherein the macroporous body and porous carrier are contained within a casing constructed of moisture-impermeable material and the means for applying sample to said macroporous body comprises 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.

15. The analytical device according to claim 1, 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.

16. The analytical device according to claim 15, 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.

17. The analytical device according to claim 16, wherein the casing 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 mean which enable an indication to be given as to whether an assay procedure has been completed.

18. The analytical device according to claim 16, comprising a removable cap or shroud which can protect the protruding porous receiving member during storage before use.

19. The analytical test device of claim 1 which comprises a further zone downstream of said detection zone to indicate completion of the assay procedure.

20. A pregnancy testing device for detecting hCG antigen in a urine sample 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 which is different from the dry porous carrier such that any urine 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 specific anti-hCG antibody bearing a coloured particulate 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 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, said nitrocellulose carrier and macroporous body overlapping at their adjacent ends to provide effective contact between these components to ensure that liquid sample applied to said application member can permeate sequentially through the macroporous body and the carrier strip.

21. A testing device for detecting LH antigen in a urine sample 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 which is different from the dry porous carrier such that any urine 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 specific anti-LH antibody bearing a coloured particulate 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 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, said nitrocellulose carrier and macroporous body overlapping at their adjacent ends to provide effective contact between these components to ensure that liquid sample applied to said application member can permeate sequentially through the macroporous body and the carrier strip.

* * * * *

EXHIBIT C

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United States Patent [19]

*May et al.

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[45] Date of Patent: Aug. 12, 1997

[54] **TEST DEVICE FOR DETECTING ANALYTES IN BIOLOGICAL SAMPLES**

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[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,602,040.

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Apr. 26, 1988	[WO]	WIPO	PCT/GB88/00322

[51] **Int. Cl.**[6] **G01N 33/558**

[52] **U.S. Cl.** **436/514**; 429/2.11; 435/7.1; 435/7.92; 435/970; 436/501; 436/515; 436/518; 436/523; 436/524; 436/530; 436/541; 436/810; 436/814; 436/525

[58] **Field of Search** 422/56–58, 60; 427/2, 2.11; 435/7.1, 7.92–7.95, 970; 436/501, 514–515, 518, 523, 524, 530, 541, 810, 814, 525

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[57] **ABSTRACT**

An analytical test device useful for example in pregnancy testing, comprises a hollow casing (**500**) constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier (**510**) which communicates indirectly with the exterior of the casing via a bibulous sample receiving member (**506**) which protrudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, wherein the mobility is facilitated by a material comprising a sugar, in an amount effective to reduce interaction between the test strip and the labelled reagent, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means, such as an aperture (**508**) in the casing, enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

64 Claims, 5 Drawing Sheets

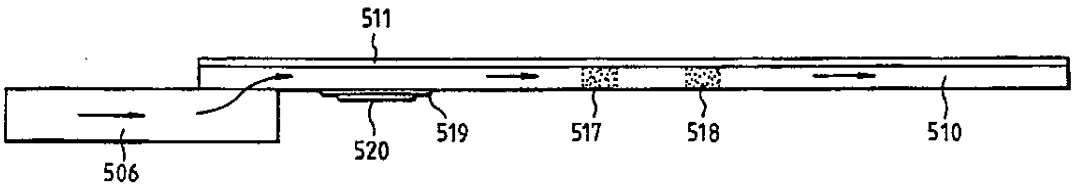

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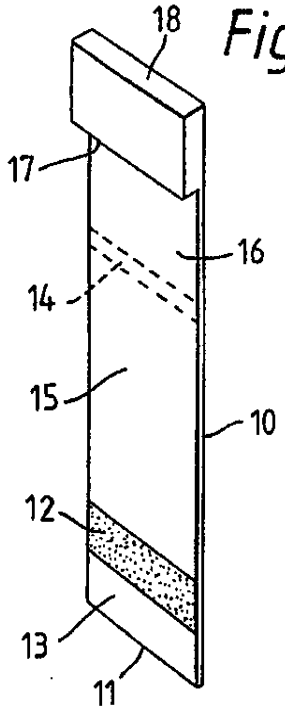
Fig.1.



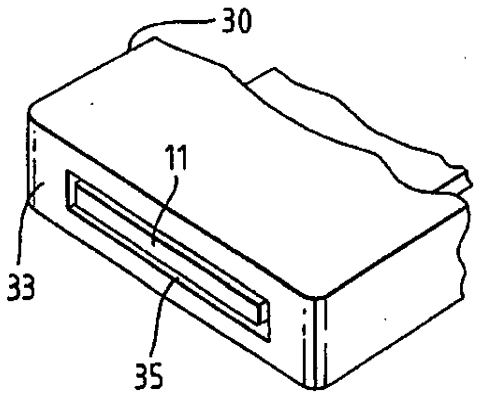
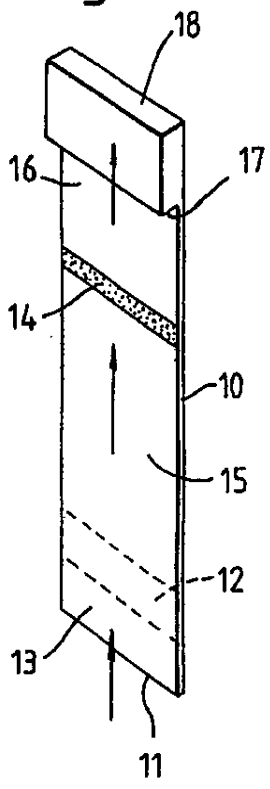
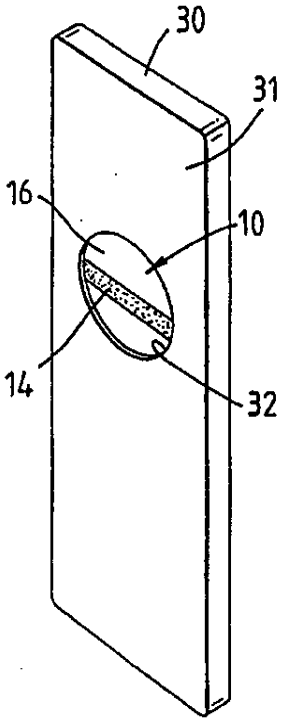


Fig.5.

Fig.2.





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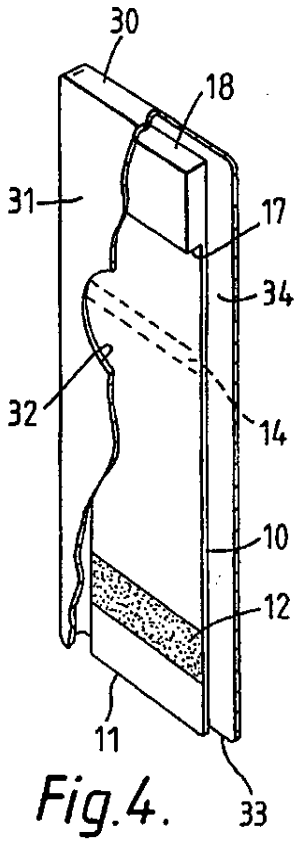

Fig.4.

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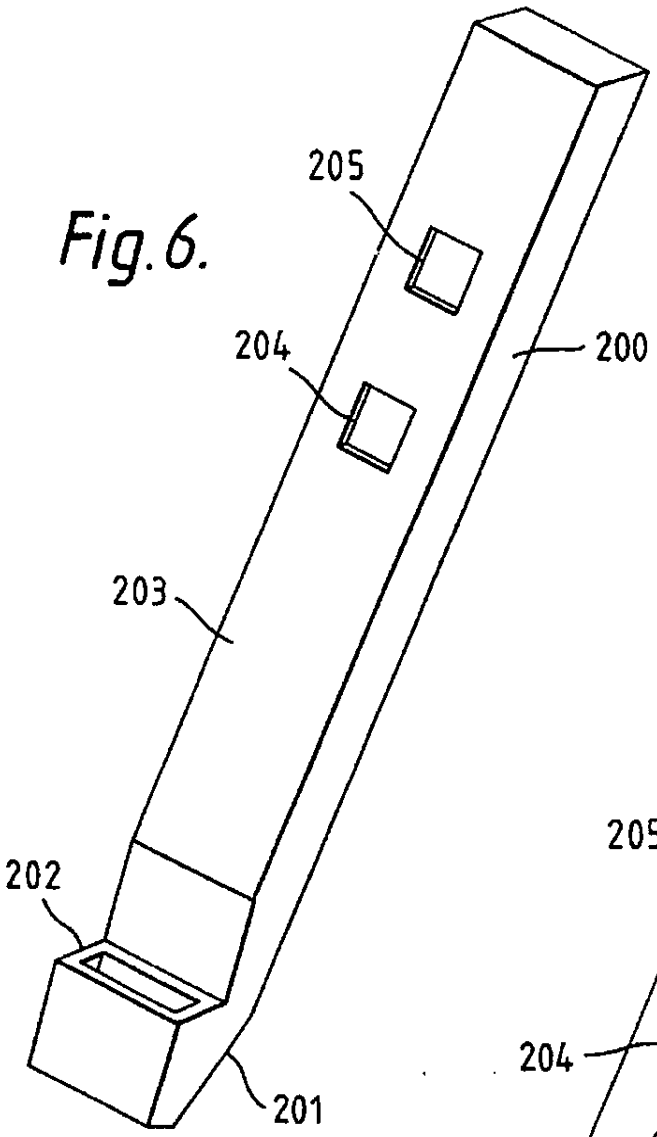
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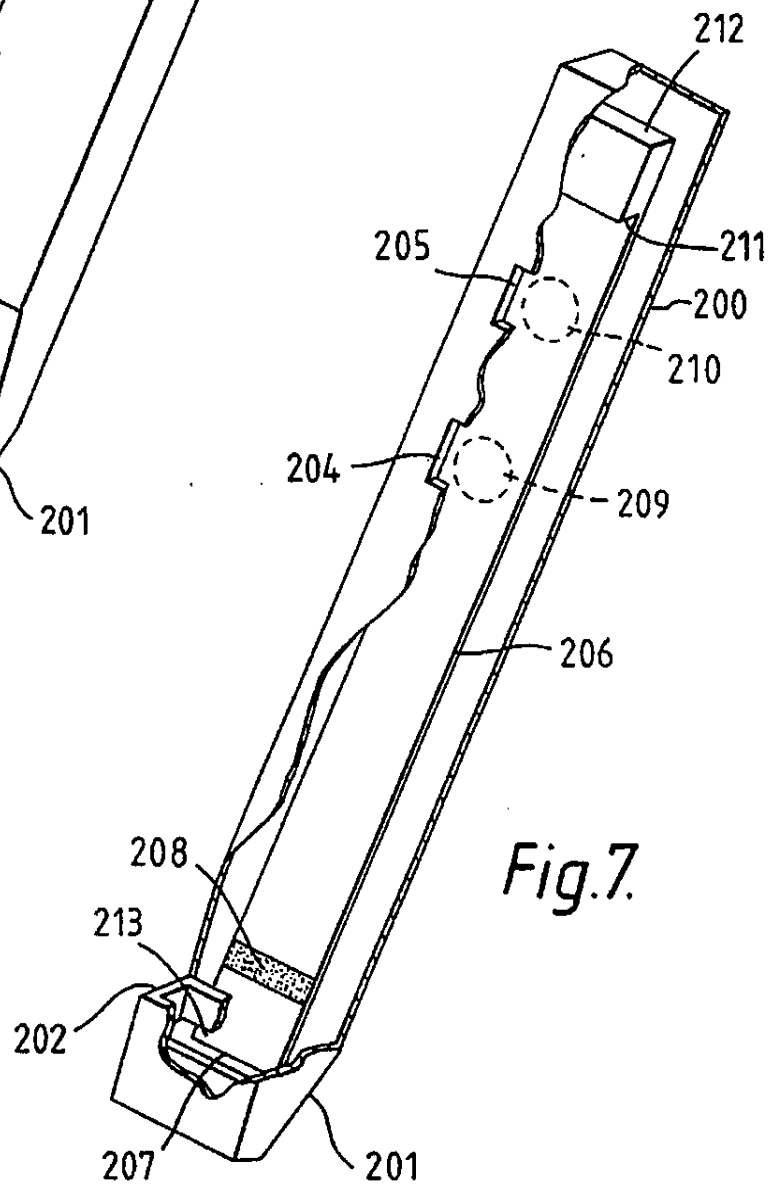
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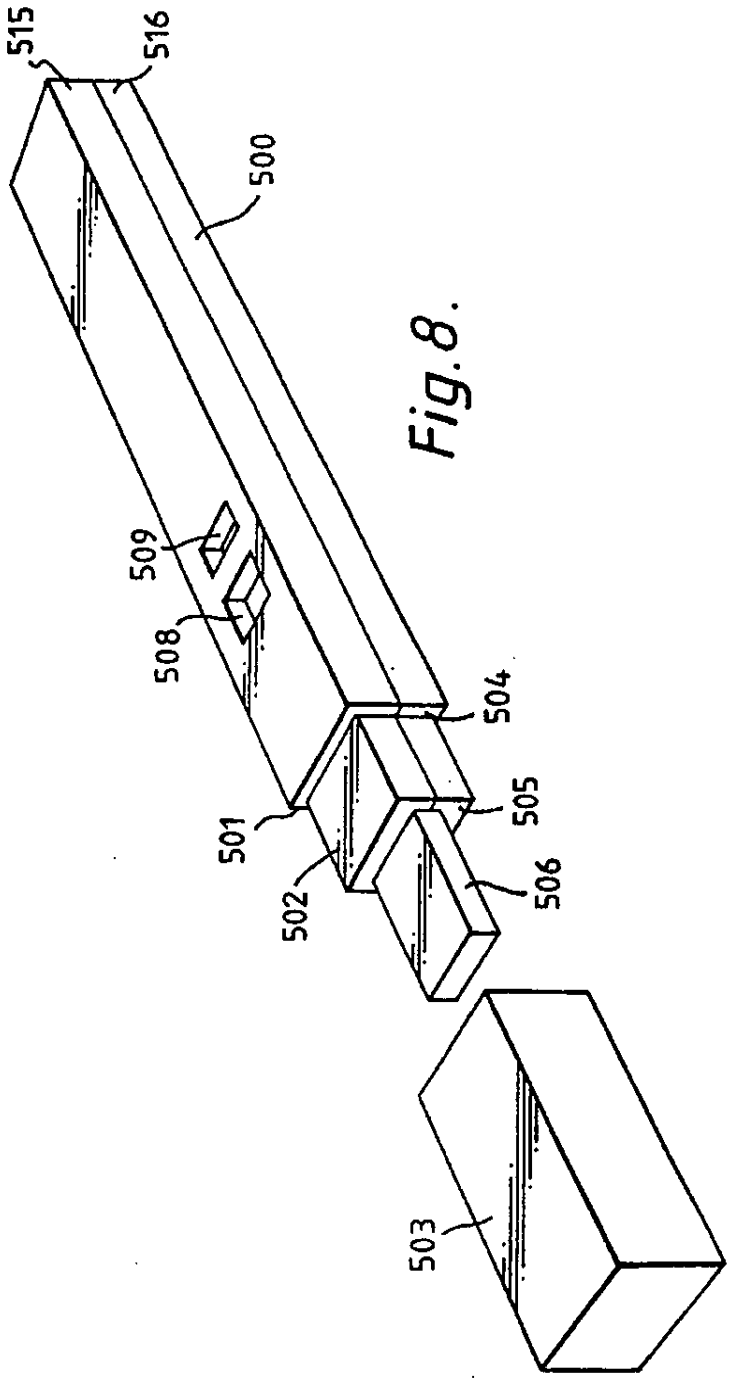
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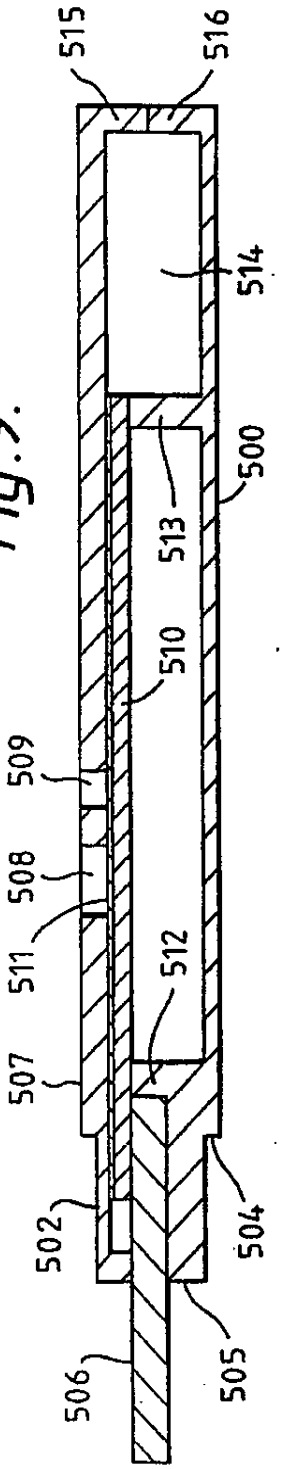
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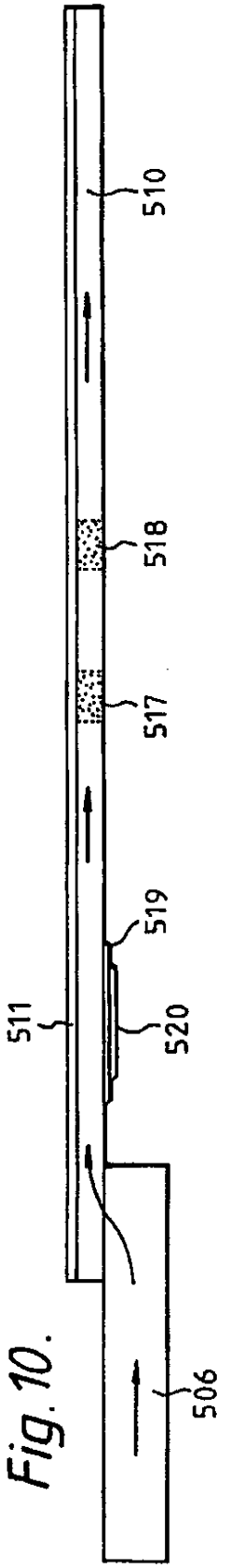
Fig. 9.



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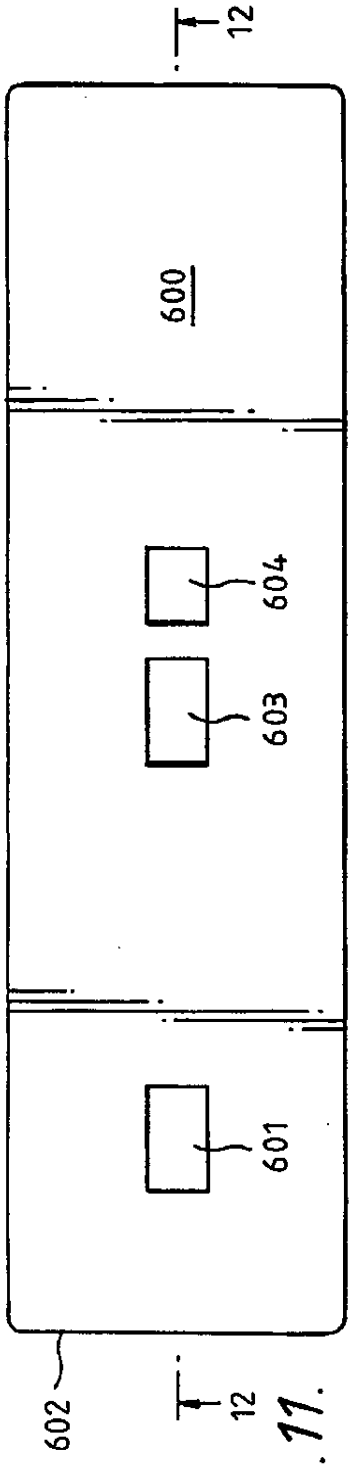

Fig. 11.

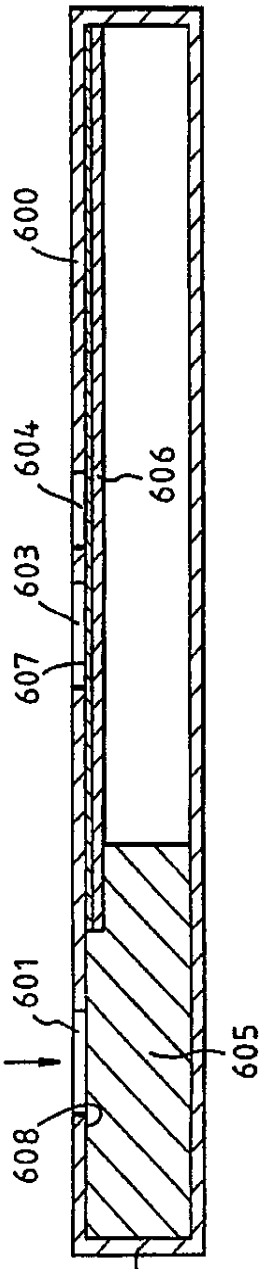

Fig. 12.

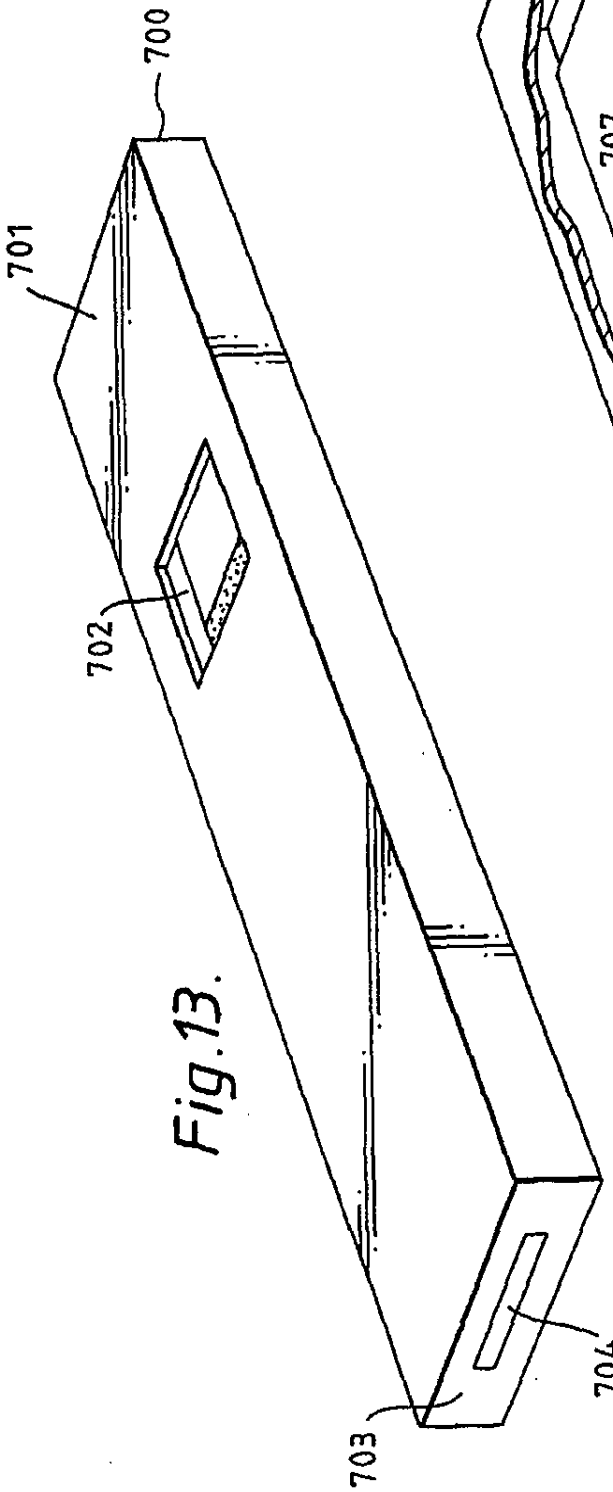

Fig. 13.

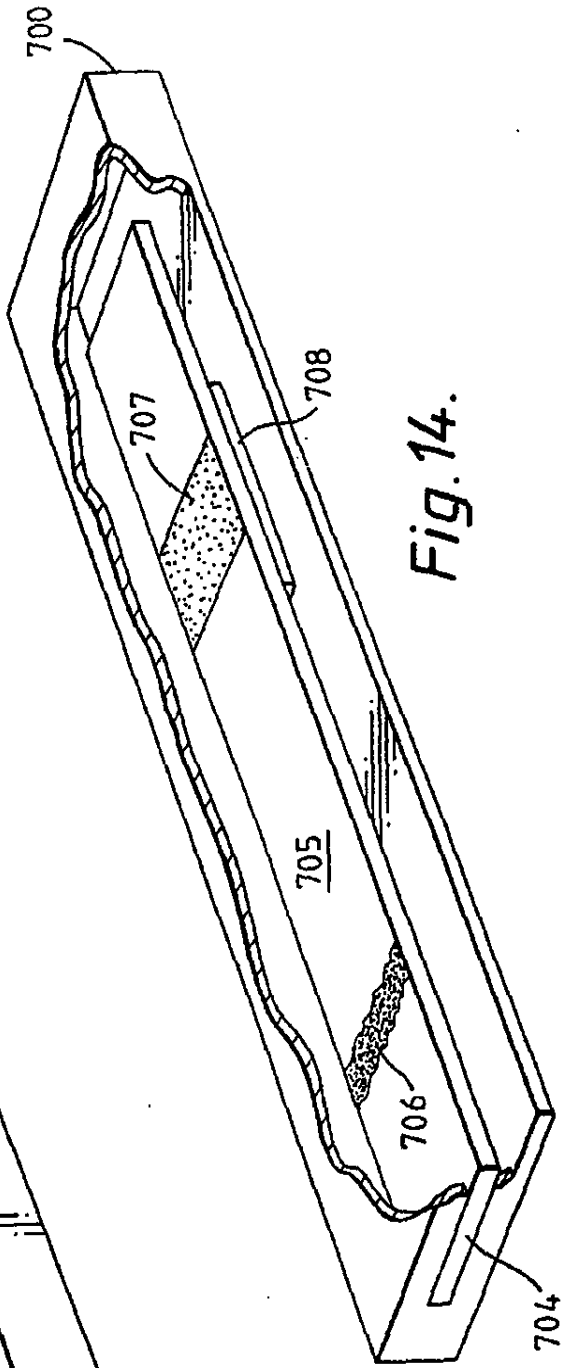

Fig. 14.

TEST DEVICE FOR DETECTING ANALYTES IN BIOLOGICAL SAMPLES

This is a division of application Ser. No. 08/102,313, filed Jul. 15, 1993 which was a continuation of application Ser. No. 07/876,449 filed Apr. 30, 1992, now abandoned which was a Divisional of application Ser. No. 07/795,266 filed Nov. 19, 1991, now abandoned which was a continuation of application Ser. No. 07/294,146 filed Feb. 27, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to assays involving specific binding, especially immunoassays. This application is based on applications filed in Great Britain, having application Ser. Nos. 8709873 (filed Apr. 27, 1987) and 8725457 (filed Oct. 30, 1987), as well as PCT application GB88/00322 (filed Apr. 26, 1988).

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 which is rapid and which requires the minimum degree of skill and involvement from the user.

2. Description of the Related Art

The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream 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. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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 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 for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices

especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is in the moist state.

The invention also provides an analytical method in which a device as set forth in the proceeding paragraph is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action through the porous solid phase material via the first zone into the second zone and the labelled reagent migrates therewith from the first zone to the second zone, the presence-of analyte in the sample being determined by observing the extent (if any) to which the labelled reagent becomes bound in the second zone.

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 together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

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 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 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

solid phase material into the second zone and bind with the immobolised 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 second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it 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 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 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.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols 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. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. 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 size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable 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 material to be observed from outside the housing and which further zone incorporates 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 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. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but this is not preferred.

An important embodiment of the invention is 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 and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone :a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone 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 two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and 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 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 multi-directional (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 flouride, ethylene vinylacetate, acrylonitrile and polytetrafluoroethylene 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 hydrophobictiy 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 nitrocellulose. 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 porous solid phase material.

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 antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a 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 second 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 horseradish 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 porous solid phase material 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 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 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 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, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away 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 to allow any unbound conjugage 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.

The presence or intensity of the signal from the label which becomes bound in the second zone can 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.

The immobilised specific binding reagent in the second 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 antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and 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 incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the 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 antibody 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 ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred

embodiment of the invention, the carrier is pre-treated with a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is 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 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 thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose 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 such that in the untreated material, aqueous liquid migrates at a rate of 1 cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the zones, 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, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second 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 present in the migrating sample.

The reagents can be applied to the 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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **2** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **3** is a perspective view of a device utilizing a porous strip of the type illustrated in FIGS. **1** and **2**;

FIG. **4** is a perspective view, partially broken away, revealing a porous strip within the device of FIG. **3**;

FIG. **5** is an enlarged view of one end of the device of FIG. **3**;

FIG. **6** is a perspective view of another test device according to the invention;

FIG. **7** is a perspective view, similar to FIG. **6**, but partially cut away to reveal the porous test strip contained within the body of the device;

FIG. **8** is an exploded perspective view of an assay device in accordance with the present invention;

FIG. **9** is a cross-sectional side elevation of the device shown in FIG. **8**;

FIG. **10** is an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**;

FIG. **11** is a plan view of another embodiment of the invention;

FIG. **12** is a schematic cross-sectional view taken along line **12**—**12** in FIG. **11**;

FIG. **13** is a perspective view of yet another embodiment of the invention; and

FIG. **14** is a partially cut away view of the device of FIG. **13**.

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

Embodiment 1

FIGS. **1** and **2** represent a typical strip of porous solid phase material for use in an assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to FIG. **1**, the assay test strip **10** is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end **11** of strip **10** is a narrow band or zone **12** extending across the entire width of the strip. A small region **13** of strip **10** lies vertically below zone **12**. Above zone **12** is a second zone **14** lying a discrete distance up strip **10** and similarly extending the entire width of the strip. The region **15** of strip **10** between zones **12** and **14** can be of any height as long as the two zones are separate. A further region **16** of the strip extends above zone **14**, and at the top **17** of the strip is a porous pad **18** firmly linked to strip **10** such that pad **18** can act as a "sink" for any liquid sample which may be rising by capillary action through strip **10**.

Zone **12** is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone **14**, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

FIG. **2** illustrates what happens when the assay strip is used in an analytical procedure. The lower end **11** of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad **18**. In so doing, the sample traverses zone **12** and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone **14**, the labelled antibody can bind to any analyte present in the sample. On reaching zone **14**, any analyte molecule should become bound to the second antibody, so immobilising the labelled "sandwich" so produced. If a

significant concentration of the analyte to be determined is present in the liquid sample, in a short period of time a distinct accumulation of the visible label should occur in zone **14**.

As an example of an analysis to which this embodiment can be applied, the analyte can be hCG, the reagents in zones **12** and **14** can be monoclonal antibodies to hCG which can participate in a "sandwich" reaction with hCG, and the label can be a particulate dye, a gold sol or coloured latex particles.

Although described above in relation to a "sandwich" reaction, it will be readily apparent to the skilled reader that this can be modified to a "competition" reaction format if desired, the labelled reagent in zone **12** being the analyte or an analogue of the analyte.

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 vital 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 (estrone-3-glucoronide) P-3-G (progesterone-3-glucuronide).

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 A_1 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. By way of example, a dual analyte test for apoliproteins A_1 and B may be prepared by depositing, as two spacially distinct zones, antibody specific for apolipoprotein A_1 throughout a first zone and depositing a second antibody specific for apolipoprotein B, throughout the second zone of a porous carrier matrix. Following the application of both antibodies to each of their respective zones via a suitable application procedure (e.g.-ink-jet printing, metered pump and pen, or airbrush), the remainder of the porous material should be treated with a reagent, e.g. bovine serum albumin, polyvinyl alcohol, or ethanolamine, to block any remaining binding sites elsewhere. A third and fourth reagent, bearing a label, may then be dispensed onto the dry carrier in one or more zones near to one end of the strip, the strip being allowed to dry between applications of the two reagents to the same zone. Reagent **3** and Reagent **4** may comprise conjugates of anti-apolipoprotein A_1 antibody and anti-apolipoprotein B antibody respectively. Both of these conjugates will become mobile in and on the carrier when in the moist state. Reagents **3** and **4** can migrate with the solvent flow when an aqueous sample is applied to the first end of the carrier strip. While migrating towards the two zones further along the strip, reagent **3** may bind any apolipoprotein A_1 present in the sample and reagent **4** may bind any apolipoprotein B present in the sample. On reaching the first second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein A_1 molecules should become bound to the second antibody, immobilising the labelled 'sandwich' so produced. No labelled apoliprotein B molecules will bind to this first zone. On reaching the second second-antibody zone (anti-apolipoprotein B antibody zone) any apolipoprotein B molecules should become bound to the second antibody (solid-phase antibody), immobilising the labelled 'sandwich' so produced. No labelled apolipoprotein A_1 molecules will bind to the second zone. An accumulation of each of the direct label may occur at both or either zones to a lesser or greater extent resulting in a visible signal at either or both of the solid phase antibody zones. Excess unbound conjugate (of both reagent **3** and reagent **4**) can pass freely over the two antibody zones and will be washed into the distal end of the strip.

The development of a quantifiable colour in both of the second-antibody zones may be assessed with an appropriate form of instrumentation, yielding a ratio of colour density between the two sites.

The determination of the presence of more than two (is 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 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 1 mm 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. Conjugates of label, e.g. a dye sol and each immunochemically-reactive component specific for each bacterial group may then be dispensed either onto a single zone at the bottom end of the strip, proximal to the sample application zone, or as a series of separate zones.

FIGS. **3**, **4** and **5** of the accompanying drawings depict a complete device utilising a porous strip as just described above. FIG. **3** represents the complete device viewed from the front, FIG. **4** shows the same device partially cut away to reveal the details of the strip inside, and FIG. **5** shows the underside of the device.

Referring to FIG. **3**, the device comprises a flat rectangular body **30** the front face **31** of which is perforated by a circular hole or window **32** which reveals the porous test strip **10** within the body. The region of the test strip **10** visible through the window **32** incorporates a narrow horizontal zone **14**.

Referring to FIG. **4**, the device comprises a dry rectangular test strip **10** made from porous material which extends from the bottom end **33** of the body **30** within the body between the front **31** and back **34** of the body. Near the bottom end **11** of the strip **10** is a horizontal zone **12** bearing a labelled specific binding reagent for an analyte, the binding reagent being mobile in the test strip in the moist state. Further up the test strip is the narrow horizontal zone **14** which is visible through the window **32**. At the top **17** of the test strip **10** is a porous 'sink' **18** which can absorb any liquid sample that has permeated upwards through the strip.

Referring to FIG. **5**, the bottom edge **35** of the body **30** incorporates a lateral aperture in which the bottom end **11** of the strip lies.

In operation, the bottom end **33** of the body **30** is immersed in a liquid sample (eg urine) so that the liquid

sample can be absorbed by the bottom end **11** of the test strip **20** and rise by capillary action to the top **17** of the test strip and into the sink **18**. In so doing, the liquid sample progresses via zone **12** to zone **14**. Specific binding reactions as described above occur, and the test result is visible to the user through the window **32**.

Embodiment 2

FIGS. **6** and **7** of the accompanying drawings illustrate another test device according to the invention. FIG. **6** illustrates the complete device viewed from the front, and FIG. **7** depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to FIG. **6**, the device comprises an elongate body **200** terminating at its lower end **201** in a small integral receptacle **202** which can hold a predetermined volume of a liquid sample, eg urine. The front face **203** of the body **200** incorporates two square small square apertures or windows **204** and **205** located one above the other.

Referring to FIG. **7**, the elongate portion of the body **200** is hollow and incorporates a test strip **206** running almost the full height of the body. This test strip is of similar construction to those described under Embodiment 1, and incorporates near its lower end **207** a horizontal zone **208** boating a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones **209** and **210** adjacent to the windows **204** and **205** and visible therethrough. The strip terminates at its top end **211** in a porous sink **212**. At the bottom end **201** of the device, the receptacle **202** communicates with the hollow body via a lateral aperture **213**.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacles **202**. From the receptacle **202** the liquid sample rises by capillary action through the test strip **206** and conveys the labelled reagent from zone **208** to the two circular zones **209** and **210**. A series of specific binding reactions as described in relation to Embodiment 1 above occur. In this embodiment the second circular zone **210** can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone **209**. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone **210** can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone **208** by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone **209** by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone **208**, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Embodiment 3

FIG. **8** of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and FIG. **9** represents a cross-sectional side elevation of the device shown in FIG. **8**.

Referring to FIG. **8**, the device comprises a housing or casing **500** of elongate rectangular form having at one end **501** a portion **502** of reduced cross-sectional area. A cap **503** can be fitted onto portion **502** and can abut against the shoulder **504** at end **501** of the housing. Cap **503** is shown separated from housing **500**. Extending beyond end **505** of portion **502** is a porous member **506**. When cap **503** is fitted onto portion **502** of the housing, it covers porous member **506**. Upper face **507** of housing **500** incorporates two apertures **508** and **509**.

Referring to FIG. **9**, it can be seen that housing **500** is of hollow construction. Porous member **506** extends into housing **500** and contacts a strip of porous carrier material **510**. Porous member **506** and strip **510** overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member **506** can permeate member **506** and progress into strip **510**. Strip **510** extends further into housing **500**. Strip **510** is "backed" by a supporting strip **511** formed of transparent moisture-impermeable plastics material. Strip **510** extends beyond apertures **508** and **509**. Means are provided within housing **500** by webbs **512** and **513** to hold strip **510** firmly in place. In this respect, the internal 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, and porous member **506** is firmly retained in the housing and adequate fluid permeable contact is maintained between member **506** and strip **510**. The transparent backing strip **511** lies between strip **510** and apertures **508** and **509** and can act as a seal against ingress of moisture from outside the housing **500** via these apertures. If desired, the residual space **514** within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip **510** in the dry state during storage. The reagent-containing zones in strip **510** are not depicted in FIG. **8**, but the first zone containing the labelled reagent which is mobile when the strip is moistened will lie in the region between the porous member **506** and aperture **508**. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture **508** in order that when the device has been used in an assay, the result can be observed through aperture **508**. Aperture **509** provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap **503** is removed from the holder and member **506** is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member **506** to the liquid sample for a time sufficient to ensure that member **506** is saturated with the sample, the cap **503** 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 **510** to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures **508** and **509** and can ascertain whether the assay has been completed by observing the control zone through aperture **509**, and can ascertain the result of the assay by observing the second zone through aperture **508**.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing **500** being moulded in two parts (e.g. upper and lower halves **515** and **516**) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member 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 porous member and test strip together to ensure adequate contact between them. Cap **503** can be moulded as a separate

complete item. If desired, apertures **508** and **509** 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 **505** of housing **500** and the protruding porous member **506**, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member **506**. Member **506** 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 as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with FIGS. **8** and **9** 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 then (after optionally replacing the cap) can observe the test result through aperture **508** within a matter of a few minutes.

Although described with particular reference to 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 **509** 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 from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member **506**.

FIG. **10** of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**.

The porous receiving member **506** is linked to the porous test strip **510**, backed by the transparent plastics sheet **511**, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone **517** incorporates the immobilised specific binding reagent, and control zone **518** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip **511** and adjacent the porous receiving member **506**, carries a glaze **519** on which is deposited a layer **520** of labelled specific binding reagent. The thickness of these two layers as depicted in FIG. **10** is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member **506** can flow therefrom along the length of strip **510** and in so doing, will dissolve glaze **519** and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone **517**.

Embodiment 4

FIGS. **11** and **12** illustrate another embodiment of the invention, which is seen in plan view in FIG. **11** and in cross-section in FIG. **12**, the cross-section being an elevation on the line **12—12** seen in FIG. **11**.

Referring to FIG. **11**, the test device comprises a flat rectangular casing **600** incorporating a centrally disposed rectangular aperture **601**, adjacent the left hand end **602**, and two further apertures **603** and **604** near the mid point of the device and arranged such that apertures **601**, **603** and **604** lie on the central longitudinal axis of the device corresponding to line **12—12**. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **12**, the device is hollow and incorporates within it a porous sample receiving member adjacent end **602** of casing **600** and lying directly beneath aperture **601**. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip **606** backed by a transparent plastics sheet **607** is also contained within casing **600**, and extends from the porous receiving member **602**, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet **607** is in firm contact with the upper inner surface **608** of casing **600**, and provides a seal against apertures **603** and **604** to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip **606** will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures **603** and **604**, in a manner analagous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture **601**, e.g. by means of a syringe, to saturate porous receiving member **605**. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures **603** and **604**.

Embodiment 5

A yet further embodiment of the invention is illustrated in FIGS. **13** and **14** of the accompanying drawings. FIG. **13** shows a device comprising a rectangular casing **700** having in its upper surface **701** a rectangular aperture **702**. One end wall **703** of the device **703** incorporates an aperture **704** through which a porous test element communicates with the exterior of the device. Aperture **702** is situated in surface **701** at a point relatively remote from the end **703** containing the aperture **704**.

FIG. **14** shows a partially cut-away view of the device in FIG. **13**. The hollow device incorporates a porous test strip **705**, running almost the entire length of casing **700** from aperture **704**. Test strip **705** incorporates a first zone **706** containing a labelled specific binding reagent and a further zone **707**, remote from aperture **704**, incorporating an immobilised specific reagent. Zone **706** lies directly beneath aperture **702** is therefore observable from outside casing. Beneath strip **705** and adjacent zone **707**, is a crushable element **708** containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone **707**, if labelled reagent from **706** has become bound in zone **707** following use of the device. Release of the reagents from member **708** can be effected by applying pressure to the outside of the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end **703** of casing **700** into a vessel containing the sample. The liquid sample will then permeate the length of test strip **705**, taking labelled reagent from zone **706** and passing through zone **707** where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the

sample has permeated the test strip, reagent can be released from the crushable member 708 and the result of the test observed through aperture 702.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45 mm (minutes)
Schleicher + Schuell nitrocellulose (unbacked)	3μ	3.40
	5μ	3.30
	8μ	3.00
	12μ	2.20
polyester-backed	8μ (nominal)	3.40
Whatman Nitrocellulose	5	19.20
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

PREPARATION OF LABELS

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20 nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22μ filtered 0.1M K_2CO_3, and 20 mls is added to a clean glass beaker. 200 μl of anti-alpha hCG antibody, prepared in 2 mM borax buffer pH9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2 mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000 g, 30 minutes, and 4° C., with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20 mM Tris, 150 mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specification No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grams of dye is dispersed in 1 liter of distilled water by mixing on a magnetic stirrer for 2–3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500 g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000 g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500 mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100 mls distilled water.

The spectra of dye sols prepared as described above. can be measured, giving lambda-max values of approximately 657 nm for Foron Blue, and 690 nm for Resolin Blue. The absorbance at lambda-max, for 1 cm path length, is used as an arbitrary man arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoassays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the preformed particles. In the latter route, the dye should be dissolved in a water-immisicible solvent, such a chloroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5 ml (12.5 mg solids) of suspension is diluted with 1 ml of 0.1M berate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1 ml borate buffer, mixed with 300 μg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16–20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000 rpm, the supernatant is discarded and the pellet resuspended in 1.5 mls borate buffer containing 0.5 milligrams bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5 mg/ml BSA in phosphate buffered saline pH7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5 mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4° C. until used.

(A) Anti—hCG—Dye Sol Preparation

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an

antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction mixture is prepared which contains 100 µl antibody solution, 2 mls dye sol, 2 mls 0.1M phosphate buffer pH 5.8 and 15.9 mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4 mls of 150 mg/ml BSA in 5 mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000 g for 10 minutes, and the pellet resuspended in 10 mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate. buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH—Dye Sol Preparation

Due to the structural homology between the alpha submits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8µ nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30° C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5 cm in length and 1 cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8µ nitrocellulose, 25 cm in length and 20 cm in width with zones of immobilised antibody at 5 cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6 cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5 cm in length and 1 cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100 µl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% TRITON X305 (Trademark—obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100 µl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1 mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA—Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of E-3-G (2 nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10 nM) in dry DMF were equilibrated separately at 4° C. Using pre-cooled glassware E-3-G in DMF (1.25 ml) and TnB in DMF (0.25 ml) were added to a pre-cooled 5 ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10 nM) was prepared and an aliquot (0.25 ml) was cooled to 4° C. and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4° C. and a solution of BSA (1 mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5 ml) and stirred on a magnetic stirrer for 4 hours at 4° C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 SEPHADEX G-25 column, transferred to an amber glass storage bottle and stored at 4° C.

Preparation of BSA—E-3-G dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850 rpm (1500 g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10 μg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 a the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5 mM, pH7.4) to yield a final BSA concentration of 25 mg/ml. The reaction mixture was centrifuged at 4850 rpm (3000 g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH5.8) buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA—E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to all combinations and subcombinations of the features of this description and the accompanying drawings.

We claim:

1. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample selected from the group consisting of urine and serum, said device comprising:

a) a hollow casing having a liquid biological sample application aperture and a test result observation aperture;

b) a bibulous liquid biological sample receiving member within said hollow casing to receive said liquid biological sample applied to said sample application aperture;

c) a test strip comprising a dry porous carrier within said casing and extending from said bibulous liquid biological sample receiving member to and beyond said test result observation aperture, said dry porous carrier having a test result zone observable through said observation aperture;

at least one of said bibulous liquid biological sample receiving member and said test strip containing upstream from said test result zone a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte;

said label being a particulate direct label wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said dry porous carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

migration of said liquid biological sample from said bibulous sample receiving member into and through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable through said test result observation aperture, thereby to indicate the present of said analyte in said liquid biological sample.

2. The test device according to claim 1, wherein said particulate direct label is a sol.

3. The test device according to claim 1, wherein said sol is a dye sol or a metallic sol.

4. The device according to claim 1, wherein said labelled reagent comprises a label selected from the group consisting of dye sols, metallic sols and colored latex particles of maximum diameter not greater than 0.5 microns.

5. The device according to claim 1, wherein said labelled reagent comprises an anti-hCG antibody and said liquid biological sample is urine.

6. The device according to claim 1, wherein said labelled reagent comprises an anti-LH antibody and said liquid biological sample is urine.

7. The analytical test device according to claim 1, wherein said labelled reagent is contained in a first zone of said dry porous carrier upstream from said test result zone, and said means for binding is an unlabelled reagent which is immobilized in said test result zone, said test result zone being spaced from said first zone, said first zone and said test result zone being arranged such that said liquid biological sample applied to said sample aperture is conveyed by capillarity through said first zone into said test result zone.

8. The test device according to claim 1, wherein said hollow casing is molded from plastics material.

9. The test device according to claim 1, wherein said dry porous carrier comprises a strip or sheet of porous material.

10. The test device according to claim 1, wherein said dry porous carrier comprises a strip or sheet of porous material backed with a layer of transparent moisture-impervious material, said transparent layer being in contact with the inside of said hollow casing adjacent said test result observation aperture to inhibit ingress of moisture or said liquid biological sample.

11. The test device according to claim 10, wherein said layer of transparent moisture-impervious material is transparent plastics material.

12. The test device according to claim 1, wherein said dry porous carrier material is nitrocellulose.

13. The test device according to claim 12, wherein said nitrocellulose has a pore size of at least about one micron.

14. The test device according to claim 13, wherein said pore size is about greater than 5 microns.

15. The test device according to claim 14, wherein said pore size is about 8–12 microns.

16. The test device according to claim 1, further comprising a control zone downstream from said test result zone in said dry porous carrier, which control zone comprises means to indicate that said liquid biological sample has been conveyed by capillarity beyond said test result zone, said control zone being observable from outside said hollow casing.

17. The test device according to claim 1, further comprising an absorbent sink at a distal end of said dry porous carrier, said sink having sufficient absorptive capacity to absorb any unbound labelled reagent washed out of said test result zone.

18. The test device according to claim 1, wherein said labelled reagent has been applied to said dry porous carrier as a surface layer.

19. The test device according to claim 18, wherein said dry porous carrier has been pre-treated with a material comprising sugar in the region to which said labelled reagent is applied.

20. The test device according to claim 1, wherein said immobilized reagent in said test result zone is impregnated throughout of said dry porous carrier in said test result zone.

21. The device according to claim 1, wherein said binding means binds directly with said first complex.

22. The device according to claim 1, in combination with a cap for being selectively received over a longitudinal end of said hollow casing to selectively cover said sample application aperture.

23. The device according to claim 1, wherein said bibulous liquid biological sample receiving member protrudes from said hollow casing through said sample application aperture.

24. The device according to claim 1, wherein said dry porous carrier contains said labelled reagent.

25. The device according to claim 1, wherein said bibulous sample receiving member contains said labeled reagent.

26. The test device according to claim 1, wherein said material comprising sugar further comprises a protein.

27. The test device according to claim 26, wherein said protein is bovine serum albumin.

28. The test device according to claim 1, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

29. The test device according to claim 1, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

30. The test device according to claim 1, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

31. The test device according to claim 30, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

32. The test device according to claim 1, wherein said bibulous liquid biological sample receiving member has unidirectional porosity parallel to its longitudinal axis.

33. An analytical method for detecting an analyte suspected of being present in a liquid biological sample selected from the group consisting of urine and serum, which comprises utilizing an analytical test device comprising:

providing an analytical test device having:

a) a hollow casing having a liquid biological sample application aperture and a test result observation aperture;

b) a bibulous liquid biological sample receiving member within said hollow casing to receive said liquid biological sample applied to said sample application aperture;

c) a test strip comprising a dry porous carrier within said hollow casing and extending from said liquid biological sample receiving member to and beyond said test result observation aperture, said dry porous carrier having a test result zone observable through said observation aperture;

said test strip containing in a first zone upstream from said test result zone a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said label being a particulate direct label wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

migration of said applied liquid biological sample from said bibulous sample receiving member into and through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable through said test result observation aperture, thereby to indicate the presence of said analyte in said liquid biological sample;

applying said liquid biological sample to said bibulous receiving member;

permitting said liquid biological sample to convey by capillary action through said dry porous carrier via said first zone into said test result zone with said labelled reagent and

detecting the presence of said analyte in said liquid biological sample by visually observing the presence of any labelled reagent bound in said test result zone.

34. The method according to claim 33, wherein said material comprising sugar further comprises a protein.

35. The method according to claim 34, wherein said protein is bovine serum albumin.

36. The method according to claim 33, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

37. The method according to claim 33, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

38. The method according to claim 33, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

39. The method according to claim 38, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

40. The method according to claim 33, wherein said bibulous liquid biological sample receiving member has unidirectional porosity parallel to its longitudinal axis.

41. The method according to claim 40, wherein the bibulous liquid biological sample receiving member protrudes from said hollow casing through said sample application aperture.

42. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample selected from the group consisting of urine and serum, said device comprising:

a) a hollow casing having a liquid biological sample application aperture and a test result observation aperture;

b) a bibulous liquid biological sample receiving member within said hollow casing to receive said liquid biological sample applied to said sample application aperture;

c) a test strip comprising a dry porous carrier within said hollow casing and extending from said bibulous liquid biological sample receiving member to and beyond said test result observation aperture, said dry porous carrier having a test result zone observable through said observation aperture;

at least one of said bibulous liquid biological sample receiving member and said test strip containing upstream from said test result zone a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and analyte;

said label being a particulate direct label which is dry prior to use of the device and is released into mobile form by said applied liquid biological sample, wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

(d) a control zone downstream from said test result zone in said dry porous carrier for binding labelled reagent to indicate that said applied liquid biological sample has been conveyed by capillarity beyond said test result zone, said hollow casing having a control zone observation window defined therein, whereby said control zone is observable from outside said hollow casing;

(e) an absorbent sink at a distal end of said carrier, said sink having sufficient absorptive capacity to allow any unbound labelled reagent to wash out of said test result zone;

migration of said applied liquid biological sample from said bibulous sample receiving member into and through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable through said test result observation aperture, thereby to indicate the presence of said analyte in said applied liquid biological sample.

43. The test device according to claim 42, wherein said material comprising sugar further comprises a protein.

44. The test device according to claim 43 wherein said protein is bovine serum albumin.

45. The test device according to claim 42, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

46. The test device according to claim 42, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

47. The test device according to claim 42, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

48. The test device according to claim 47, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

49. The test device according to claim 42, wherein said bibulous liquid biological sample receiving member has unidirectional porosity parallel to its longitudinal axis.

50. The test device according to claim 49, wherein the bibulous liquid biological sample receiving member protrudes from said hollow casing through said sample application aperture.

51. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device comprising:

a) a hollow casing having a liquid biological sample application aperture and a test result observation aperture;

b) a test strip comprising a dry porous carrier contained within said hollow casing, said carrier communicating directly or indirectly with the exterior of said hollow casing through said liquid biological sample application aperture to receive applied said liquid biological sample, said carrier having a test result zone observable through said test result observation aperture, said dry porous carrier comprising a strip or sheet of porous material backed with a layer of transparent moisture-impervious material, said transparent layer being in contact with the inside of said hollow casing adjacent said test result observation aperture to inhibit ingress of moisture or liquid biological sample, said test strip, in the dry unused state, containing upstream from said test result zone a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said label being a particulate direct label which is dry prior to the use of the device and is released into mobile form by said liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

migration of said applied liquid biological sample through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable through said test result observation aperture, thereby to indicate the presence of said analyte in said liquid biological sample.

52. The test device according to claim **51**, wherein said backing material is transparent plastics material.

53. The test device according to claim **51**, wherein said dry porous carrier material is nitrocellulose.

54. The test device according to claim **53**, wherein said nitrocellulose has a pore size of at least one micron.

55. The test device according to claim **51**, wherein said labelled reagent has been applied to said dry porous carrier as a surface layer.

56. The test device according to claim **55**, wherein said dry porous carrier has been pre-treated with said material comprising sugar in the region to which said labelled reagent is applied.

57. The test device according to claim **51**, wherein said analyte is hCG.

58. The test device according to claim **51**, wherein said analyte is LH.

59. The test device according to claim **51**, wherein said material further comprises a protein.

60. The test device according to claim **59** wherein said protein is bovine serum albumin.

61. The test device according to claim **51**, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

62. The test device according to claim **51**, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

63. The test device according to claim **51**, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

64. The test device according to claim **63**, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

* * * * *

EXHIBIT D

US006187598B1

(12) **United States Patent**
May et al.

(10) **Patent No.: US 6,187,598 B1**
(45) **Date of Patent: *Feb. 13, 2001**

(54) **CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS**

(75) Inventors: **Keith May**, Bedfordshire; **Michael Evans Prior**, Northamptonshire; **Ian Richards**, Bedford, all of (GB)

(73) Assignee: **Conopco Inc.**, New York, NY (US)

(*) Notice: Under 35 U.S.C. 154(b), the term of this patent shall be extended for 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/487,319**

(22) Filed: **Jun. 7, 1995**

Related U.S. Application Data

(62) Division of application No. 08/102,313, filed on Jul. 15, 1993, now Pat. No. 5,622,871, which is a continuation of application No. 07/876,449, filed on Apr. 30, 1992, now abandoned, which is a division of application No. 07/795,266, filed on Nov. 19, 1991, now abandoned, which is a continuation of application No. 07/294,146, filed as application No. PCT/GB88/00322 on Apr. 26, 1998, now abandoned.

(30) **Foreign Application Priority Data**

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Oct. 30, 1988 (GB) 8725457

(51) **Int. Cl.**[7] **G01N 33/558**

(52) **U.S. Cl.** **436/514**; 422/55; 422/56; 422/57; 422/58; 435/7.92; 435/287.1; 435/287.2; 435/805; 435/810; 435/962; 435/969; 435/970; 436/518; 436/531; 436/533; 436/534; 436/525; 436/810; 436/814; 436/817; 436/818

(58) **Field of Search** 422/55, 56, 57, 422/58; 427/2.13; 435/7.92, 962, 969, 970, 287.1, 287.2, 805, 810; 436/514, 518, 531, 533, 534, 525, 810, 814, 817, 818

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Primary Examiner—Christopher L. Chin
(74) *Attorney, Agent, or Firm*—Pillsbury Madison & Sutro LLP

(57) **ABSTRACT**

An analytical test device useful for example in pregnancy testing, comprises a hollow casing **(500)** constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier **(510)** which communicates indirectly with the exterior of the casing via a bibulous sample receiving member **(506)** which protrudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating means, such as an aperture **(508)** in the casing, enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

38 Claims, 5 Drawing Sheets

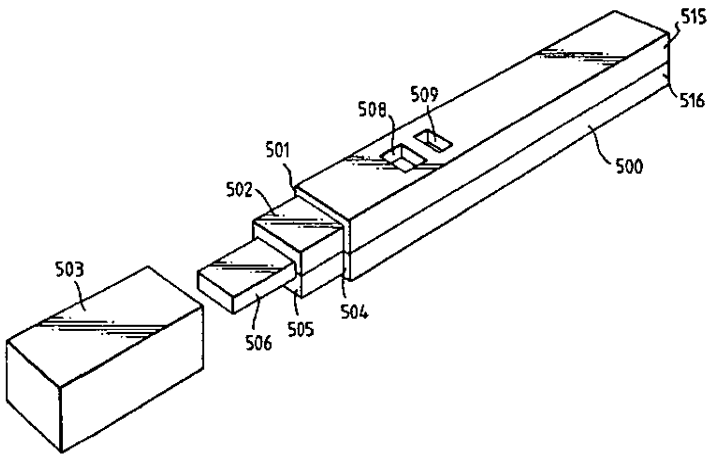

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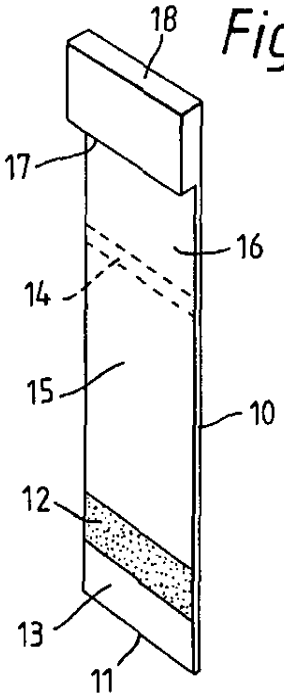
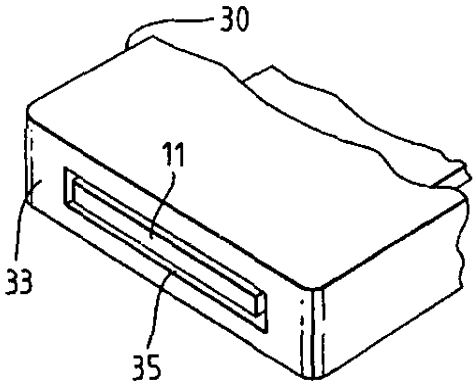
Fig.1.





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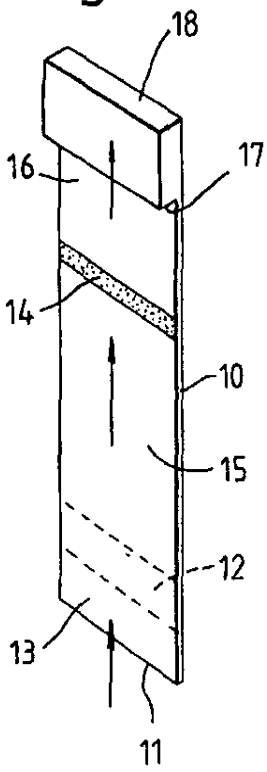

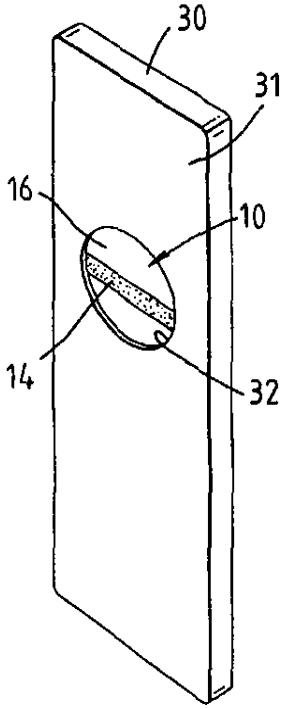

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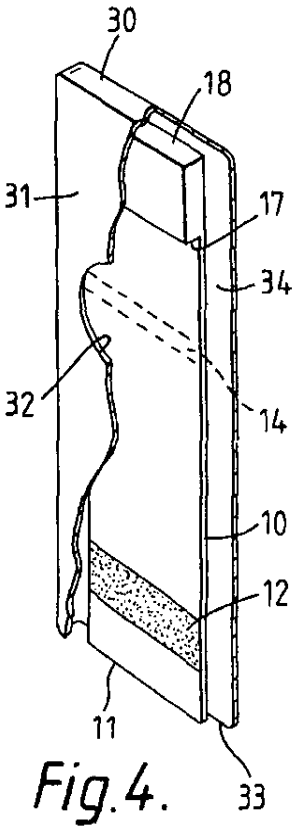

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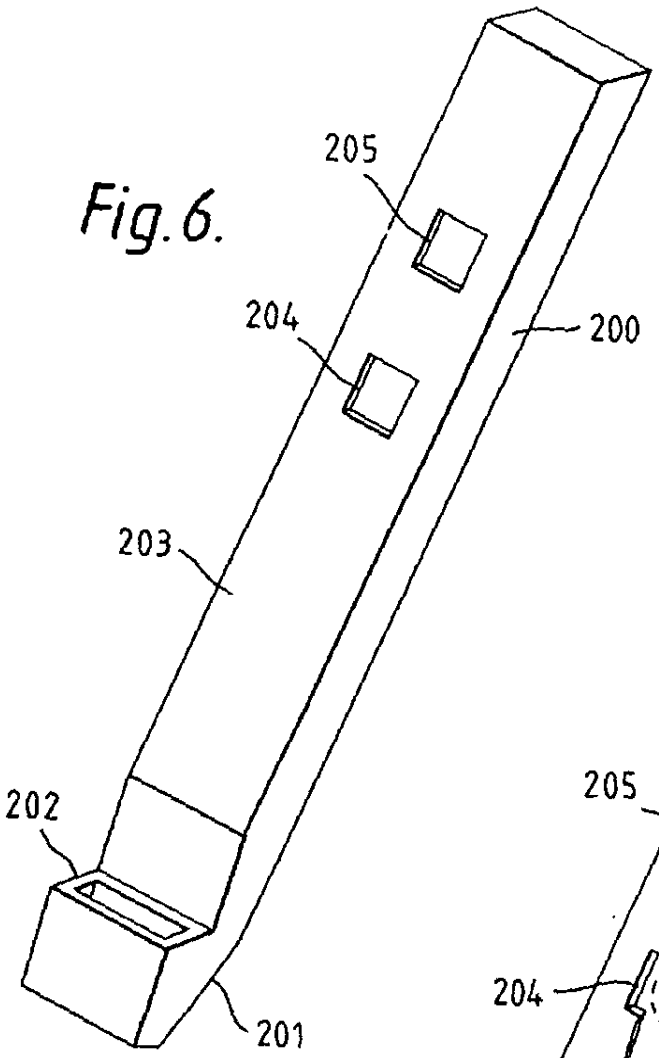
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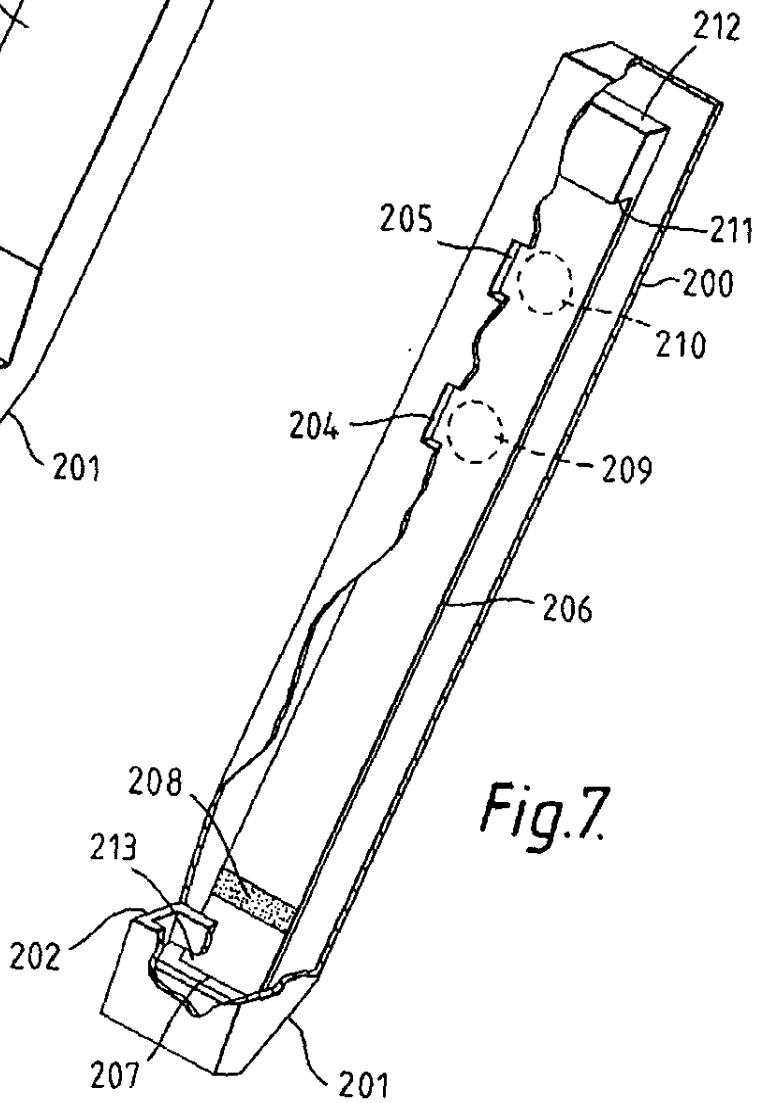
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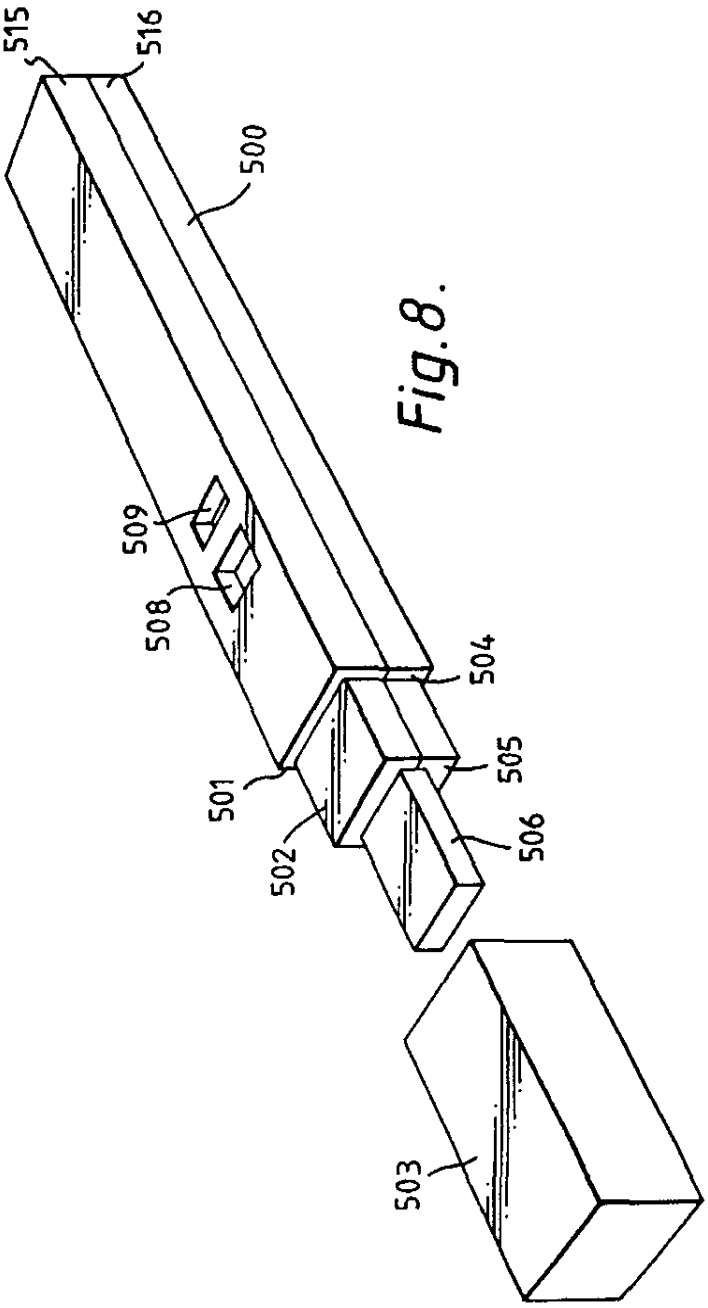
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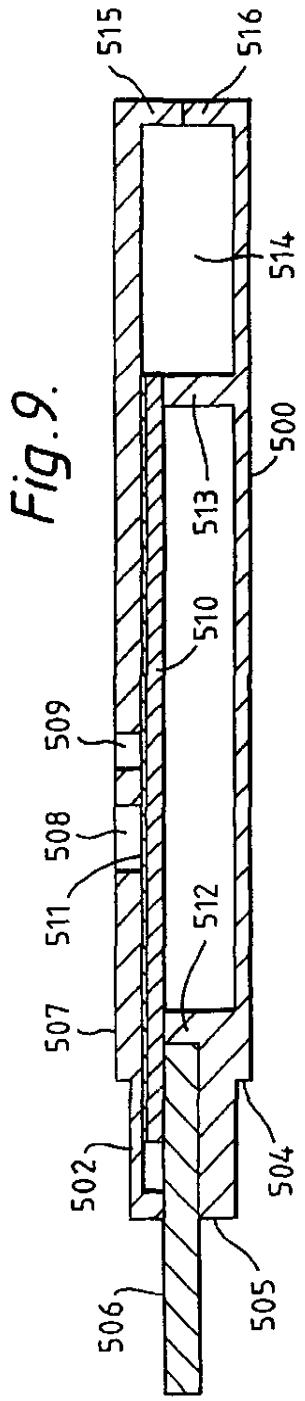
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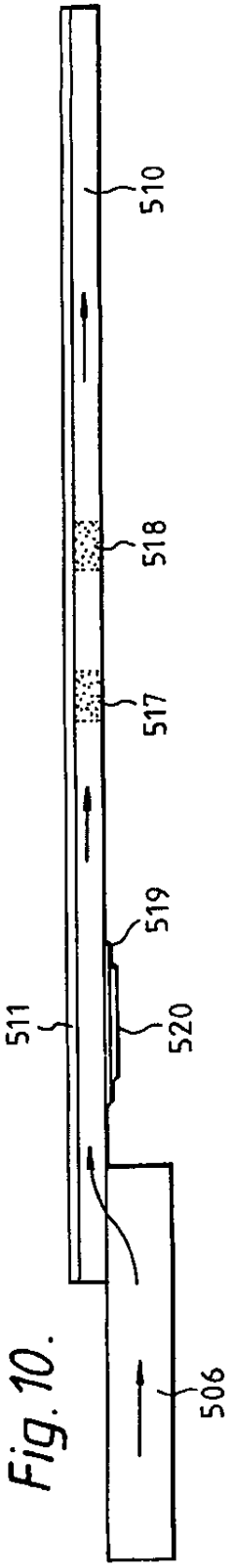
Fig. 9.



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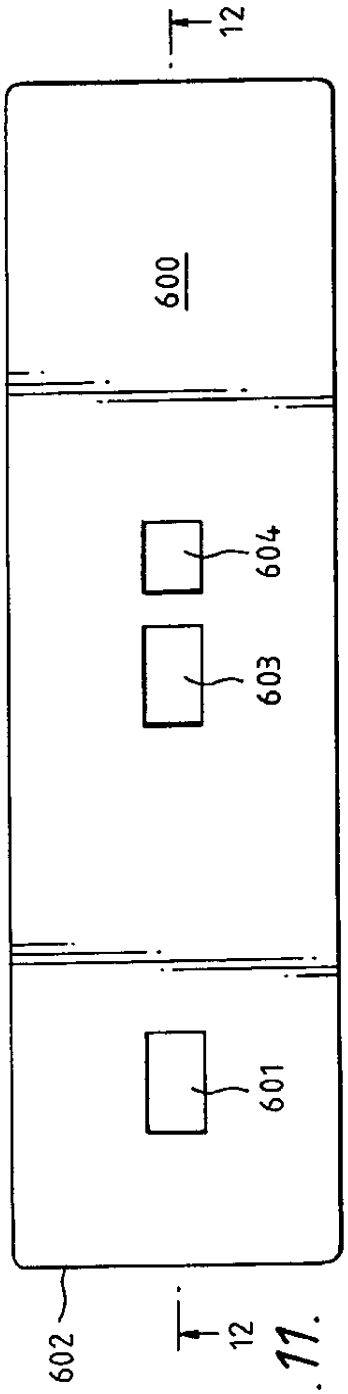

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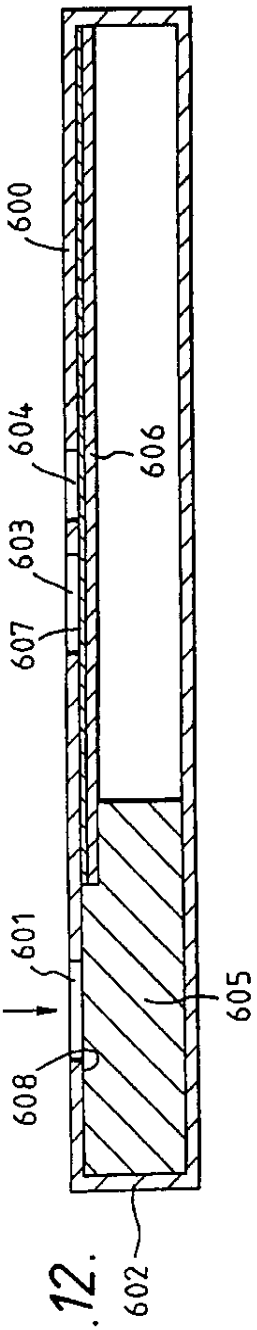

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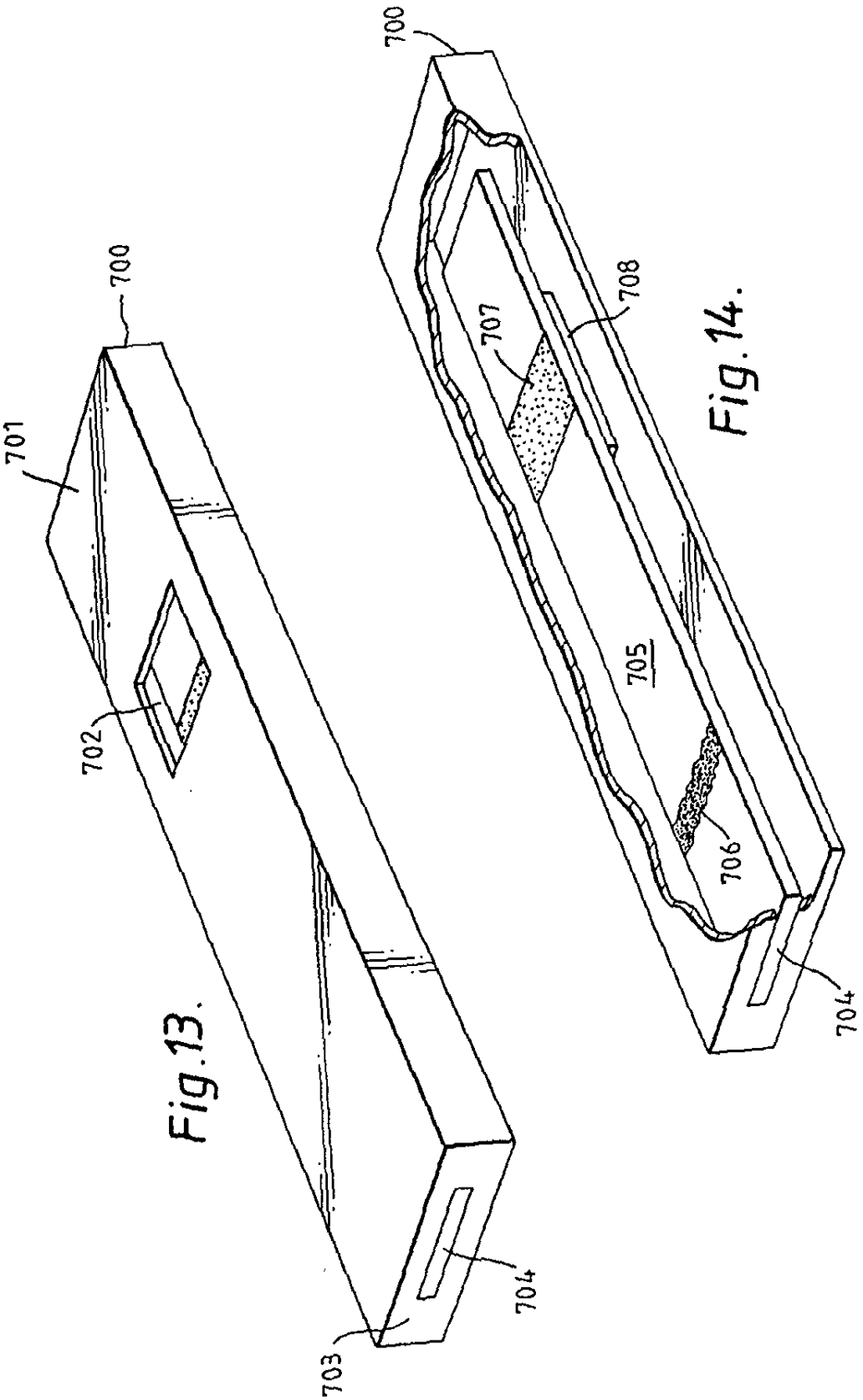

Fig. 13.

Fig. 14.

CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS

This is a division of application Ser. No. 08/102,313, filed Jul. 15, 1993, now U.S. Pat. No. 5,622,871, which was a continuation of 07/876,449 filled Apr. 30, 1992, now abandoned; which was a division of application Ser. No. 07/795,266, filed Nov. 19, 1991, now abandoned; which was a continuation of application Ser. No. 07/294,146, filed Feb. 27, 1989, now abandoned, which was a U.S. national phase of PCT/GB88/00322, filed Apr. 26, 1988. Priority is claimed in this case from each of the above-listed applications.

BACKGROUND OF THE INVENTION

1. Field of the Invention

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

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 which is rapid and which requires the minimum degree of skill and involvement from the user.

2. Description of the Related Art

The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream 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. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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 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 for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which commicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is in the moist state.

The invention also provides an analytical method in which a device as set forth in the proceeding paragraph is contacted with an aqueous liquid sample suspected of containing the analyte, such that the sample permeates by capillary action through the porous solid phase material via the first zone into the second zone and the labelled reagent migrates therewith from the first zone to the second zone, the presence-of analyte in the sample being determined by observing the extent (if any) to which the labelled reagent becomes bound in the second zone.

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 together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

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 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 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 solid phase material into the second zone and bind with the

immobolized 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 second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it 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 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 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.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols 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. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. 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 size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the

porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable 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 material to be observed from outside the housing and which further zone incorporates 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 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. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but this is not preferred.

An important embodiment of the invention is 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 and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone 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 two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and 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 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 multi-directional (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 flouride, 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 porous solid phase material.

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 antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a 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 second 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 horseradish 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 porous solid phase material 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 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 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 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, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away from the detection zone by this continuing flow. If desired, an absorbent "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 to allow any unbound conjugage 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.

The presence or intensity of the signal from the label which becomes bound in the second zone can 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.

The immobilised specific binding reagent in the second 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 antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and 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 incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the 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 antibody 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 ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with

a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is 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 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 thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose 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 such that in the untreated material, aqueous liquid migrates at a rate of 1 cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the zones, 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, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second 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 present in the migrating sample.

The reagents can be applied to the 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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **2** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **3** is a perspective view of a device utilizing a porous strip of the type illustrated in FIGS. **1** and **2**;

FIG. **4** is a perspective view, partially broken away, revealing a porous strip within the device of FIG. **3**;

FIG. **5** is an enlarged view of one end of the device of FIG. **3**;

FIG. **6** is a perspective view of another test device according to the invention;

FIG. **7** is a perspective view, similar to FIG. **6**, but partially cut away to reveal the porous test strip contained within the body of the device;

FIG. **8** is an exploded perspective view of an assay device in accordance with the present invention;

FIG. **9** is a cross-sectional side elevation of the device shown in FIG. **8**;

FIG. **10** is an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**;

FIG. **11** is a plan view of another embodiment of the invention;

FIG. **12** is a schematic cross-sectional view taken along line **12**—**12** in FIG. **11**;

FIG. **13** is a perspective view of yet another embodiment of the invention; and

FIG. **14** is a partially cut away view of the device of FIG. **13**.

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

EMBODIMENT 1

FIGS. **1** and **2** represent a typical strip of porous solid phase material for use in an assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to FIG. **1**, the assay test strip **10** is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end **11** of strip **10** is a narrow band or zone **12** extending across the entire width of the strip. A small region **13** of strip **10** lies vertically below zone **12**. Above zone **12** is a second zone **14** lying a discrete distance up strip **10** and similarly extending the entire width of the strip. The region **15** of strip **10** between zones **12** and **14** can be of any height as long as the two zones are separate. A further region **16** of the strip extends above zone **14**, and at the top **17** of the strip is a porous pad **18** firmly linked to strip **10** such that pad **18** can act as a "sink" for any liquid sample which may be rising by capillary action through strip **10**.

Zone **12** is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone **14**, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

FIG. **2** illustrates what happens when the assay strip is used in an analytical procedure. The lower end **11** of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad **18**. In so doing, the sample traverses zone **12** and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone **14**, the labelled antibody can bind to any analyte present in the sample. On reaching zone **14**, any analyte

molecule should become bound to the second antibody, so immobilising the labelled "sandwich" so produced. If a significant concentration of the analyte to be determined is present in the liquid sample, in a short period of time a distinct accumulation of the visible label should occur in zone **14**.

As an example of an analysis to which this embodiment can be applied, the analyte can be hCG, the reagents in zones **12** and **14** can be monoclonal antibodies to hCG which can participate in a "sandwich" reaction with hCG, and the label can be a particulate dye, a gold sol or coloured latex particles.

Although described above in relation to a "sandwich" reaction, it will be readily apparent to the skilled reader that this can be modified to a "competition" reaction format if desired, the labelled reagent in zone **12** being the analyte or an analogue of the analyte.

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 Streptoccus, 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 (estrane-3-glucuronide) and P-3-G (progesterone-3-glucuronide).

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 A_1 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. By way of example, a dual analyte test for apolipoproteins A_1 and B may be prepared by depositing, as two spacially distinct zones, antibody specific for apolipoprotein A_1 throughout a first zone and depositing a second antibody specific for apolipoprotein B, throughout the second zone of a porous carrier matrix. Following the application of both antibodies to each of their respective zones via a suitable application procedure (e.g. ink-jet printing, metered pump and pen, or airbrush), the remainder of the porous material should be treated with a reagent, e.g. bovine serum albumin, polyvinyl alcohol, or ethanolamine, to block any remaining binding sites elsewhere. A third and fourth reagent, bearing a label, may then be dispensed onto the dry carrier in one or more zones near to one end of the strip, the strip being allowed to dry between applications of the two reagents to the same zone. Reagent **3** and Reagent **4** may comprise conjugates of anti-apolipoprotein A_1 antibody and anti-apolipoprotein B antibody respectively. Both of these conjugates will become mobile in and on the carrier when in the moist state. Reagents **3** and **4** can migrate with the solvent flow when an aqueous sample is applied to the first end of the carrier strip. While migrating towards the two zones further along the strip, reagent 3 may bind any apolipoprotein A_1 present in the sample and reagent 4 may bind any apolipoprotein B present in the sample. On reaching the first second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein A_1 molecules should become bound to the second antibody, immobilising the labelled 'sandwich' so produced. No labelled apoliprotein B molecules will bind to this first zone. On reaching the second second-antibody zone (anti-apolipoprotein B antibody zone) any apolipoprotein B molecules should become bound to the second antibody (solid-phase antibody), immobilising the labelled 'sandwich' so produced. No labelled apolipoprotein A_1 molecules will bind to the second zone. An accumulation of each of the direct label may occur at both or either zones to a lesser or greater extent resulting in a visible signal at either or both of the solid phase antibody zones. Excess unbound conjugate (of both reagent 3 and reagent 4) can pass freely over the two antibody zones and will be washed into the distal end of the strip.

The development of a quantifiable colour in both of the second-antibody zones may be assessed with an appropriate form of instrumentation, yielding a ratio of colour density between the two sites.

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 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 1 mm 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. Conjugates of label, e.g. a dye sol, and each immunochemically-reactive component specific for each bacterial group may then be dispensed either onto a single zone at the bottom end of the strip, proximal to the sample application zone, or as a series of separate zones.

FIGS. **3**, **4** and **5** of the accompanying drawings depict a complete device utilising a porous strip as just described above. FIG. **3** represents the complete device viewed from the front, FIG. **4** shows the same device partially cut away to reveal the details of the strip inside, and FIG. **5** shows the underside of the device.

Referring to FIG. **3**, the device comprises a flat rectangular body **30** the front face **31** of which is perforated by a circular hole or window **32** which reveals the porous test strip **10** within the body. The region of the test strip **10** visible through the window **32** incorporates a narrow horizontal zone **14**.

Referring to FIG. **4**, the device comprises a dry rectangular test strip **10** made from porous material which extends from the bottom end **33** of the body **30** within the body between the front **31** and back **34** of the body. Near the bottom end **11** of the strip **10** is a horizontal zone **12** bearing a labelled specific binding reagent for an analyte, the binding reagent being mobile in the test strip in the moist state. Further up the test strip is the narrow horizontal zone **14** which is visible through the window **32**. At the top **17** of the test strip **10** is a porous 'sink' **18** which can absorb any liquid sample that has permeated upwards through the strip.

Referring to FIG. **5**, the bottom edge **35** of the body **30** incorporates a lateral aperture in which the bottom end **11** of the strip lies.

In operation, the bottom end **33** of the body **30** is immersed in a liquid sample (eg urine) so that the liquid sample can be absorbed by the bottom end **11** of the test strip **20** and rise by capillary action to the top **17** of the test strip and into the sink **18**. In so doing, the liquid sample progresses via zone **12** to zone **14**. Specific binding reactions as described above occur, and the test result is visible to the user through the window **32**.

EMBODIMENT 2

FIGS. **6** and **7** of the accompanying drawings illustrate another test device according to the invention. FIG. **6** illustrates the complete device viewed from the front, and FIG. **7** depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to FIG. **6**, the device comprises an elongate body **200** terminating at its lower end **201** in a small integral receptacle **202** which can hold a predetermined volume of a liquid sample, eg urine. The front face **203** of the body **200** incorporates two square small square apertures or windows **204** and **205** located one above the other.

Referring to FIG. **7**, the elongate portion of the body **200** is hollow and incorporates a test strip **206** running almost the full height of the body. This test strip is of similar construction to those described under Embodiment **1**, and incorporates near its lower end **207** a horizontal zone **208** bearing a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones **209** and **210** adjacent to the windows **204** and **205** and visible therethrough. The strip terminates at its top end **211** in a porous sink **212**. At the bottom end **201** of the device, the receptacle **202** communicates with the hollow body via a lateral aperture **213**.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacle **202**. From the receptacle **202** the liquid sample rises by capillary action through the test strip **206** and conveys the labelled reagent from zone **208** to the two circular zones **209** and **210**. A series of specific binding reactions as described in relation to Embodiment 1 above occur. In this embodiment the second circular zone **210** can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone **209**. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone **210** can contain immobilized HCG which will bind a labelled antibody which is carried upwards from zone **208** by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone **209** by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone **208**, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

EMBODIMENT 3

FIG. **8** of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and FIG. **9** represents a cross-sectional side elevation of the device shown in FIG. **8**.

Referring to FIG. **8**, the device comprises a housing of casing **500** of elongate rectangular form having at one end **501** a portion **502** of reduced cross-sectional area. A cap **503** can be fitted onto portion **502** and can abut against the shoulder **504** at end **501** of the housing. Cap **503** is shown separated from housing **500**. Extending beyond end **505** of portion **502** is a porous member **506**. When cap **503** is fitted onto portion **502** of the housing, it covers porous member **506**. Upper face **507** of housing **500** incorporates two apertures **508** and **509**.

Referring to FIG. **9**, it can be seen that housing **500** is of hollow construction. Porous member **506** extends into housing **500** and contacts a strip of porous carrier material **510**. Porous member **506** and strip **510** overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member **506** can permeate member **506** and progress into strip **510**. Strip **510** extends further into housing **500**. Strip **510** is "backed" by a supporting strip **511** formed of transparent moisture-impermeable plastics material. Strip **510** extends beyond apertures **508** and **509**. Means are provided within housing **500** by webbs **512** and **513** to hold strip **510** firmly in place. In this respect, the internal 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, and porous member **506** is firmly retained in the housing and adequate fluid permeable contact is maintained between member **506** and strip **510**. The transparent backing strip **511** lies between strip **510** and apertures **508** and **509** and can act as a seal against ingress of moisture from outside the housing **500** via these apertures. If desired, the residual space **514** within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip **510** in the dry state during storage. The reagent-containing zones in strip **510** are not depicted in FIG. **8**, but the first zone containing the labelled reagent which is mobile when the strip is moistened will lie in the region between the porous member **506** and aperture **508**. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture **508** in order that when the device has been used in an assay, the result can be observed through aperture **508**. Aperture **509** provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap **503** is removed from the holder and member **506** is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member **506** to the liquid sample for a time sufficient to ensure that member **506** is saturated with the sample, the cap **503** 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 **510** to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures **508** and **509** and can ascertain whether the assay has been completed by observing the control zone through aperture **509**, and can ascertain the result of the assay by observing the second zone through aperture **508**.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing **500** being moulded in two parts (e.g. upper and lower halves **515** and **516**) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member and test strip have been placed within one of the halves and then sand-

wiched between the two halves. The act of forming this sandwich construction can be used to "crimp" the porous member and test strip together to ensure adequate contact between them. Cap **503** can be moulded as a separate complete item. If desired, apertures **508** and **509** 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 **505** of housing **500** and the protruding porous member **506**, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member **506**. Member **506** 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 as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with FIGS. **8** and **9** 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 then (after optionally replacing the cap) can observe the test result through aperture **508** within a matter of a few minutes.

Although described with particular reference to 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 **509** 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 from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member **506**.

FIG. **10** of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**.

The porous receiving member **506** is linked to the porous test strip **510**, backed by the transparent plastics sheet **511**, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone **517** incorporates the immobilised specific binding reagent, and control zone **518** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip **511** and adjacent the porous receiving member **506**, carries a glaze **519** on which is deposited a layer **520** of labelled specific binding reagent. The thickness of these two layers as depicted in FIG. **10** is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member **506** can flow therefrom along the length of strip **510** and in so doing, will dissolve glaze **519** and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone **517**.

EMBODIMENT 4

FIGS. **11** and **12** illustrate another embodiment of the invention which is seen in plan view in FIG. **11** and in cross-section in FIG. **11**, the cross-section being an elevation on the line **12**—**12** seen in FIG. **12**.

Referring to FIG. **11**, the test device comprises a flat rectangular casing **600** incorporating a centrally disposed rectangular aperture **601**, adjacent the left hand end **602**, and two further apertures **603** and **604** near the mid point of the device and arranged such that apertures **601**, **603** and **604** lie on the central longitudinal axis of the device corresponding to line A. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **12**, the device is hollow and incorporates within it a porous sample receiving member adjacent end **602** of casing **600** and lying directly beneath aperture **601**. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip **606** backed by a transparent plastics sheet **607** is also contained within casing **600**, and extends from the porous receiving member **602**, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet **607** is in firm contact with the upper inner surface **608** of casing **600**, and provides a seal against apertures **603** and **604** to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip **606** will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures **603** and **604**, in a manner analogous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture **601**, e.g. by means of a syringe, to saturate porous receiving member **605**. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures **603** and **604**.

EMBODIMENT 5

A yet further embodiment of the invention is illustrated in FIGS. **13** and **14** of the accompanying drawings. FIG. **13** shows a device comprising a rectangular casing **700** having in its upper surface **701** a rectangular aperture **702**. One end wall **703** of the device **703** incorporates an aperture **704** through which a porous test element communicates with the exterior of the device. Aperture **702** is situated in surface **701** at a point relatively remote from the end **703** containing the aperture **704**.

FIG. **14** shows a partially cut-away view of the device in FIG. **13**. The hollow device incorporates a porous test strip **705**, running almost the entire length of casing **700** from aperture **704**. Test strip **705** incorporates a first zone **706** containing a labelled specific binding reagent and a further zone **707**, remote from aperture **704**, incorporating an immobilised specific reagent. Zone **706** lies directly beneath aperture **702** is therefore observable from outside casing. Beneath strip **705** and adjacent zone **707**, is a crushable element **708** containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone **707**, if labelled reagent from **706** has become bound in zone **707** following use of the device. Release of the reagents from member **708** can be effected by applying pressure to the outside of the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end **703** of casing **700** into

a vessel containing the sample. The liquid sample will then permeate the length of test strip **705**, taking labelled reagent from zone **706** and passing through zone **707** where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the sample has permeated the test strip, reagent can be released from the crushable member **708** and the result of the test observed through aperture **702**.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45 mm (minutes)
Schleicher + Schuell nitrocellulose (unbacked)	3μ	3.40
	5μ	3.30
	8μ	3.00
	12μ	2.20
polyester-backed	8μ (nominal)	3.40
Whatman Nitrocellulose	5	19.20
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Imunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20 nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22μ filtered 0.1M K_2CO_3, and 20 mls is added to a clean glass beaker. 200 μl of anti-alpha hCG antibody, prepared in 2 mM borax buffer pH9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2 mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000 g, 30 minutes, and 4° C., with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20 mM Tris, 150 mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specification No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 liter of distilled water by mixing on a magnetic stirrer for 2–3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500 g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000 g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500 mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100 mls distilled water.

The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657 nm for Foron Blue, and 690 nm for Resolin Blue. The absorbance at lambda-max, for 1 cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoassays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the preformed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chloroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in imunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5 ml (12.5 mg solids) of suspension is diluted with 1 ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1 ml borate buffer, mixed with 300 μg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16–20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000 rpm, the supernatant is discarded and the pellet resuspended in 1.5 mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5 mg/ml BSA in phosphate buffered

saline pH 7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5 mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4° C. until used.

(A) Anti-hCG—Dye Sol Preparation

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction mixture is prepared which contains 100 μl antibody solution, 2 mls dye sol, 2 mls 0.1M phosphate buffer pH 5.8 and 15.9 mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4 mls of 150 mg/ml BSA in 5 mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000 g for 10 minutes, and the pellet resuspended in 10 mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LR—Dye Sol Preparation

Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8μ nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30° C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5 cm in length and 1 cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material, when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8μ nitrocellulose, 25 cm in length and 20 cm in width with zones of immobilised antibody at 5 cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6 cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled. antibody prepared in 1% sethacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5 cm in length and 1 cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% TRITON X305 (Trademark—obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1 mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of

estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA—Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of Z-3-G (2 nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10 nM) in dry DMF were equilibrated separately at 4° C. Using pre-cooled glassware E-3-G in DMF (1.25 ml) and TnB in DMF (0.25 ml) were added to a pre-cooled 5 ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10 nM) was prepared and an aliquot (0.25 ml) was cooled to 4° C. and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4° C. and a solution of BSA (1 mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5 ml) and stirred on a magnetic stirrer for 4 hours at 4° C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 SEPHADEX G-25 column, transferred to an amber glass storage bottle and stored at 4° C.

Preparation of BSA—E-3-G Dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850 rpm (1500 g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10 μg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5 mM, pH 7.4) to yield a final BSA concentration of 25 mg/ml. The reaction mixture was centrifuged at 4850 rpm (3000 g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH 5.8) buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA—E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to all combinations and subcombinations of the features of this description and the accompanying drawings.

What is claimed is:

1. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device comprising a test strip comprising a dry porous carrier comprising a test result zone, said test strip, in a dry unused state, comprises a labeled reagent capable of specifically binding with said analyte to form a first complex of said labeled reagent and said analyte, said labeled reagent comprising a particulate direct label, wherein said labeled reagent is dry on a portion of said test strip upstream from said test result zone prior to use and is released into mobile form by application of said liquid biological sample,

wherein said carrier comprises in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized

in said test result zone,

wherein migration of said applied liquid biological sample through said carrier conveys by capillarity said first complex to said test result zone of said carrier where at said binding means binds said first complex thereby to form a second complex, thereby to indicate the presence of said analyte in said liquid biological sample,

wherein mobility of said labeled reagent within said test strip is facilitated by at least one of 1) coating at least said portion of said test strip with, or 2) drying said labeled reagent onto said portion of said test strip in the presence of a material which is effective to reduce interaction between said portion of said test strip and said labeled reagent.

2. The test device according to claim **1**, wherein excess binding sites within said test strip are blocked.

3. The test device according to claim **2**, wherein said excess binding sites are blocked by polyvinyl alcohol.

4. The test device according to claim **2**, wherein said excess binding sites are blocked by a blocking protein.

5. The test device according to claim **4**, wherein said blocking protein is selected from the group consisting of bovine serum albumin and milk protein.

6. The test device according to claim **1**, wherein said label is selected from the group consisting of dye sols and metallic sols.

7. The test device according to claim **1**, wherein said labeled reagent comprises an anti-human chorionic gonadotropin (HCG) antibody.

8. The test device according to claim **1**, wherein said labeled reagent comprises an anti-lutenising horomone (LH) antibody.

9. The test device according to claim **1**, wherein said carrier material is nitrocellulose.

10. The test device according to claim **9**, wherein said nitrocellulose has a pore size greater than about 5 microns.

11. The test device according to claim **9**, wherein said nitrocellulose has a pore size of about 8–12 microns.

12. The test device according to claim **1**, further comprising an absorbent sink at a distal end of said carrier, said sink having sufficient absorptive capacity to absorb any labelled reagent not bound to said analyte washed out of said test zone.

13. The test device according to claim **1**, wherein said means for binding is impregnated throughout said carrier in said test zone.

14. The device according to claim **1**, wherein said means for binding binds directly with said first complex.

15. A test device according to claim **1** wherein said material comprises a sugar.

16. The test device according to claim 15 wherein said sugar is sucrose.

17. The test device according to claim 15 wherein said sugar is lactose.

18. The test device according to claim 1 wherein said material comprises a protein.

19. The test device according to claim 18 wherein said protein is bovine serum albumin.

20. The test device according to claim 18 wherein said protein is milk protein.

21. The test device according to claim 1 wherein said test strip is disposed within a hollow casing having a liquid biological sample application aperture and at least one observation aperture for permitting observation of said test result zone.

22. The test device as in claim 21, wherein said hollow casing further comprises a test result observation aperture defined through a wall of said casing, spacially separated from said sample application aperture.

23. The test device according to claim 21 further comprising (i) a control zone downstream from said test zone in said carrier wherein said control zone indicates said applied liquid biological sample has been conveyed by said capillarity beyond said test result zone, and (ii) a control zone observation aperture in said hollow casing to observe said control zone from outside said hollow casing.

24. The test device according to claim 23, wherein said control zone comprises a means for binding said labeled reagent which comprises specific binding means and is immobilized in said control zone.

25. An analytical method comprising:

a) contacting an analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device including:

a test strip comprising a dry porous carrier comprising a test result zone, said test strip, in a dry unused state, comprises a labeled reagent capable of specifically binding with said analyte to form a first complex of said labeled reagent and said analyte, said labeled reagent comprising a particulate direct label, wherein said labeled reagent is dry on a portion of said test strip upstream from said test result zone prior to use and is released into mobile form by said contacted liquid biological sample,

wherein said carrier comprises in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone,

wherein migration of said applied liquid biological sample through said carrier conveys by capillarity said labeled reagent and said analyte to said test result zone of said carrier whereat said binding means binds said first complex thereby to form a second complex, thereby to indicate the presence of said analyte in said liquid biological sample,

wherein mobility of said labeled reagent within said test strip is facilitated by at least one of 1) coating at least said portion of said test strip with, or 2) drying said labeled reagent onto said portion of, said test strip in the presence of a material which is effective to reduce interaction between said portion of said test strip and said labeled reagent;

b) allowing said contacted liquid biological sample to be conveyed by said capillary action through said dry porous carrier into said test zone and said labeled reagent being conveyed therewith; and

c) detecting the presence of said analyte in said liquid biological sample by observing the presence of any labeled reagent-analyte complex bound in said test result zone.

26. The method according to claim 25, wherein excess binding sites within said test strip are blocked.

27. The method according to claim 26, wherein said excess binding sites is effected by polyvinyl alcohol.

28. The method according to claim 26, wherein said excess binding sites are blocked by a blocking protein.

29. The method according to claim 28, wherein said blocking protein is selected from the group consisting of bovine serum albumin and milk protein.

30. The method according to claim 25 wherein said material comprises a sugar.

31. The method according to claim 30 wherein said sugar is sucrose.

32. The method according to claim 30 wherein said sugar is lactose.

33. The method according to claim 25 wherein said material comprises a protein.

34. The method according to claim 33 wherein said protein is bovine serum albumin.

35. The method according to claim 33 wherein said protein is milk protein.

36. The method according to claim 25 wherein said test strip is disposed within a hollow casing having a liquid biological sample application aperture and at least one observation aperture for permitting observation of said test result zone.

37. The method according to claim 36, wherein said contacting step comprises contacting a device in which said carrier is a strip of dry porous material, and has a control zone downstream from said test result zone, for indicating to a user that the liquid biological sample has been conveyed by said capillarity beyond said test result zone; and said hollow casing has a second observation aperture through which said control zone is visible and wherein said liquid biological sample is a urine sample whereby urine is conveyed by said capillarity through said carrier to said test result zone and to said control zone, and said test result is obtained by observing the extent to which said labeled reagent-analyte complex becomes bound in said test result zone.

38. The method according to claim 37, wherein said control zone contains a means for binding said labelled reagent which comprises specific binding means and is immobilized in said control zone.

* * * * *

EXHIBIT E

United States Patent [19]
May et al.

[11] Patent Number: 5,602,040
[45] Date of Patent: *Feb. 11, 1997

[54] ASSAYS

[75] Inventors: **Keith May**, Bedfordshire; **Michael E. Prior**, Northamptonshire; **Ian Richards**, Bedford, all of England

[73] Assignee: **Unilever Patent Holdings B.V.**, Netherlands

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,599,721.

[21] Appl. No.: **241,675**

[22] Filed: **May 12, 1994**

Related U.S. Application Data

[60] Continuation of Ser. No. 876,448, Apr. 30, 1992, abandoned, which is a division of Ser. No. 795,266, Nov. 19, 1991, abandoned, which is a continuation of Ser. No. 294,146, filed as PCT/GB88/00322, Apr. 26, 1988, abandoned.

[30] **Foreign Application Priority Data**

Apr. 27, 1987 [GB] England 8709873
Oct. 30, 1987 [GB] United Kingdom 8725457

[51] **Int. Cl.**[6] **G01N 33/558**

[52] **U.S. Cl.** **436/514**; 436/501; 436/518; 436/523; 436/524; 436/525; 436/530; 436/541; 436/810; 436/814; 436/817; 436/818; 436/906; 435/962; 435/970; 435/975; 427/2.13; 422/60

[58] **Field of Search** 422/56–58, 60; 436/501, 530, 514, 810, 814, 515, 518, 523, 524, 541, 817, 818, 906; 435/7.92–7.95, 970, 810, 962, 975; 427/2, 2.11, 2.13

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Primary Examiner—Carol A. Spiegel
Attorney, Agent, or Firm—Cushman Darby & Cushman, LLP

[57] **ABSTRACT**

An analytical test device useful for example in pregnancy testing, includes a hollow casing (**500**) constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier (**510**) which communicates indirectly with the exterior of the casing via a bibulous sample receiving member (**506**) which protrudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, wherein the mobility is facilitated by a material comprising sugar, in a amount effective to reduce interaction between the test strip and the labelled reagent, and in a second zone spatially distinct from the first zone unlabeled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilized on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating an aperture (**508**) in the casing, enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

34 Claims, 5 Drawing Sheets

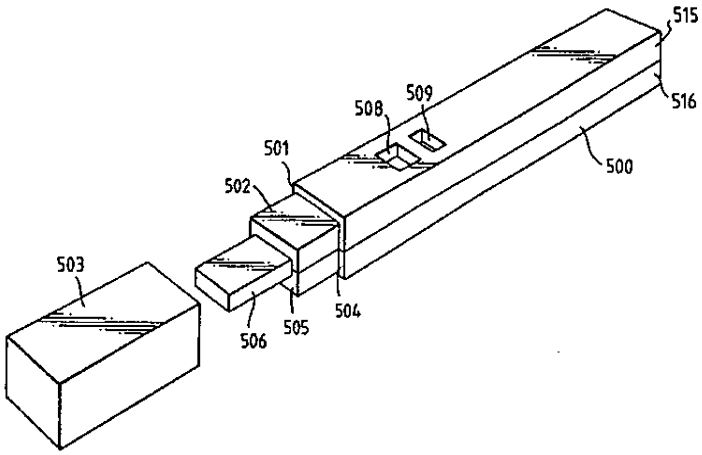

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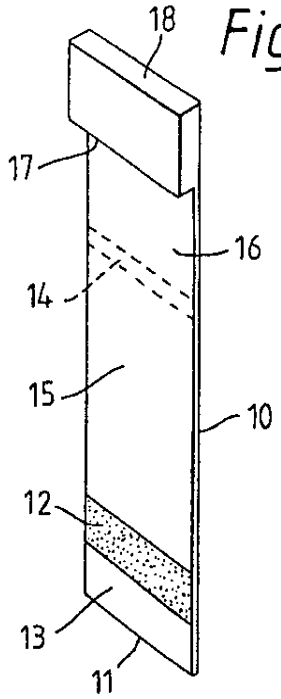
Fig.1.



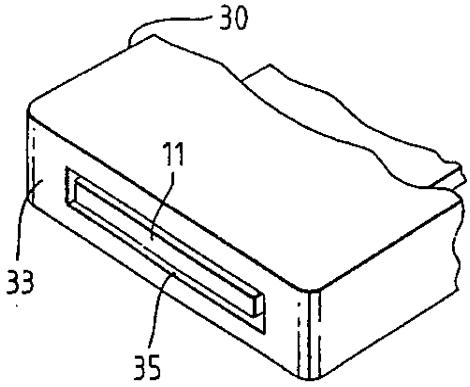


Fig.5.

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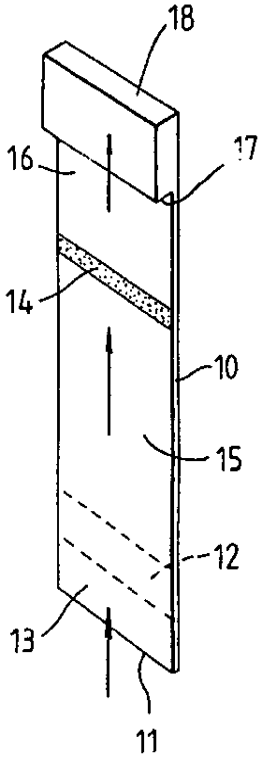

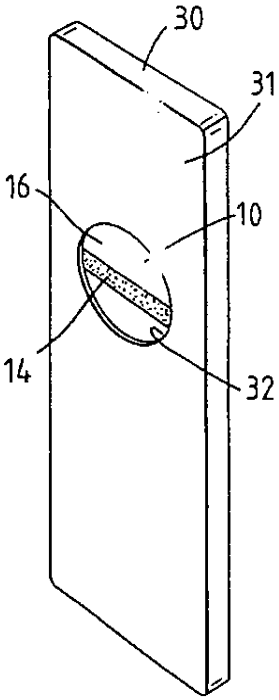

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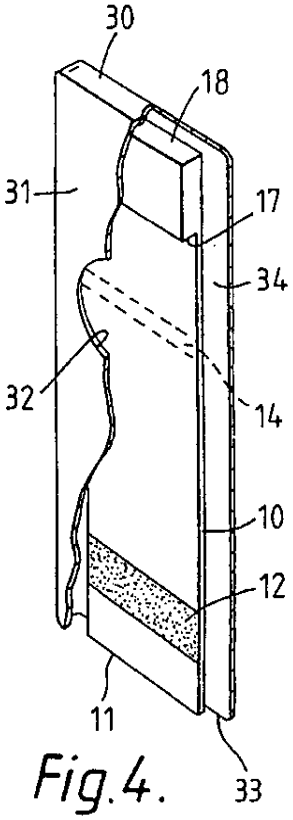
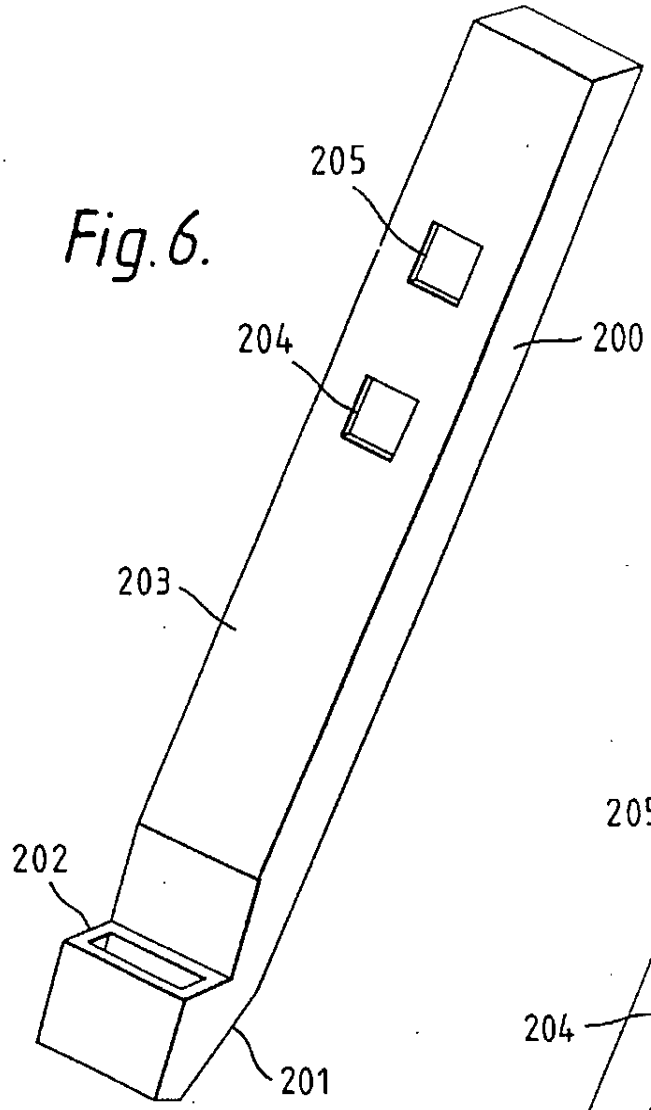


Fig.4.

Fig.6.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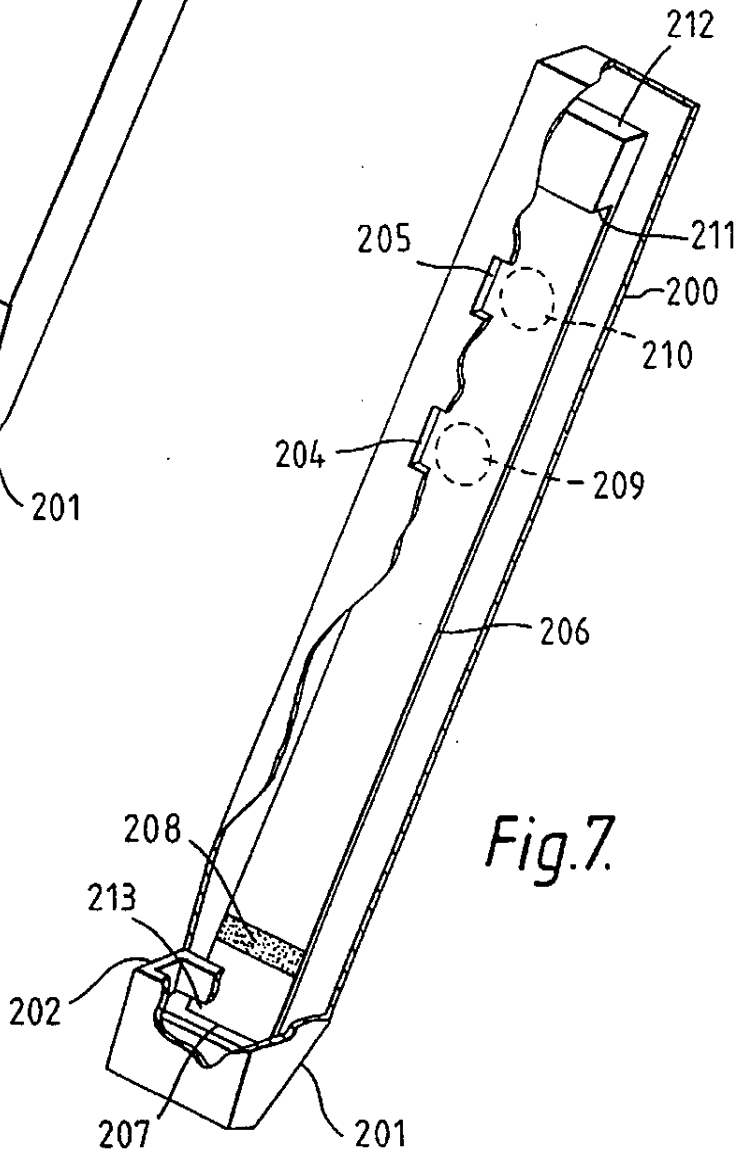
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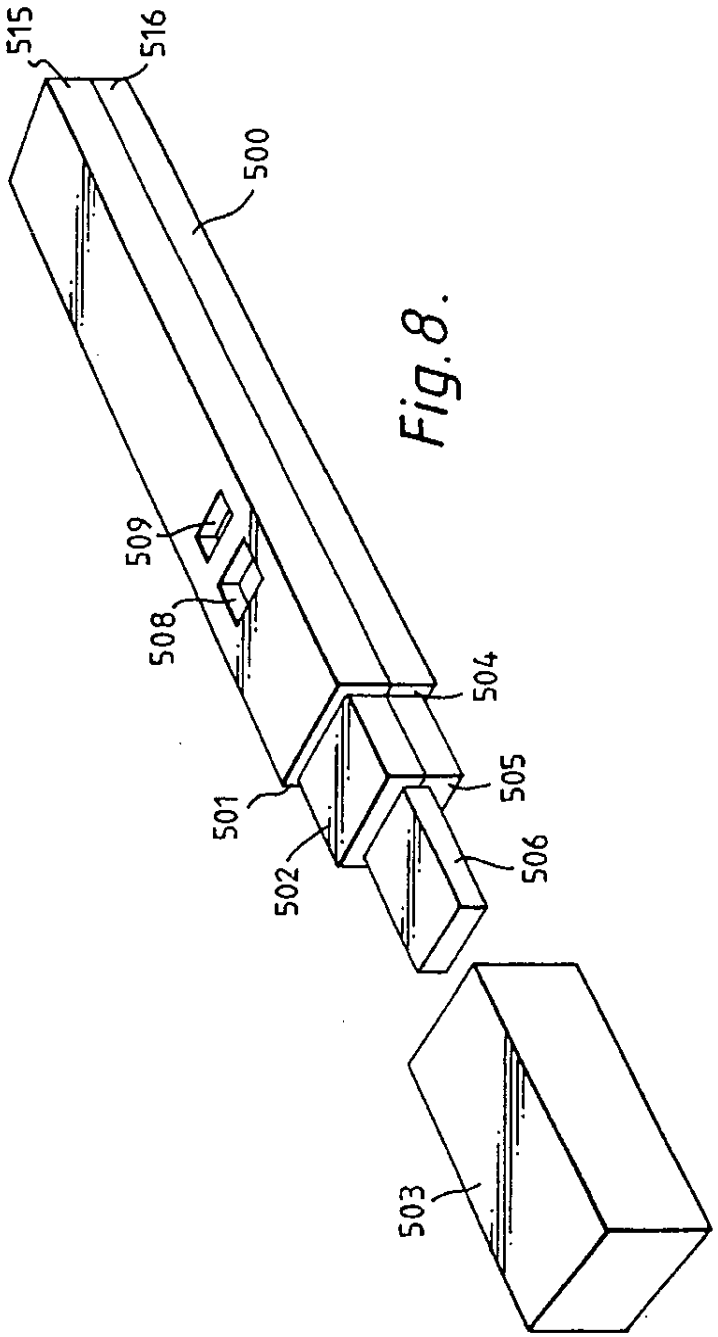
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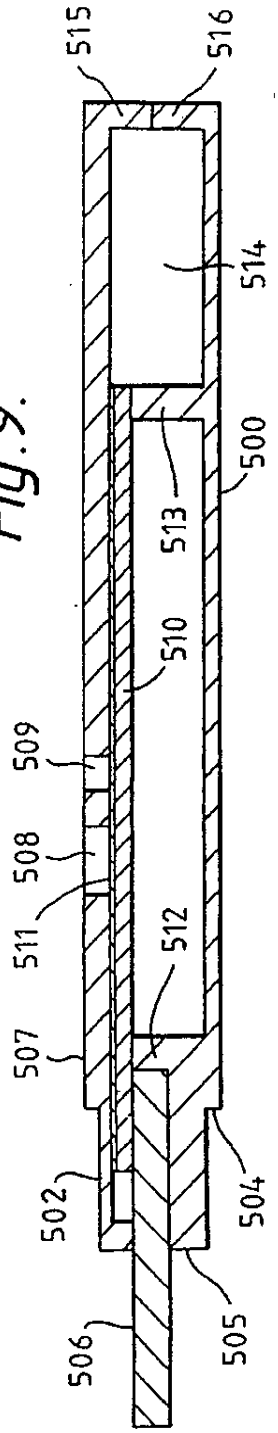
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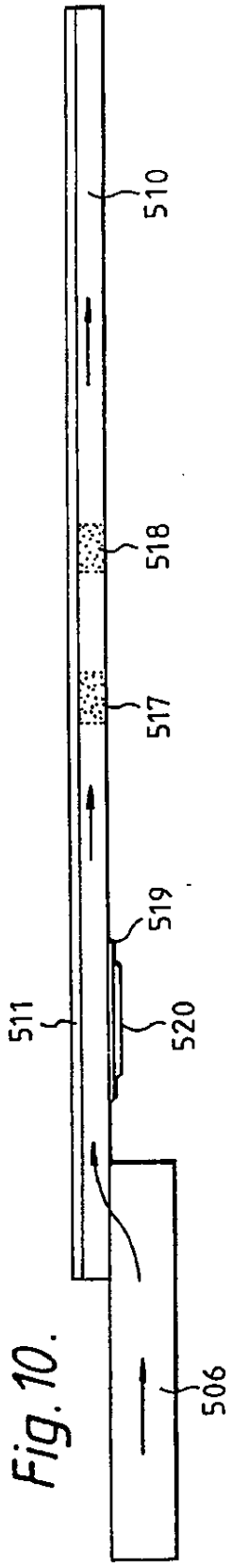
Fig. 9.



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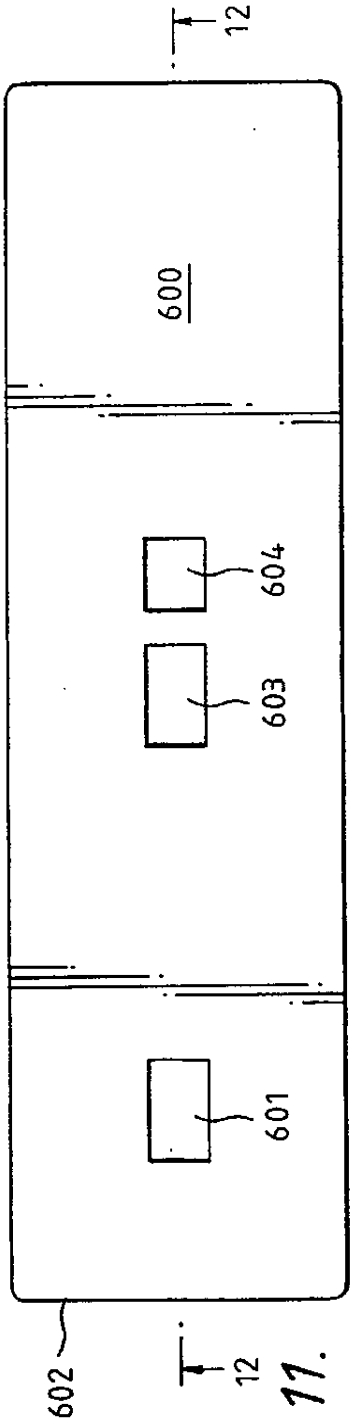

Fig. 11.

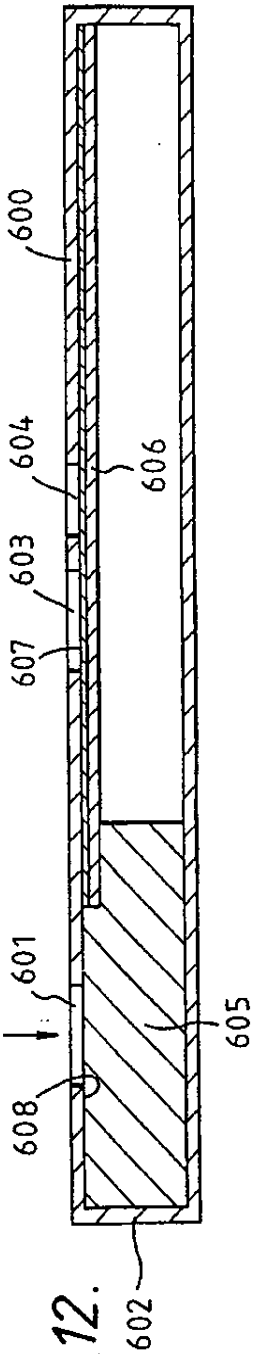

Fig. 12.

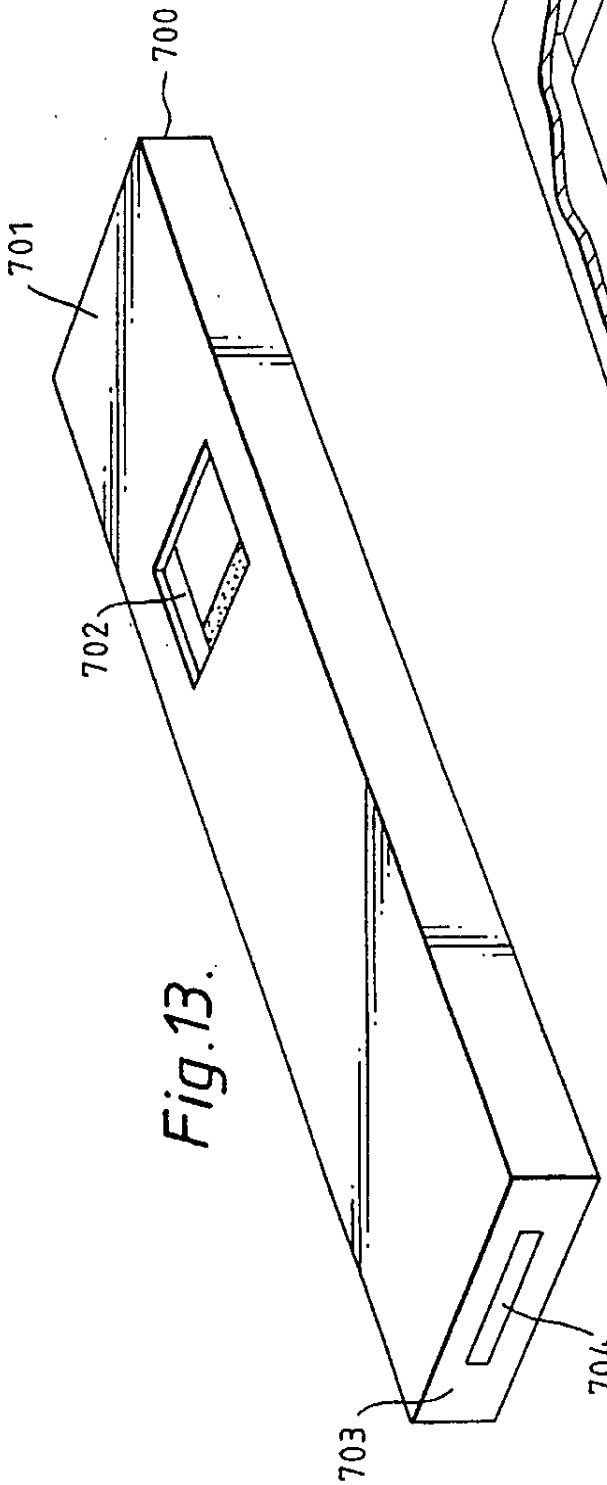

Fig. 13.

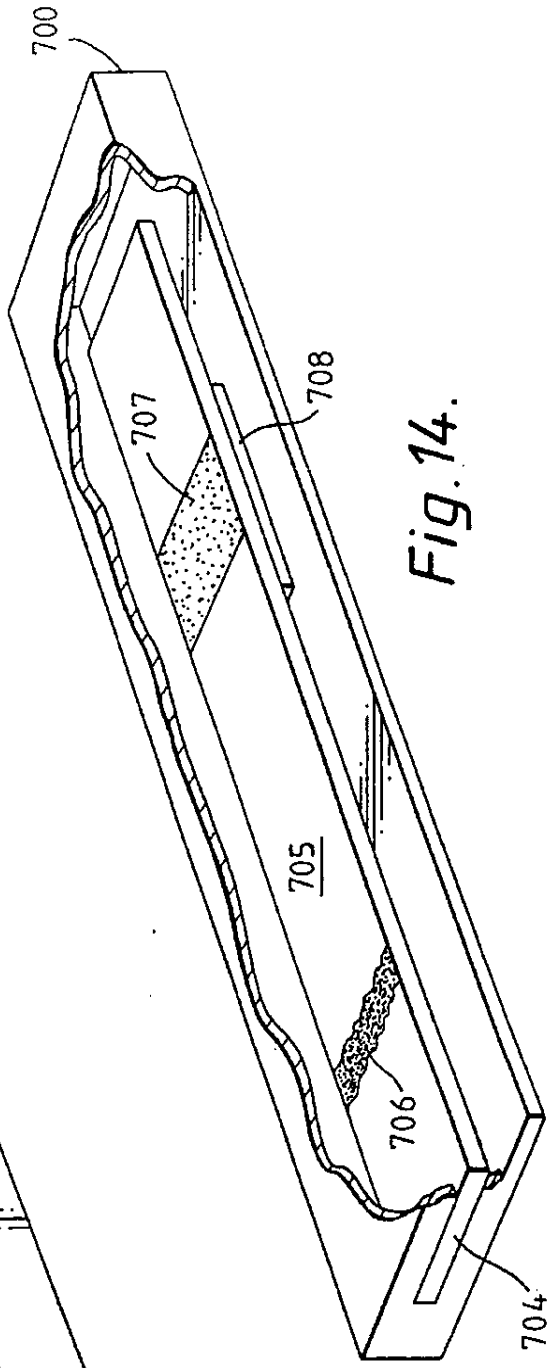

Fig. 14.

ASSAYS

This is a continuation of application Ser. No. 07/876,448, filed on Apr. 30, 1992, which was abandoned upon the filing hereof which was a Divisional of application Ser. No. 07/795,266 filed Nov. 19, 1991 which was a continuation of application Ser. No. 07/294,146 filed Feb. 27, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to assays involving specific binding, especially immunoassays. This application is based on applications filed in Great Britain, having application numbers 8709873 (filed Apr. 27, 1987) and 8725457 (filed Oct. 30, 1987), as well as PCT application GB88/00322 (filed Apr. 26, 1988).

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 which is rapid and which requires the minimum degree of skill and involvement from the user.

2. Description of the Related Art

The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream 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, Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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 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 sons in the test strip wherein a specific binding reagent for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (U.S.A.) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is in the moist state.

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

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 together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

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 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 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 solid phase material into the second zone and bind with the

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 second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it 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 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 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.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols 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. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. 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 size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable 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 material to be observed from outside the housing and which further zone incorporates 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 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. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but this is not preferred.

An important embodiment of the invention is 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 and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone 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 two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and 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 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 multi-directional (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 flouride, ethylene vinylacetate, acrylonitrile and polytetrafluoroethylene 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 nitrocellulose. 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 porous solid phase material.

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 antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a 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 second 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 horseradish 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 porous solid phase material 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 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 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 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, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away 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 to allow any unbound conjugage 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.

The presence or intensity of the signal from the label which becomes bound in the second zone can 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.

The immobilised specific binding reagent in the second 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 antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and 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 incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

The material comprising the porous solid phase is preferably nitrocellulose. This has the advantage that the antibody in the second zone can be immobilised firmly without prior chemical treatment. If the porous solid phase material comprises paper, for example, the immobilisation of the 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 antibody 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 ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with

a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is 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 commercially from Schleicher and Schuell GmbH.

Preferably, the nitrocellulose sheet is "backed", e.g. with plastics sheet, to increase its handling strenth. This can be manufactured easily by forming a thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

Alternatively, a pre-formed sheet of nitrocellulose 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 such that in the untreated material, aqueous liquid migrates at a rate of 1 cm in not more than 2 minutes, but slower flow rates can be used if desired.

The spatial separation between the zones, 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, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second 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 present in the migrating sample.

The reagents can be applied to the 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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **2** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **3** is a perspective view of a device utilizing a porous strip of the type illustrated in FIGS. **1** and **2**;

FIG. **4** is a perspective view, partially broken away, revealing a porous strip within the device of FIG. **3**;

FIG. **5** is an enlarged view of one end of the device of FIG. **3**;

FIG. **6** is a perspective view of another test device according to the invention;

FIG. **7** is a perspective view, similar to FIG. **6**, but partially cut away to reveal the porous test strip contained within the body of the device;

FIG. **8** is an exploded perspective view of an assay device in accordance with the present invention;

FIG. **9** is a cross-sectional side elevation of the device shown in FIG. **8**;

FIG. **10** is an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**;

FIG. **11** is a plan view of another embodiment of the invention;

FIG. **12** is a schematic cross-sectional view taken along line **12**—**12** in FIG. **11**;

FIG. **13** is a perspective view of yet another embodiment of the invention; and

FIG. **14** is a partially cut away view of the device of FIG. **13**.

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

Embodiment 1

FIGS. **1** and **2** represent a typical strip of porous solid phase material for use in an assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to FIG. **1**, the assay test strip **10** is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end **11** of strip **10** is a narrow band or zone **12** extending across the entire width of the strip. A small region **13** of strip **10** lies vertically below zone **12**. Above zone **12** is a second zone **14** lying a discrete distance up strip **10** and similarly extending the entire width of the strip. The region **15** of strip **10** between zones **12** and **14** can be of any height as long as the two zones are separate. A further region **16** of the strip extends above zone **14**, and at the top **17** of the strip is a porous pad **18** firmly linked to strip **10** such that pad **18** can act as a "sink" for any liquid sample which may be rising by capillary action through strip **10**.

Zone **12** is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone **14**, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

FIG. **2** illustrates what happens when the assay strip is used in an analytical procedure. The lower end **11** of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad **18**. In so doing, the sample traverses zone **12** and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone **14**, the labelled antibody can bind to any analyte

reaching zone 14, any analyte bound to the second antibody, so ...d "sandwich" so produced. If a ...n of the analyte to be determined is present in the sample, in a short period of time a molecule should ... immobilisation of the visible label should occur in ... significa... ...

... presence of an analysis to which this embodiment ... the analyte can be hCG, the reagents in zones ... be monoclonal antibodies to hCG which can ... in a "sandwich" reaction with hCG, and the label ... particulate dye, a gold sol or coloured latex ...

...hough described above in relation to a "sandwich" ...tion, it will be readily apparent to the skilled reader that ...s can be modified to a "competition" reaction format if ...esired, the labelled reagent in zone 12 being the analyte or an analogue of the analyte.

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 vital 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 (estrone-3-glucuronide) and P-3-G (progesterone-3-glucuronide).

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 A_1 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. By way of example, a dual analyte test for apolipoproteins A_1 and B may be prepared by depositing, as two specially distinct zones, antibody specific for apolipoprotein A_1 throughout a first zone and depositing a second antibody specific for apolipoprotein B, throughout the second zone of a porous carrier matrix. Following the application of both antibodies to each of their respective zones via a suitable application procedure (e.g. ink-jet printing, metered pump and pen, or airbrush), the remainder of the porous material should be treated with a reagent, e.g. bovine serum albumin, polyvinyl alcohol, or ethanolamine, to block any remaining binding sites elsewhere. A third and fourth reagent, bearing a label, may then be dispensed onto the dry carrier in one or more zones near to one end of the strip, the strip being allowed to dry between applications of the two reagents to the same zone. Reagent 3 and Reagent 4 may comprise conjugates of anti-apolipoprotein A_1 antibody and anti-apolipoprotein B antibody respectively. Both of these conjugates will become mobile in and on the carrier when in the moist state. Reagents 3 and 4 can migrate with the solvent flow when an aqueous sample is applied to the first end of the carrier strip. While migrating towards the two zones further along the strip, reagent 3 may bind any apolipoprotein A_1 present in the sample and reagent 4 may bind any apolipoprotein B present in the sample. On reaching the first second-antibody zone (anti-apolipoprotein A_1 antibody zone) anti-apolipoprotein A_1 molecules should become bound to the second antibody, immobilising the labelled 'sandwich' so produced. No labelled apoliprotein B molecules will bind to this first zone. On reaching the second second-antibody zone (anti-apolipoprotein B antibody zone) any apolipoprotein B molecules should become bound to the second antibody (solid-phase antibody), immobilising the labelled 'sandwich' so produced. No labelled apolipoprotein A_1 molecules will bind to the second zone. An accumulation of each of the direct label may occur at both or either zones to a lesser or greater extent resulting in a visible signal at either or both of the solid phase antibody zones. Excess unbound conjugate (of both reagent 3 and reagent 4) can pass freely over the two antibody zones and will be washed into the distal end of the strip.

The development of a quantifiable colour in both of the second-antibody zones may be assessed with an appropriate form of instrumentation, yielding a ratio of colour density between the two sites.

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 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 1 mm 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. Conjugates of label, e.g. a dye sol, and each immunochemically-reactive component specific for each bacterial group may then be dispensed either onto a single zone at the bottom end of the strip, proximal to the sample application zone, or as a series of separate zones.

FIGS. 3, 4 and 5 of the accompanying drawings depict a complete device utilising a porous strip as just described above. FIG. 3 represents the complete device viewed from the front, FIG. 4 shows the same device partially cut away to reveal the details of the strip inside, and FIG. 5 shows the underside of the device.

Referring to FIG. 3, the device comprises a flat rectangular body 30 the front face 31 of which is perforated by a circular hole or window 32 which reveals the porous test strip 10 within the body. The region of the test strip 10 visible through the window 32 incorporates a narrow horizontal zone 14.

Referring to FIG. 4, the device comprises a dry rectangular test strip 10 made from porous material which extends from the bottom end 33 of the body 30 within the body between the front 31 and back 34 of the body. Near the bottom end 11 of the strip 10 is a horizontal zone 12 bearing a labelled specific binding reagent for an analyte, the binding reagent being mobile in the test strip in the moist state. Further up the test strip is the narrow horizontal zone 14 which is visible through the window 32. At the top 17 of the test strip 10 is a porous 'sink' 18 which can absorb any liquid sample that has permeated upwards through the strip.

Referring to FIG. 5, the bottom edge 35 of the body 30 incorporates a lateral aperture in which the bottom end 11 of the strip lies.

In operation, the bottom end 33 of the body 30 is immersed in a liquid sample (eg urine) so that the liquid sample can be absorbed by the bottom end 11 of the test strip 20 and rise by capillary action to the top 17 of the test strip and into the sink 18. In so doing, the liquid sample progresses via zone 12 to zone 14. Specific binding reactions as described above occur, and the test result is visible to the user through the window 32.

Embodiment 2

FIGS. 6 and 7 of the accompanying drawings illustrate another test device according to the invention. FIG. 6 illustrates the complete device viewed from the front, and FIG. 7 depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to FIG. 6, the device comprises an elongate body 200 terminating at its lower end 201 in a small integral receptacle 202 which can hold a predetermined volume of a liquid sample, eg urine. The front face 203 of the body 200 incorporates two square small square apertures or windows 204 and 205 located one above the other.

Referring to FIG. 7, the elongate portion of the body 200 is hollow and incorporates a test strip 206 running almost the full height of the body. This test strip is of similar construction to those described under Embodiment 1, and incorporates near its lower end 207 a horizontal zone 208 bearing a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones 209 and 210 adjacent to the windows 204 and 205 and visible therethrough. The strip terminates at its top end 211 in a porous sink 212. At the bottom end 201 of the device, the receptacle 202 communicates with the hollow body via a lateral aperture 213.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacle 202. From the receptacle 202 the liquid sample rises by capillary action through the test strip 206 and conveys the labelled reagent from zone 208 to the two circular zones 209 and 210. A series of specific binding reactions as described in relation to Embodiment 1 above occur. In this embodiment the second circular zone 210 can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone 209. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone 210 can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone 208 by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone 209 by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone 208, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Embodiment 3

FIG. 8 of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and FIG. 9 represents a cross-sectional side elevation of the device shown in FIG. 8.

Referring to FIG. 8, the device comprises a housing or casing 500 of elongate rectangular form having at one end 501 a portion 502 of reduced cross-sectional area, A cap 503 can be fitted onto portion 502 and can abut against the shoulder 504 at end 501 of the housing. Cap 503 is shown separated from housing 500. Extending beyond end 505 of portion 502 is a porous member 506. When cap 503 is fitted onto portion 502 of the housing, it covers porous member 506. Upper face 507 of housing 500 incorporates two apertures 508 and 509.

Referring to FIG. 9, it can be seen that housing 500 is of hollow construction. Porous member 506 extends into housing 500 and contacts a strip of porous carrier material 510. Porous member 506 and strip 510 overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member 506 can permeate member 506 and progress into strip 510. Strip 510 extends further into housing 500. Strip 510 is "backed" by a supporting strip 511 formed of transparent moisture-impermeable plastics material. Strip 510 extends beyond apertures 508 and 509. Means are provided within housing 500 by webbs 512 and 513 to hold strip 510 firmly in place. In this respect, the internal 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, and porous member 506 is firmly retained in the housing and adequate fluid permeable contact is maintained between member 506 and strip 510. The transparent backing strip 511 lies between strip 510 and apertures 508 and 509 and can act as a seal against ingress of moisture from outside the housing 500 via these apertures. If desired, the residual space 514 within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip 510 in the dry state during storage. The reagent-containing zones in strip 510 are not depicted in FIG. 8, but the first zone containing the labelled reagent which is mobile when the strip is moistened will lie in the region between the porous member 506 and aperture 508. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture 508 in order that when the device has been used in an assay, the result can be observed through aperture 508. Aperture 509 provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap 503 is removed from the holder and member 506 is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member 506 to the liquid sample for a time sufficient to ensure that member 506 is saturated with the sample, the cap 503 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 510 to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures 508 and 509 and can ascertain whether the assay has been completed by observing the control zone through aperture 509, and can ascertain the result of the assay by observing the second zone through aperture 508.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing **500** being moulded in two parts (e.g. upper and lower halves **515** and **516** which can be securely fastened together (e.g. by ultrasonic welding) after the porous member 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 porous member and test strip together to ensure adequate contact between them. Cap **503** can be moulded as a separate complete item. If desired, apertures **508** and **509** 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 **505** of housing **500** and the protruding porous member **506**, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member **506**. Member **506** 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 as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with FIGS. **8** and **9** 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 then (after optionally replacing the cap) can observe the test result through aperture **508** within a matter of a few minutes.

Although described with particular reference to 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 **509** 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 from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member **506**.

FIG. **10** of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**.

The porous receiving member **506** is linked to the porous test strip **510**, backed by the transparent plastics sheet **511**, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone **517** incorporates the immobilised specific binding reagent, and control zone **518** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip **511** and adjacent the porous receiving member **506**, carries a glaze **519** on which is deposited a layer **520** of labelled specific binding reagent. The thickness of these two layers as depicted in FIG. **10** is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, the essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member **506** can flow therefrom along the length of strip **510** and in so doing, will dissolve glaze **519** and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone **517**.

Embodiment 4

FIGS. **11** and **12** illustrate another embodiment of the invention, which is seen in plan view in FIG. **11** and in cross-section in FIG. **12**, the cross-section being an elevation on the line **12—12** seen in FIG. **11**.

Referring to FIG. **11**, the test device comprises a flat rectangular casing **600** incorporating a centrally disposed rectangular aperture **601**, adjacent the left hand end **602**, and two further apertures **603** and **604** near the mid point of the device and arranged such that apertures **601**, **603** and **604** lie on the central longitudinal axis of the device corresponding to line **12—12**. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **12**, the device is hollow and incorporates within it a porous sample receiving member adjacent end **602** of casing **600** and lying directly beneath aperture **601**. A test strip of similar construction to that described with reference to Embodiment 4, comprising a porous strip **606** backed by a transparent plastics sheet **607** is also contained within casing **600**, and extends from the porous receiving member **602**, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet **607** is in firm contact with the upper inner surface **608** of casing **600**, and provides a seal against apertures **603** and **604** to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip **606** will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures **603** and **604**, in a manner analagous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture **601**, e.g. by means of a syringe, to saturate porous receiving member **605**. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures **603** and **604**.

Embodiment 5

A yet further embodiment of the invention is illustrated in FIGS. **13** and **14** of the accompanying drawings. FIG. **13** shows a device comprising a rectangular casing **700** having in its upper surface **701** a rectangular aperture **702**. One end wall **703** of the device **703** incorporates an aperture **704** through which a porous test element communicates with the exterior of the device. Aperture **702** is situated in surface **701** at a point relatively remote from the end **703** containing the aperture **704**.

FIG. **14** shows a partially cut-away view of the device in FIG. **13**. The hollow device incorporates a porous test strip **705**, running almost the entire length of casing **700** from aperture **704**. Test strip **705** incorporates a first zone **706** containing a labelled specific binding reagent and a further zone **707**, remote from aperture **704**, incorporating an immobilised specific reagent. Zone **706** lies directly beneath aperture **702** is therefore observable from outside casing. Beneath strip **705** and adjacent zone **707**, is a crushable

element 708 containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone 707, if labelled reagent from 706 has become bound in zone 707 following use of the device. Release of the reagents from member 708 can be effected by applying pressure to the outside of the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end 703 of casing 700 into a vessel containing the sample. The liquid sample will then permeate the length of test strip 705, taking labelled reagent from zone 706 and passing through zone 707 where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the sample has permeated the test strip, reagent can be released from the crushable member 700 and the result of the test observed through aperture 702.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein; speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5μ). Examples of flow rates obtained with various materials are given below:

	Pore size	Time to Flow 45 mm (minutes)
Schleicher + Schuell	3μ	3.40
nitrocellulose (unbacked)	5μ	3.30
	8μ	3.00
	12μ	2.20
polyester-backed	8μ (nominal)	3.40
Whatman Nitrocellulose	5	19.20
Pall "Immunodyne" (nylon)	3	4.00
	5	3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20 nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22μ filtered 0.1M K_2CO_3, and 20 mls is added to a clean glass beaker. 200 μl of anti-alpha hCG antibody, prepared in 2 mM borax buffer pH9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to 9, and 2 mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000 g, 30 minutes, and 4° C., with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20 mM Tris, 150 mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specification No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 liter of distilled water by mixing on a magnetic stirrer for 2–3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500 g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000 g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500 mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100 mls distilled water.

The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657 nm for Foron Blue, and 690 nm for Resolin Blue. The absorbance at lambda-max, for 1 cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoassays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the preformed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chloroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may

be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5 ml (12.5 mg solids) of suspension is diluted with 1 ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1 ml borate buffer, mixed with 300 µg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16–20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000 rpm, the supernatant is discarded and the pellet resuspended in 1.5 mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5 mg/ml BSA in phosphate buffered saline pH7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5 mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4° C. until used.

(A) Anti-hCG—Dye Sol Preparation

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml. A reaction mixture is prepared which contains 100 µl antibody solution, 2 mls dye sol, 2 mls 0.1M phosphate buffer pH 5.8 and 15.9 mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites may be blocked by the addition of, for example, bovine serum albumin: 4 mls of 150 mg/ml BSA in 5 mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000 g for 10 minutes, and the pellet resuspended in 10 mls of 0.25% (w/v dextran/0.5% (w/v lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH—Dye Sol Preparation

Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Materials

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8µ nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10^9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30° C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5 cm in length and 1 cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8µ nitrocellulose, 25 cm in length and 20 cm in width with zones of immobilised antibody at 5 cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6 cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5 cm in length and 1 cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100 µl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% TRITON X305 (Trademark—obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100 µl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1 mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA—Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of E-3-G (2 nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10 nM) in dry DMF were equilibrated separately at 4° C. Using pre-cooled glassware E-3-G in DMF (1.25 ml) and TnB in DMF 0.25 ml) were added to a pre-cooled 5 ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10 nM) was prepared and an aliquot (0.25 ml) was cooled to 4° C. and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4° C. and a solution of BSA (1 mg/ml in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5 ml) and stirred on a magnetic stirrer for 4 hours at 4° C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 SEPHADEX G-25 column, transferred to an amber glass storage bottle and stored at 4° C.

Preparation of BSA—E-3-G dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850 rpm 1500 g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10 µg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5 mM, pH7.4) to yield a final BSA concentration of 25 mg/ml. The reaction mixture was centrifuged at 4850 rpm (3000 g) for 10 minutes in a benchtop centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH5.8) buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA - E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to combinations and subcombinations of the features of this description and the accompanying drawings.

We claim:

1. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device comprising:

a) a hollow casing having a first end, a second end, a longitudinal axis, and a liquid biological sample application aperture adjacent to said first end;

b) a displaceable shroud disposed on said first end for shielding said liquid biological sample application aperture;

c) a test strip comprising a dry porous carrier disposed within said hollow casing, said dry porous carrier communicating directly or indirectly with the exterior of said hollow casing through said liquid biological sample application aperture to receive applied said liquid biological sample; said dry porous carrier having a detection zone; said hollow casing including means permitting observation of said detection zone from outside said casing; said test strip, in the dry unused state, containing upstream from said detection zone a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said labelled reagent being labelled with a particulate direct label, wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said detection zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream of said detection zone in the presence of, a material comprising sugar, in an

amount effective to reduce interaction between said test strip and said labelled reagent;

said dry porous carrier containing in said detection zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said detection zone;

migration of said liquid biological sample through said dry porous carrier conveying by capillarity said first complex to said detection zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable via said detection zone observation means, thereby to indicate the presence of said analyte in said liquid biological sample.

2. The test device according to claim 1, wherein said particulate direct label is selected from the group consisting of a dye sol, a metallic sol, and colored latex particles having a maximum diameter of less than or equal to about 0.5 micron.

3. The test device according to claim 1, wherein said means permitting observation comprises at least one aperture through which the second complex may be observed.

4. The test device according to claim 3, wherein said dry porous carrier comprises a strip or sheet of porous material backed with a layer of transparent moisture-impervious material, said transparent layer being in contact with the inside of said hollow casing adjacent said at least one aperture to inhibit ingress of moisture or said liquid biological sample.

5. The test device according to claim 1, wherein said hollow casing and said shroud are molded from a plastic material.

6. The test device according to claim 1, wherein said dry porous carrier comprises a strip or sheet of porous material.

7. The test device according to claim 1, wherein said dry porous carrier material is nitrocellulose.

8. The test device according to claim 7, wherein said nitrocellulose has a pore size of at least about one micron.

9. The test device according to claim 7, wherein said pore size is greater than about 5 microns.

10. The test device according to claim 7, wherein said pore size is about 8–12 microns.

11. The test device according to claim 1, incorporating a control zone downstream from said detection zone in said dry porous carrier, which control zone comprises means to indicate that said liquid biological sample has been conveyed by capillarity beyond said detection zone, said hollow casing further including means permitting observation of said control zone from outside said hollow casing.

12. The test device according to claim 1, further comprising an absorbent sink at a distal end of said dry porous carrier, said sink having sufficient absorptive capacity to allow any labelled reagent not bound with said analyte to wash out of said detection zone.

13. The test device according to claim 1, wherein said labelled reagent is applied to said dry porous carrier as a surface layer.

14. The test device according to claim 13, wherein said dry porous carrier has been pre-treated with said material comprising sugar in the upstream portion to which said labelled reagent is applied.

15. The test device according to claim 1, wherein said means for binding in said detection zone is impregnated throughout the thickness of said dry porous carrier in said detection zone.

16. The test device according to claim 1, wherein said analyte is hCG.

17. The test device according to claim 1, wherein said analyte is LH.

18. The test device according to claim 1, wherein

(a) said dry porous carrier
(i) is nitrocellulose and
(ii) communicates indirectly with the exterior of said hollow casing via a bibulous urine receiving member which protrudes from said hollow casing and which acts as a reservoir from which urine is released;

(b) contains said labelled reagent in a first zone upstream from said detection zone, wherein said labelled reagent
(i) comprises an anti-LH antibody conjugated to a colored particulate label and
(ii) is freely mobile within said dry porous carrier when wetted with said urine;

(c) said detection zone is spatially distinct from said first zone;

(d) said means for binding said first complex is an unlabelled anti-LH antibody which is permanently immobilized in said detection zone, wherein said unlabelled anti-LH antibody specifically binds a different LH epitope than the anti-LH antibody of said labelled reagent; and

(e) said first zone and said detection zone being arranged such that said urine applied to said protruding bibulous urine receiving member, and consequently thereby to said dry porous carrier, is conveyed by capillarity via said first zone into said detection zone, said hollow casing having at least one aperture through which said second complex can be observed, whereby said test device is a fertile period prediction device.

19. The test device according to claim 1, wherein

(a) said dry porous carrier
(i) is nitrocellulose and
(ii) communicates indirectly with the exterior of said hollow casing via a bibulous urine receiving member which protrudes from said hollow casing and which acts as a reservoir from which urine is released;

(b) contains said labelled reagent in a first zone upstream from said detection zone, wherein said labelled reagent
(i) comprises an anti-hCG antibody conjugated to a colored particulate label and
(ii) is freely mobile within said dry porous carrier when wetted with said urine;

(c) said detection zone is spatially distinct from said first zone;

(d) said means for binding said first complex is an unlabelled anti-hCG antibody which is permanently immobilized in said detection zone, wherein said unlabelled anti-hCG antibody specifically binds a different hCG epitope than the anti-hCG antibody of said labelled reagent; and,

(e) said first zone and said detection zone being arranged such that said urine applied to said protruding bibulous urine receiving member, and consequently thereby to said dry porous carrier, is conveyed by capillarity via said first zone into said detection zone, said hollow casing having at least one aperture through which said second complex can be observed, whereby said test device is a pregnancy test device.

20. The device according to claim 1, wherein said test strip further comprises a bibulous sample receiving member for initially receiving said liquid biological sample, said bibulous sample receiving member thereafter releasing said liquid biological sample into said dry porous carrier, and

wherein said dry porous carrier contains said labelled reagent.

21. The test device according to claim **20**, wherein said bibulous sample receiving member protrudes from said housing through said sample aperture, and wherein said shroud surrounds said protruding bibulous member.

22. The test device according to claim **1**, which further comprises a control zone downstream from said detection zone in said dry porous carrier, which control zone comprises means to indicate that said liquid biological sample has been conveyed by capillarity beyond said detection zone, said hollow casing further including means permitting observation of said control zone from outside said hollow casing.

23. The test device according to claim **22**, wherein said means permitting observation of said control zone comprises a control zone observation aperture.

24. The test device according to claim **1**, wherein said means permitting observation of said detection zone comprises a detection zone observation aperture.

25. The test device according to claim **1**, wherein said shroud is constructed of opaque or translucent material.

26. The test device according to claim **1**, wherein said material comprising sugar further comprises a protein.

27. The test device according to claim **26**, wherein said protein is bovine serum albumin.

28. The test device according to claim **1**, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

29. The test device according to claim **1**, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

30. The test device according to claim **1**, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

31. The test device according to claim **30**, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

32. The test device according to claim **20**, wherein said bibulous sample receiving member has unidirectional porosity parallel to its longitudinal axis.

33. A kit comprising a packaged pregnancy test device for detecting hCG suspected of being present in a urine sample, said device comprising:

a) a hollow casing having a first end, a second end, a longitudinal axis, and a urine sample application aperture adjacent to said first end;

b) a displaceable shroud disposed on said first end for shielding said urine sample application aperture;

c) a test strip comprising a dry porous carrier disposed within said hollow casing, wherein said dry porous carrier is nitrocellulose and communicates indirectly with the exterior of said hollow casing via a bibulous urine sample receiving member which protrudes from said hollow casing and which acts as a reservoir from which said urine sample is released into said dry porous carrier,

said dry porous carrier having a detection zone; said hollow casing including means permitting observation of said detection zone from outside said casing;

said dry porous carrier containing in a first zone upstream from said detection zone a labelled reagent comprising a colored particulate direct label conjugated to an anti-hCG antibody, wherein said anti-hCG antibody present in said labelled reagent is capable of specifically binding with said hCG to form a first complex of said labelled reagent and said hCG,

wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said urine sample, said labelled reagent being freely mobile within said test strip when said test strip is wetted with said urine sample, said detection zone being spatially distinct from said first zone;

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least said first zone with, or 2) drying said labelled reagent onto a portion of said first zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said dry porous carrier containing in said detection zone a means for specifically binding said first complex, said means for specifically binding said first complex being an unlabelled anti-hCG antibody which is impregnated throughout the thickness of said dry porous carrier in said detection zone and is permanently immobilized in said detection zone,

wherein said anti-hCG antibody of said labelled reagent and said unlabelled anti-hCG antibody specifically bind to different hCG epitopes,

said first zone and said detection zone being arranged such that said urine sample applied to said protruding bibulous urine sample receiving member and consequently to said dry porous carrier can migrate via said first zone into said detection zone,

said migration of said urine sample through said dry porous carrier conveying by capillarity said first complex to said detection zone whereat said means for specifically binding binds said first complex thereby to form a second complex;

said second complex being observable via said means permitting observation in said detection zone, thereby to indicate the presence of said hCG in said urine sample.

34. A kit comprising a packaged fertile period prediction test device for detecting LH suspected of being present in a urine sample, said device comprising:

a) a hollow casing having a first end, a second end, a longitudinal axis, and a urine sample application aperture adjacent to said first end;

b) a displaceable shroud disposed on said first end for shielding said urine sample application aperture;

c) a test strip comprising a dry porous carrier disposed within said hollow casing, wherein said dry porous carrier is nitrocellulose communicates indirectly with the exterior of said hollow casing via a bibulous urine sample receiving member which protrudes from said hollow casing and which acts as a reservoir from which said urine sample is released into said dry porous carrier,

said dry porous carrier having a detection zone; said hollow casing including means permitting observation of said detection zone from outside said casing;

said dry porous carrier containing in a first zone upstream from said detection zone a labelled reagent comprising a colored particulate direct label conjugated to an anti-LH antibody, wherein said anti-LH antibody present in said labelled reagent is capable of specifically binding with said LH to form a first complex of said labelled reagent and said LH,

wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said urine sample, said labelled reagent being freely mobile within said test strip when said test strip is wetted with said urine sample, said detection zone being spatially distinct from said first zone;

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least said first zone with, or 2) drying said labelled reagent onto a portion of said first zone in the presence of, a material comprising sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said dry porous carrier containing in said detection zone a means for specifically binding said first complex, said means for specifically binding said first complex being an unlabelled anti-LH antibody which is impregnated throughout the thickness of said dry porous carrier in said detection zone and is permanently immobilized in said detection zone.

wherein said anti-LH antibody of said labelled reagent and said unlabelled anti-LH antibody specifically bind to different LH epitopes,

said first zone and said detection zone being arranged such that said urine sample applied to said protruding bibulous urine sample receiving member and consequently to said dry porous carrier can migrate via said first zone into said detection zone,

said migration of said urine sample through said dry porous carrier conveying by capillarity said first complex to said detection zone whereat said means for specifically binding binds said first complex thereby to form a second complex;

said second complex being observable via said means permitting observation in said detection zone, thereby to indicate the presence of said LH in said urine sample.

* * * * *

EXHIBIT E

US005622871A

United States Patent [19]

May et al.

[11] Patent Number: 5,622,871

[45] Date of Patent: Apr. 22, 1997

[54] **CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS**

[75] Inventors: **Keith May**, Bedfordshire; **Michael E. Prior**, Northamptonshire; **Ian Richards**, Bedford, all of England

[73] Assignee: **Unilever Patent Holdings B.V.**, Netherlands

[21] Appl. No.: **102,313**

[22] Filed: **Jul. 15, 1993**

Related U.S. Application Data

[60] Continuation of Ser. No. 876,449, Apr. 30, 1992, abandoned, which is a division of Ser. No. 795,266, Nov. 19, 1991, abandoned, which is a continuation of Ser. No. 294,146, filed as PCT/GB88/00322, Apr. 26, 1988, abandoned.

[30] **Foreign Application Priority Data**

Apr. 27, 1987 [GB] United Kingdom 8709873
Oct. 30, 1987 [GB] United Kingdom 8725457

[51] **Int. Cl.**[6] **G01N 33/558**

[52] **U.S. Cl.** **436/514**; 422/56; 422/58; 422/60; 435/7.1; 435/962; 435/970; 435/971; 435/973; 436/510; 436/518; 436/541; 436/805; 436/810; 436/817; 436/818

[58] **Field of Search** 722/56–58; 436/501, 436/530, 810, 814, 514, 515, 518, 523, 524, 541, 510, 538, 817, 818; 435/810, 7.92–7.95, 970; 422/60; 427/2, 2.11

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Primary Examiner—Carol A. Spiegel
Attorney, Agent, or Firm—Cushman Darby & Cushman, LLP

[57] **ABSTRACT**

An analytical test device useful for example in pregnancy testing, includes a hollow casing (**500**) constructed of moisture-impervious solid material, such as plastics materials, containing a dry porous carrier (**510**) which communicates indirectly with the exterior of the casing via a bibulous sample receiving member (**506**) which protrudes from the casing such that a liquid test sample can be applied to the receiving member and permeate therefrom to the porous carrier, the carrier containing in a first zone a labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the two zones being arranged such that liquid sample applied to the porous carrier can permeate via the first zone into the second zone, and the device incorporating an aperture (**508**) in the casing, enabling the extent (if any) to which the labelled reagent becomes bound in the second zone to be observed. Preferably the device includes a removable cap for the protruding bibulous member.

36 Claims, 5 Drawing Sheets

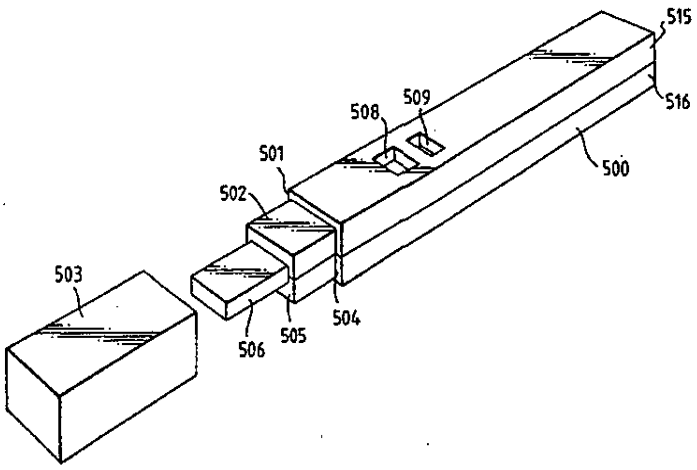

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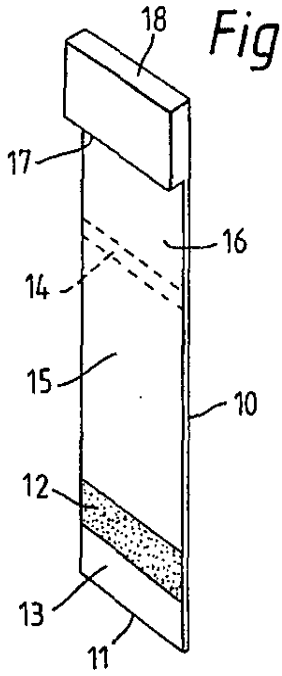
Fig.1.



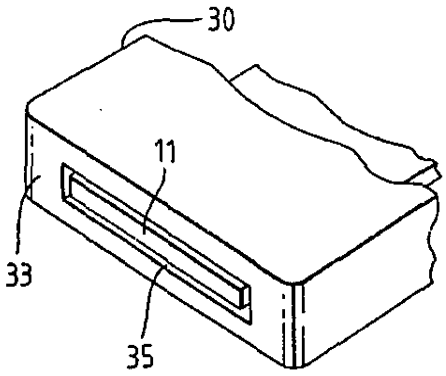
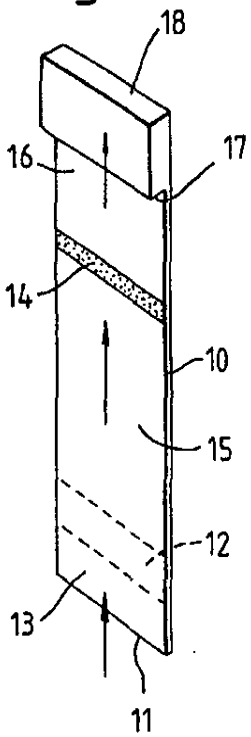
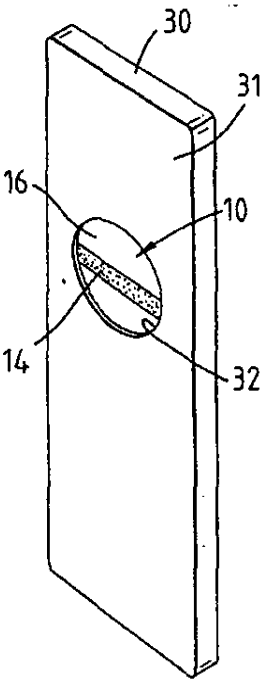


Fig.5.

Fig.2.





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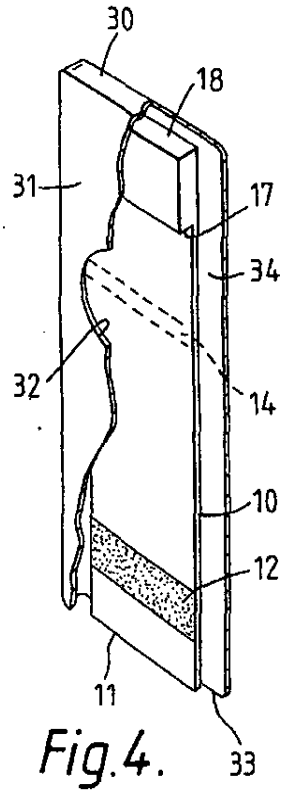
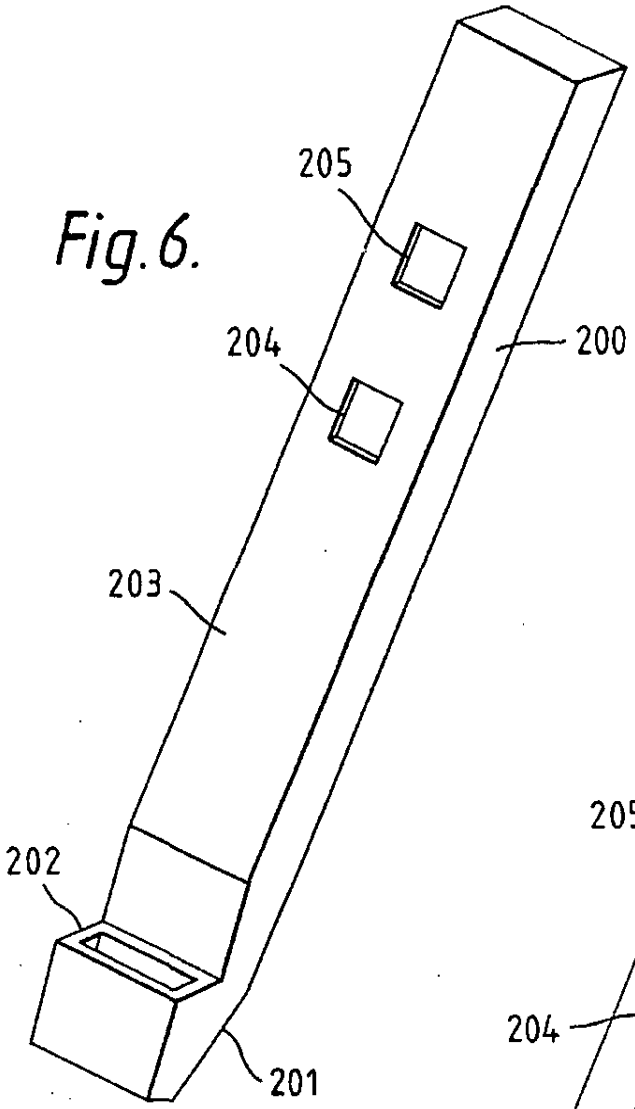


Fig.4.

Fig.6.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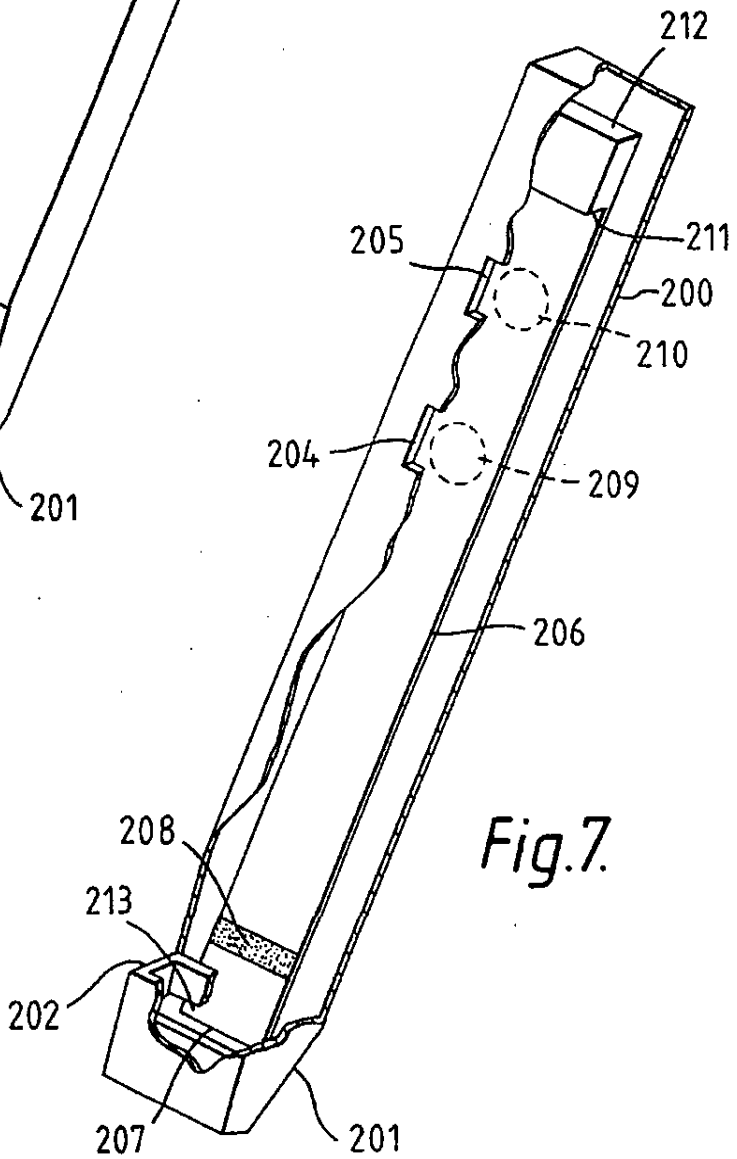
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Fig.7.

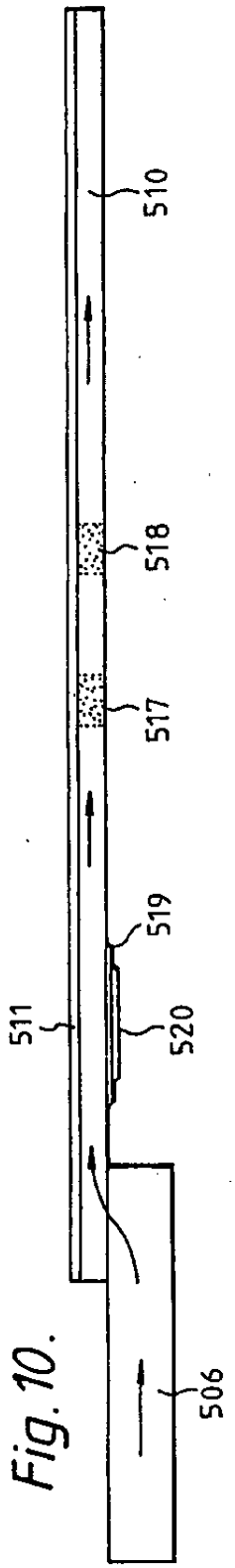

Fig. 10.

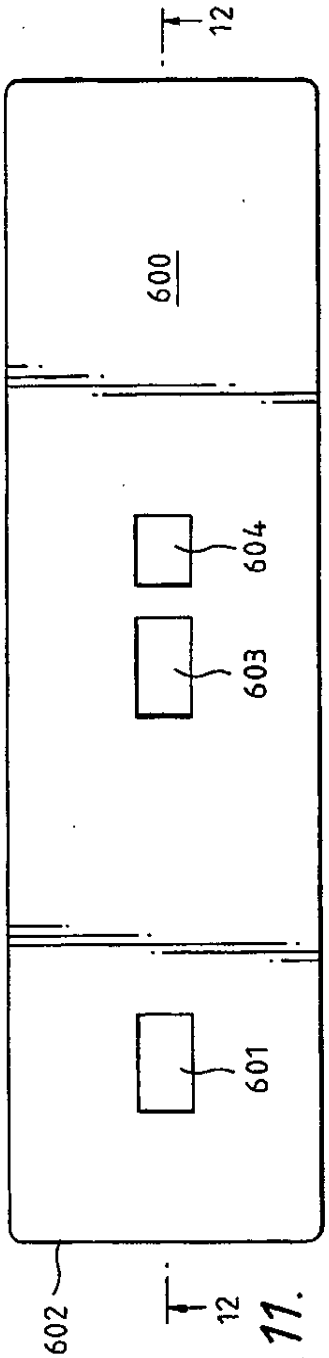

Fig. 11.

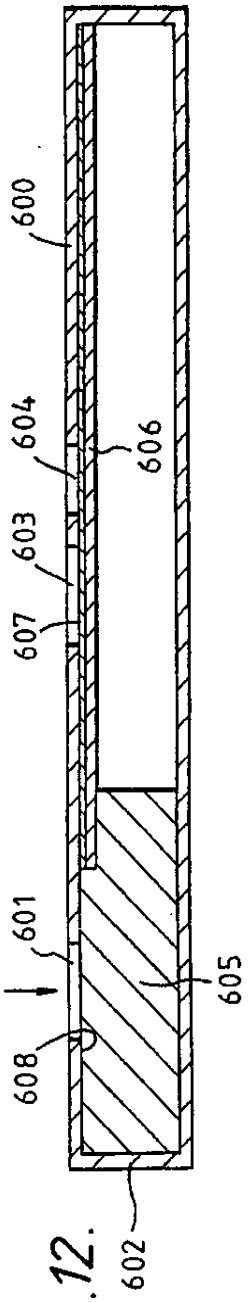

Fig. 12.

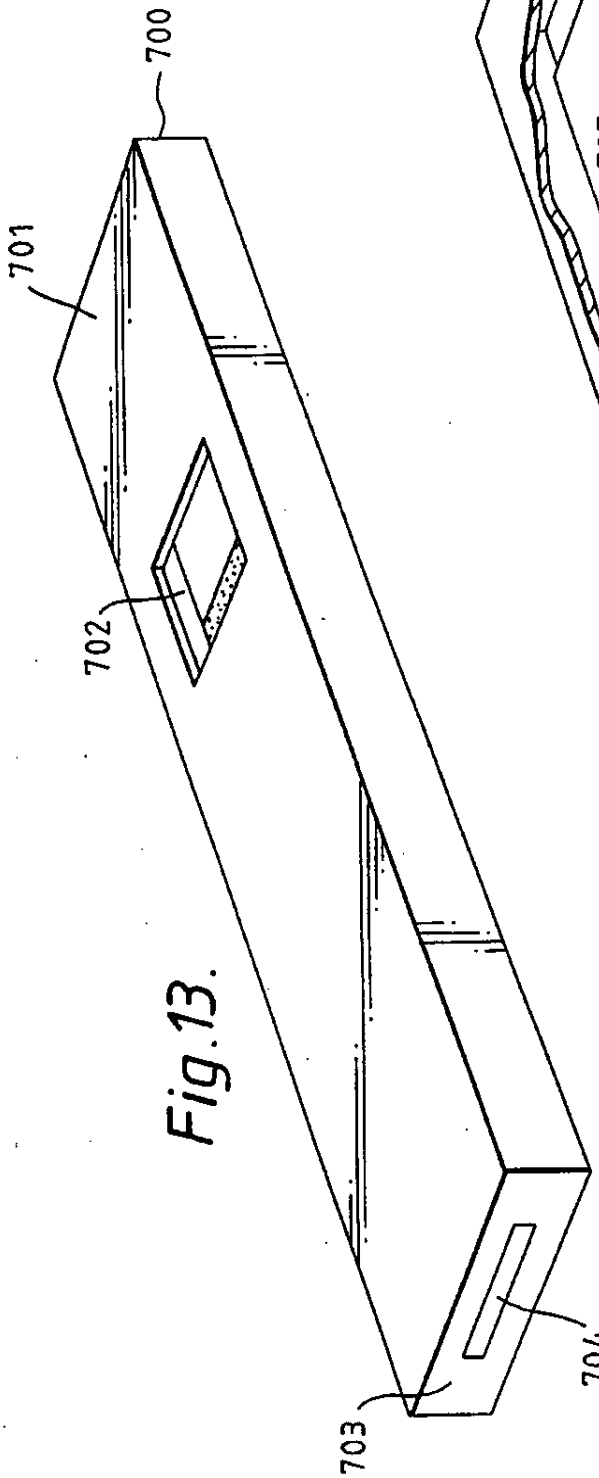

Fig. 13.

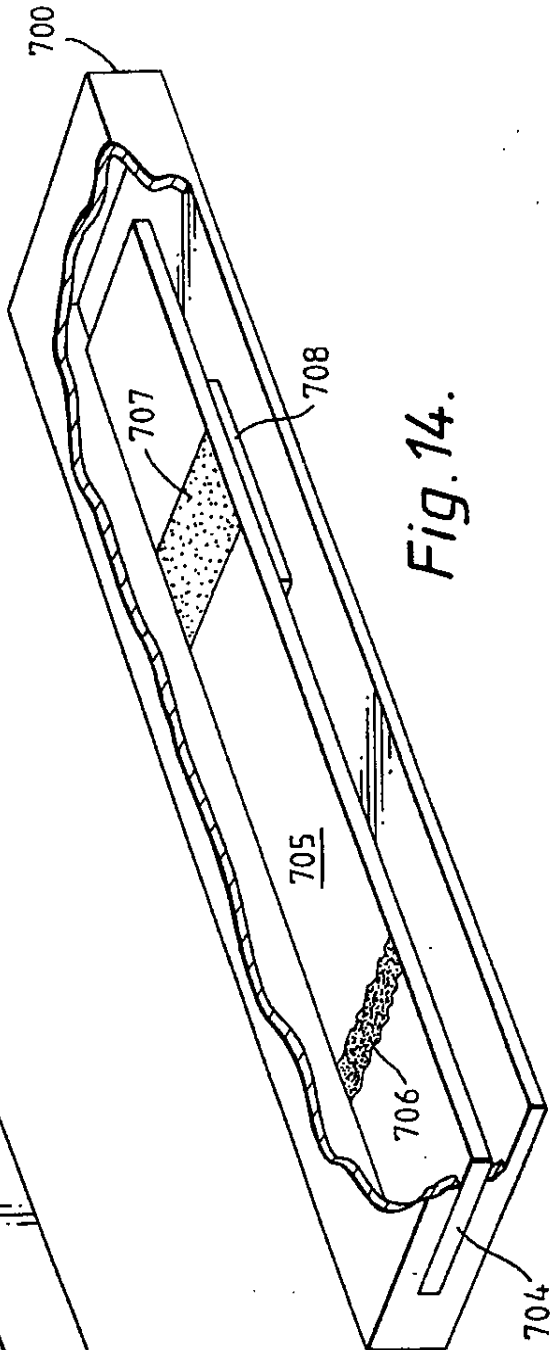

Fig. 14.

CAPILLARY IMMUNOASSAY AND DEVICE THEREFOR COMPRISING MOBILIZABLE PARTICULATE LABELLED REAGENTS

This is a continuation of application Ser. No. 07/876,449 filed on Apr. 30, 1992, which was abandoned upon filing hereof, which is a Divisional of Appln. Ser. No. 07/795,266 filed Nov. 19, 1991, now abandoned which is a continuation of Appln. Ser. No. 07/294,146, filed as PCT/GB88/00322, Apr. 26, 1988, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

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

In particular, he 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 which is rapid and which requires the minimum degree of skill and involvement from the user.

2. Description of the Related Art The use of test devices in the home to test for pregnancy and fertile period (ovulation) is now commonplace, and a wide variety of test devices and kits are available commercially. Without exception, the commercially-available devices all require the user to perform a sequence of operations before the test result is observable. These operations necessarily involve time, and introduce the possibility of error.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a test device which is readily usable by an unskilled person and which preferably merely requires that some portion of the device is contacted with the sample (e.g. a urine stream 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. Ideally the analytical result should be observable within a matter of minutes following sample application, e.g. ten minutes or less.

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 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 for an analyte suspected of being in the sample is immobilised. Analyte present in the sample can therefore become bound within the detection zone. The extent to which the analyte becomes bound in that zone can be determined with the aid of labelled reagents which can also be incorporated in the test strip or applied thereto subsequently. Examples of prior proposals utilising these principles are given in Thyroid Diagnostics Inc GB 1589234, Boots-Celltech Diagnostics Limited EP 0225054, Syntex (USA) Inc EP 0183442, and Behringwerke AG EP 0186799.

The present invention is concerned with adapting and improving the known techniques, such as those referred to in the above publications, to provide diagnostic test devices especially suitable for home use which are quick and convenient to use and which require the user to perform as few actions as possible.

A typical embodiment of the invention is an analytical test device comprising a hollow casing constructed of moisture-impervious solid material containing a dry porous carrier which communicates directly or indirectly with the exterior of the casing such that a liquid test sample can be applied to the porous carrier, the device also containing a labelled specific binding reagent for an analyte which labelled specific binding reagent is freely mobile within the porous carrier when in the moist state, and unlabelled specific binding reagent for the same analyte which unlabelled reagent is permanently immobilised in a detection zone on the carrier material and is therefore not mobile in the moist state, the relative positioning of the labelled reagent and detection zone being such that liquid sample applied to the device can pick up labelled reagent and thereafter permeate into the detection zone, and the device incorporating means enabling the extent (if any) to which the labelled reagent becomes in the detection zone to be observed.

Another embodiment of the invention is a device for use in an assay for an analyte, incorporating a porous solid phase material carrying in a first zone a labelled reagent which is retained in the first zone while the porous material is in the dry state but is free to migrate through the porous material when the porous material is moistened, for example by the application of an aqueous liquid sample suspected of containing the analyte, the porous material carrying in a second zone, which is spatially distinct from the first zone, an unlabelled specific binding reagent having specificity for the analyte, and which is capable of participating with the labelled reagent in either a "sandwich" or a "competition" reaction, the unlabelled specific binding reagent being firmly immobilised on the porous material such that it is not free to migrate when the porous material is in the moist state.

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

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 together in a "sandwich" reaction. This results in the labelled reagent being bound in the second zone if analyte is present in the sample. The two binding reagents must have specificities for different epitopes on the analyte.

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 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 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 solid phase material into the second zone and bind with the immobolised 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 second zone, and a consequent decrease in the intensity of the signal observed in the second zone in comparison with the signal that is observed in the absence of analyte in the sample.

An important preferred embodiment of the invention is the selection of nitrocellulose as the carrier material. This has considerable advantage over conventional strip materials, such as paper, because it 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 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 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.

Another important preferred embodiment of the invention is the use of so called "direct labels", attached to one of the specific binding reagents. Direct labels such as gold sols 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. Their release on contact with an aqueous sample can be modulated, for example by the use of soluble glazes.

An important aspect of the invention is the selection of technical features which enable a direct labelled specific binding reagent to be used in a carrier.-based analytical device, e.g. one based on a strip format, to give a quick and clear result. Ideally, the result of the assay should be discernable by eye and to facilitate this, it is necessary for the direct label to become concentrated in the detection zone. To achieve this, the direct labelled reagent should be transportable easily and rapidly by the developing liquid. Furthermore, it is preferable that the whole of the developing sample liquid is directed through a comparatively small detection zone in order that the probability of an observable result being obtained in increased.

Another important aspect of the invention is the use of a directly labelled specific binding reagent on a carrier material comprising nitrocellulose. Preferably the nitrocellulose has a pore size of at least one micron. Preferably the nitrocellulose has a pore size not greater than about 20 microns. 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 size range for such particles is from about 0.05 to about 0.5 microns.

In a further embodiment of the present invention, the porous solid phase material is linked to a porous receiving member to which the liquid sample can be applied and from which the sample can permeate into the porous solid phase material. Preferably, the porous solid phase material is contained within a moisture-impermeable casing or housing and the porous receiving member, with which the porous solid phase material is linked, extends out of the housing and can act as a means for permitting a liquid sample to enter the housing and permeate the porous solid phase material. The housing should be provided with means, e.g. appropriately placed apertures, which enable the second zone of the porous solid phase material (carrying the immobilised unlabelled specific binding reagent) to be observable 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 material to be observed from outside the housing and which further zone incorporates 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 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. Optionally, the labelled reagent can be incorporated elsewhere within the device, e.g. in the bibulous sample collection member, but this is not preferred.

An important embodiment of the invention is 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 and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone 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 two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and 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 absorbing liquid rapidly. The porosity of the material can be unidirectional (is with pores or fibres running wholly or predominantly parallel to an axis of the member) or multi-directional (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 flouride, 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 porous solid phase material.

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 antibody from the first zone, e.g. an "anti-mouse" antibody if the labelled body is one that has been derived using a 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 second 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 horseradish 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 porous solid phase material 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 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 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 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, the flow of sample continues beyond the detection zone and sufficient sample is applied to the porous material in order that this may occur, and that any excess labelled reagent from the first zone which does not participate in any binding reaction in the second zone is flushed away 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 3 MM chromatography paper, and should provide sufficient absorptive capacity 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.

The presence or intensity of the signal from the label which becomes bound in the second zone can 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.

The immobilised specific binding reagent in the second 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 antibody, and more preferably a monoclonal antibody.

Preferably the carrier material is in the form of a strip or sheet to which the reagents are applied in spacially distinct zones, and 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 incorporate two or more discrete bodies of porous solid phase material, e.g. separate strips or sheets, each carrying mobile and immobilised reagents. These discrete bodies can be arranged in parallel, for example, such that a single application of liquid sample to the device 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 of analytes in a single sample can be made. Alternatively, multiple samples can be applied individually to an array of carriers and analysed simultaneously.

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

Following the application of the antibody 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 ethanolamine, or any combination of these agents, for example. The labelled reagent for the first zone can then be dispensed onto the dry carrier and will become mobile in the carrier when in the moist state. Between each of these various process steps (sensitisation, application of unlabelled reagent, blocking and application of the labelled reagent), the porous solid phase material should be dried.

To assist the free mobility of the labelled reagent when the porous carrier is moistened with the sample, it is preferable for the labelled reagent to be applied to the carrier as a surface layer, rather than being impregnated in the thickness of the carrier. This can minimise interaction between the carrier material and the labelled reagent. In a preferred embodiment of the invention, the carrier is pre-treated with a glazing material in the region to which the labelled reagent is to be applied. Glazing can be achieved, for example, by depositing an aqueous sugar or cellulose solution, e.g. of

sucrose or lactose, on the carrier at the relevant portion, and drying. The labelled reagent can then be applied to the glazed portion. The remainder of the carrier material should not be glazed.

Preferably the porous solid phase material is 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 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 thin layer of nitrocellulose on a sheet of backing material. The actual pore size of the nitrocellulose when backed in this manner will tend to be, lower than that of the corresponding unbacked material.

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

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

The spatial separation between the zones, 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, and to allow the labelled reagent in the first zone to dissolve or disperse in the liquid sample and migrate through the carrier. Further control over these parameters can be achieved by the incorporation of viscosity modifiers (e.g. sugars and modified celluloses) in the sample to slow down the reagent migration.

Preferably, the immobilised reagent in the second zone is impregnated throughout the thickness of the carrier in the second 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 present in the migrating sample.

The reagents can be applied to the 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 using metered pumps, direct printing and ink-jet printing, and any of these techniques can be used in the 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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **2** a perspective view of a strip of porous solid phase material for use in an assay test in accordance with the invention;

FIG. **3** is a perspective view of a device utilizing a porous strip of the type illustrated in FIGS. 1 and 2;

FIG. 4 is a perspective view, partially broken away, revealing a porous strip within the device of FIG. **3**;

FIG. **5** is an enlarged view of one end of the device of FIG. **3**;

FIG. **6** is a perspective view of another test device according to the invention;

FIG. **7** is a perspective view, similar to FIG. **6**, but partially cut away to reveal the porous test strip contained within the body of the device;

FIG. **8** is an exploded perspective view of an assay device in accordance with the present invention;

FIG. **9** is a cross-sectional side elevation of the device shown in FIG. **8**;

FIG. **10** is an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**;

FIG. **11** is a plan view of another embodiment of the invention;

FIG. **12** is a schematic cross-sectional view taken along line **12—12** in FIG. **11**;

FIG. **13** is a perspective view of yet another embodiment of the invention; and

FIG. **14** is a partially cut away view of the device of FIG. **13**.

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

Embodiment 1

FIGS. **1** and **2** represent a typical strip of porous solid phase material for use in an assay test in accordance with the invention, and illustrate the underlying principle upon which the invention operates.

Referring to FIG. **1**, the assay test strip **10** is seen as a rectangular strip having (for the purpose of this description) its longitudinal axis in a vertical situation. Adjacent the lower end **11** of strip **10** is a narrow band-or zone **12** extending across the entire width of the strip. A small region **13** of strip **10** lies vertically below zone **12**. Above zone **12** is a second zone **14** lying a discrete distance up strip **10** and similarly extending the entire width of the strip. The region **15** of strip **10** between zones **12** and **14** can be of any height as long as the two zones are separate. A further region **16** of the strip extends above zone **14**, and at the top **17** of the strip is a porous pad **18** firmly linked to strip **10** such that pad **18** can act as a "sink" for any liquid sample which may be rising by capillary action through strip **10**.

Zone **12** is loaded with a first antibody bearing a visible ("direct") label (e.g. coloured latex particle, dye sol or gold sol). This reagent can freely migrate through the strip in the presence of a liquid sample. In zone **14**, the strip is impregnated with a second antibody having specificity for a different epitope on the same analyte as the first antibody. The second antibody is firmly immobilised on the strip.

FIG. **2** illustrate what happens when the assay strip is used in an analytical procedure. The lower end **11** of the dry strip is contacted with a liquid sample (not shown) which may contain the analyte to be determined. Capillary action causes the fluid to rise through the strip and eventually reach pad **18**. In so doing, the sample traverses zone **12** and the labelled antibody will dissolve or disperse in the sample and migrate with it through the strip. While migrating towards zone **14**, the labelled antibody can bind to any analyte present in the sample. On reaching zone **14**, any analyte molecule should become bound to the second antibody, so immobilising the labelled "sandwich" so produced. If a significant concentration of the analyte to be determined is present in the liquid sample, in a short period of time a

sample can be absorbed by the bottom end **11** of the test strip **20** and rise by capillary action to the top **17** of the test strip and into the sink **18**. In so doing, the liquid sample progresses via zone **12** to zone **14**. Specific binding reactions as described above occur, and the test result is visible to the user through the window **32**.

Embodiment 2

FIGS. **6** and **7** of the accompanying drawings illustrate another test device according to the invention. FIG. **6** illustrates the complete device viewed from the front, and FIG. **7** depicts the same device partially cut away to reveal details of a porous test strip contained within the body of the device.

Referring to FIG. **6**, the device comprises an elongate body **200** terminating at its lower end **201** in a small integral receptacle **202** which can hold a predetermined volume of a liquid sample, eg urine. The front face **203** of the body **200** incorporates two square small square apertures or windows **204** and **205** located one above the other.

Referring to FIG. **7**, the elongate portion of the body **200** is hollow and incorporates a test strip **206** running almost the full height of the body. This test strip is of similar construction to those described under Embodiment 1, and incorporates near its lower end **207** a horizontal zone **208** bearing a labelled specific binding reagent that can freely migrate in the strip in the moist state. There are two circular zones **209** and **210** adjacent to the windows **204** and **205** and visible therethrough. The strip terminates at its top end **211** in a porous sink **212**. At the bottom end **201** of the device, the receptacle **202** communicates with the hollow body via a lateral aperture **213**.

In operation, a liquid sample is applied to the bottom end of the device and a predetermined volume of the sample fills the receptacle **202**. From the receptacle **202** the liquid sample rises by capillary action through the test strip **206** and conveys the labelled reagent from zone **208** to the two circular zones **209** and **210**. A series of specific binding reactions as described in relation to Embodiment 1 above occur. In this embodiment the second circular zone **210** can act as a control (giving rise, for example, to a coloured signal irrespective of whether or not the sample contains the analyte to be determined) and the determination of the analyte takes place in the first circular zone **209**. The user can determine whether the analyte is present in the sample by comparing the signal produced in the two zones.

For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone **210** can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone **208** by the migrating liquid sample. The same labelled antibody can engage in a 'sandwich' reaction with hCG in the sample and be bound in the first circular zone **209** by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that he device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone **208**, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Embodiment 3

FIG. **8** of the accompanying drawings represents an isometric view of an assay device in accordance with the invention, and FIG. **9** represents a cross-sectional side elevation of the device shown in FIG. **8**.

Referring to FIG. **8**, the device comprises a housing or casing **500** of elongate rectangular form having at one end **501** a portion **502** of reduced cross-sectional area. A cap **503** can be fitted onto portion **502** and can abut against the shoulder **504** at end **501** of the housing. Cap **503** is shown separated from housing **500**. Extending beyond end **505** of portion **502** is a porous member **506**. When cap **503** is fitted onto portion **502** of the housing, it covers porous member **506**. Upper face **507** of housing **500** incorporates two apertures **508** and **509**.

Referring to FIG. **9**, it can be seen that housing **500** is of hollow construction. Porous member **506** extends into housing **500** and contacts a strip of porous carrier material **510**. Porous member **506** and strip **510** overlap to ensure that there is adequate contact between these two materials and that a liquid sample applied to member **506** can permeate member **506** and progress into strip **510**. Strip **510** extends further into housing **500**. Strip **510** is "backed" by a supporting strip **511** formed of transparent moisture-impermeable plastics material. Strip **510** extends beyond apertures **508** and **509**. Means are provided within housing **500** by webbs **512** and **513** to hold strip **510** firmly in place. In this respect, the internal 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, and porous member **506** is firmly retained in the housing and adequate fluid permeable contact is maintained between member **506** and strip **510**. The transparent backing strip **511** lies between strip **510** and apertures **508** and **509** and can act as a seal against ingress of moisture from outside the housing **500** via these apertures. If desired, the residual space **514** within the housing can contain moisture-absorbant material, such as silica gel, to help maintain the strip **510** in the dry state during storage. The reagent-containing zones in strip **510** are not depicted in FIG. **8**, but the first zone containing the labelled reagent which is mobile when the strip is moistened will lie in the region between the porous member **506** and aperture **508**. The second zone containing the immobilised unlabelled reagent will lie in the region exposed through aperture **508** in order that when the device has been used in an assay, the result can be observed through aperture **508**. Aperture **509** provides means through which a control zone containing further reagents which may enable the adequate permeation of sample through the strip to be observed.

In operation, the protective cap **503** is removed from the holder and member **506** is exposed to a liquid sample e.g. by being placed in a urine stream in the case of a pregnancy test. After exposing member **506** to the liquid sample for a time sufficient to ensure that member **506** is saturated with the sample, the cap **503** can be replaced and the device placed aside by the user for an appropriate time (e.g. two or three minutes) while the sample permeates test strip **510** to provide the analytical result. After the appropriate time, the user can observe the test strip through apertures **508** and **509** and can ascertain whether the assay has been completed by observing the control zone through aperture **509**, and can ascertain the result of the assay by observing the second zone through aperture **508**.

During manufacture, the device can be readily assembled from, for example, plastics material with the housing **500** being moulded in two parts (e.g. upper and lower halves **515** and **516**) which can be securely fastened together (e.g. by ultrasonic welding) after the porous member 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 'scrimp' the porous member and test strip together to ensure adequate contact between them. Cap **503** can be moulded as a separate complete item. If desired, apertures **508** and **509** 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 **505** of housing **500** and the protruding porous member **506**, the application of sample to the protruding member will not result in sample entering the device directly and by-passing member **506**. Member **506** 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 as a whole therefore combines the functions of samples and analyser.

By using the test strip materials and reagents as hereinafter described, a device in accordance with FIGS. **8** and **9** 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 then (after optionally replacing the cap) can observe the test result through aperture **508** within a matter of a few minutes.

Although described with particular reference to 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 **509** 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 from the spirit of the invention.

A further option is the omission of the labelled reagent from the test strip, this reagent being added to the sample prior to application of the sample to the test device. Alternatively, the labelled reagent can be contained in the protruding porous member **506**.

FIG. **10** of the accompanying drawings shows an enlarged view of the porous receiving member and test strip in the device illustrated in FIGS. **8** and **9**.

The porous receiving member **506** is linked to the porous test strip **510**, backed by the transparent plastics sheet **511**, such that liquid can flow in the direction shown by the arrows through the porous receiving member and into the porous strip. Test zone **517** incorporates the immobilised specific binding reagent, and control zone **518** contains a reagent to indicate that the sample has permeated a sufficient distance along the test strip. A portion of the test strip surface opposite the backing strip **511** and adjacent the porous receiving member **506**, carries a glaze **519** on which is deposited a layer **520** of labelled specific binding reagent. The thickness of these two layers as depicted in FIG. **10** is grossly exaggerated purely for the purpose of illustration. It will be appreciated that, in practice, the glaze may not form a true surface layer and the glazing material will penetrate the thickness of the strip to some extent. Similarly, the subsequently applied labelled reagent may also penetrate the strip. Nevertheless, he essential objective of reducing any interaction between the labelled reagent and the carrier material forming the strip will be achieved. An aqueous sample deposited in receiving member **506** can flow therefrom among the length of strip **510** and in so doing, will dissolve glaze **519** and mobilise the labelled reagent, and carry the labelled reagent along the strip and through zone **517**.

Embodiment 4

FIGS. **11** and **12** illustrate another embodiment of the invention, which is seen in plan view in FIG. **11** and in cross-section in FIG. **12**, the cross-section being an elevation on the line **1212** seen in FIG. **11**.

Referring to FIG. **11**, the test device comprises a flat rectangular casing **600** incorporating a centrally disposed rectangular aperture **601**, adjacent the left hand end **602**, and two further apertures **603** and **604** near the mid point of the device and arranged such that apertures **601**, **603** and **604** lie on the central longitudinal axis of the device corresponding to line **1212**. Although all three apertures are illustrated as being rectangular, their actual shape is not critical.

Referring to the cross-section seen in FIG. **12**, the device is hollow and incorporates within it a porous sample receiving member adjacent end **602** of casing **600** and lying directly beneath aperture **601**. A test strip of similar construction to tat described with reference to Embodiment 4, comprising a porous strip **606** backed by a transparent plastics sheet **607** is also contained within casing **600**, and extends from the porous receiving member **602**, with which the porous carrier is in liquid permeable contact, to the extreme other end of the casing. The transparent backing sheet **607** is in firm contact with the upper inner surface **608** of casing **600**, and provides a seal against apertures **603** and **604** to prevent ingress of moisture or sample into the casing. Although not shown in the drawings, the porous test strip **606** will incorporate a labelled specific binding reagent, and a test zone and a control zone placed appropriately in relation to apertures **603** and **604**, in a manner analagous to that described in Embodiment 3.

In operation, an aqueous sample can be applied through aperture **601**, e.g. by means of a syringe, to saturate porous receiving member **605**. Thereafter, the aqueous sample can permeate the test strip and after an appropriate time the test result can be observed through apertures **403** and **604**.

Embodiment 5

A yet further embodiment of the invention is illustrated in FIGS. **13** and **14** of the accompanying drawings. FIG. **13** shows a device comprising a rectangular casing **700** having in its upper surface **701** a rectangular aperture **702**. One end wall **703** of the device **703** incorporates an aperture **704** through which a porous test element communicates with the exterior of the device. Aperture **702** is situated in surface **701** at a point relatively remote from the end **703** containing the aperture **704**.

FIG. **14** shows a partially cut-away view of the device in FIG. **13**. The hollow device incorporates a porous test strip **705**, running almost the entire length of casing **700** from aperture **704**. Test strip **705** incorporates a first zone **706** containing a labelled specific binding reagent ad a further zone **707**, remote from aperture **704**, incorporating an immobilised specific reagent. Zone **706** lies directly beneath aperture **702** is therefore observable from outside casing. Beneath strip **705** and adjacent zone **707**, is a crushable element **708** containing one or more substrates or reagents which can be used to produce a detectable signal when released into zone **707**, if labelled reagent from **706** has become bound in zone **707** following use of the device. Release of the reagents from member **708** can be effected by applying pressure to the outside of the casing at that point in order to crush the member and express the reagent therefrom.

In operation, the first test element can be exposed to an aqueous sample, e.g. by dipping end **703** of casing **700** into a vessel containing the sample. The liquid sample will then permeate the length of test strip **705**, taking labelled reagent from zone **706** and passing through zone **707** where the labelled reagent can become bound e.g. through a "sandwich" reaction involving an analyte in the sample. When the sample has permeated the test strip, reagent can be released from the crushable member **708** and the result of the test observed through aperture **702**.

By way of example only, certain preferred test strip materials, reagents, and methods for their production will now be described.

1. Selection of Liquid Conductive Material

Representative examples of liquid conductive materials include paper, nitrocellulose and nylon membranes. Essential features of the material are its ability to bind protein speed of liquid conduction; and, if necessary after pretreatment, its ability to allow the passage of labelled antibodies along the strip. It this is a direct label, it may be desirable for the material to allow flow of particles of size up to a few microns (usually less than 0.5 μ). Examples of flow rates obtained with various materials are given below:

	Pore size		Time to Flow 45 mm (minutes)
Schleicher + Schuell	3μ		3.40
nitrocellulose (unbacked)	5μ		3.30
	8μ		3.00
	12μ		2.20
polyester-backed	8μ	(nominal)	3.40
Whatman Nitrocellulose	5		19.20
Pall "Immunodyne" (nylon)	3		4.00
	5		3.20

The speed of a test procedure will be determined by the flow rate of the material employed and while any of the above materials can be used some will give faster tests than others.

Nitrocellulose had the advantage of requiring no activation and will immobilise proteins strongly by absorbtion. "Immunodyne" is pre-activated and requires no chemical treatment. Papers, such as Whatman 3 MM, require chemical activation with for example carbonyldiimidazole in order to successfully immobilise antibody.

2. Labels

Preparation of Labels

A selection of labels which may be used are described below. This list is not exhaustive.

A) Gold Sol Preparation

Gold sols may be prepared for use in immunoassay from commercially-available colloidal gold, and an antibody preparation such as anti-alpha human chorionic gonadotrophin. Metallic sol labels are described, for example, in European patent specification No. EP 7654.

For example, colloidal gold G20 (20 nm particle size, supplied by Janssen Life Sciences Products) is adjusted to pH 7 with 0.22 μ filtered 0.1 M K_2CO_3, and 20 mls is added to a clean glass beaker. 200 μl of anti-alpha hCG antibody, prepared in 2 mM borax buffer pH 9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture stirred continuously for two minutes. 0.1M K_2CO_3 is used to adjust the pH of the antibody gold sol mixture to **9**, and 2 mls of 10% (w/v) BSA is added.

The antibody-gold is purified in a series of three centrifugation steps at 12000 g, 30 minutes, and 4° C., with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20 mM Tris, 150 mM NaCl pH 8.2.

B) Dye Sol Preparation

Dye sols (see, for example, European patent specification No. EP 32270) may be prepared from commercially-available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). For example, fifty grammes of dye is dispersed in 1 litre of distilled water by mixing on a magnetic stirrer for 2–3 minutes. Fractionation of the dye dispersion can be performed by an initial centrifugation step at 1500 g for 10 minutes at room temperature to remove larger sol particles as a solid pellet, with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000 g for 10 minutes at room temperature, the supernatant being discarded and the pellet resuspended in 500 mls distilled water. This procedure is repeated a further three times, with the final pellet being resuspended in 100 mls distilled water.

The spectra of dye sols prepared as described above can be measured, giving lambda-max values of approximately 657 nm for Foron Blue, and 690 nm for Resolin Blue. The absorbance at lambda-max, for 1 cm path length, is used as an arbitrary measure of the dye sol concentration.

C) Coloured Particles

Latex (polymer) particles for use in immunoasays are available commerically. These can be based on a range of synthetic polymers, such as polystyrene, polyvinyltoluene, polystyrene-acrylic acid and polyacrolein. The monomers used are normally water-insuluble, and are emulsified in aqueous surfactant so that monomer mycelles are formed, which are then induced to polymerise by the addition of initiator to the emulsion. Substantially sperical polymer particles are produced.

Coloured latex particles can be produced either by incorporating a suitable dye, such as anthraquinone, in the emulsion before polymerisation, or by colouring the preformed particles. In the latter route, the dye should be dissolved in a water-immiscible solvent, such a chloroform, which is then added to an aqueous suspension of the latex particles. The particles take up the non-aqueous solvent and the dye, and can then be dried.

Preferably such latex particles have a maximum dimension of less than about 0.5 micron.

Coloured latex particles may be sensitised with protein, and in particular antibody, to provide reagents for use in immunoassays. For example, polystyrene beads of about 0.3 micron diameter, (supplied by Polymer Laboratories) may be sensitised with anti-alpha human chorionic gonadotrophin, in the process described below:

0.5 ml (12.5 mg solids) of suspension is diluted with 1 ml of 0.1M borate buffer pH 8.5 in an Eppendorf vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for 3 minutes at 13000 rpm in an MSE microcentrifuge at room temperature. The final pellet is resuspended in 1 ml borate buffer, mixed with 3002 μg of anti-alpha hCG antibody, and the suspension is rotated end-over-end for 16–20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000 rpm, the supernatant is discarded and the pellet resuspended in 1.5 mls borate buffer containing 0.5 milligrammes bovine serum albumin. Following rotation end-over-end for 30 minutes at room temperature, the suspension is washed three times in 5 mg/ml BSA in phosphate buffered saline pH 7.2, by centrifugation at 13000 rpm for 5 minutes. The pellet is resuspended in 5 mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4° C. until used.

(A) Anti-hCG-Dye Sol Preparation

Protein may be coupled to dye sol in a process involving passive adsorption. The protein may, for example, be an antibody preparation such as anti-alpha human chorionic gonadotrophin prepared in phosphate buffered saline pH 7.4 at 2 milligram/mi. A reaction mixture is prepared which contains 100 μl antibody solution, 2 mls dye sol, 2 mls 0.1M phosphate buffer pH 5.8 and 15.9 mls distilled water. After gentle mixing of this solution, the preparation is left for fifteen minutes at room temperature. Excess binding sites

may be blocked by the addition of, for example, bovine serum albumin: 4 mls of 150 mg/ml BSA in 5 mM NaCl pH 7.4 is added to the reaction mixture, and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000 g for 10 minutes, and the pellet resuspended in 10 mls of 0.25% (w/v) dextran/0.5% (w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate is best stored in a freeze dried form.

(B) LH-Dye Sol Preparation

Due to the structural homology between the alpha subunits of hCG and LH, alpha hCG antibody can be used to detect LH in a cross-reactive immunoassay. Thus, a labelled antibody may be prepared for use in an LH assay in an identical manner to that described in Example 1, using anti-alpha hCG antibody.

3. Preparation of Reagent Strip

Zonal Impregnation of Liquid-conductive Material

Liquid-conducting material with a restricted zone of immobilised protein, particularly antibody, can be prepared for example as follows:

A rectangular sheet of Schleicher and Schuell backed 8 μ nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin of affinity Ka at 10^9 prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes at 30° C.

In one embodiment, the liquid conductive material can then be cut up into numerous strips 5 cm in length and 1 cm in width, each strip carrying a limited zone of the immobilised antibody to function as an immunosorbent part way (e.g. about half way) along its length. In this example the test strip is used with a liquid label which is mixed with sample. In use, this limited zone then becomes a test reaction zone in which the immunoassay reactions take place.

In another embodiment, the label may be dispensed/deposited into/on a restricted zone before cutting up the liquid-conductive material into strips. By way of example, this reagent may be dye sol or dye polymer-conjugated anti-hCG antibody prepared as described under dye sol preparation, said reagent being retained in the zone when the material is in the dry state but which is free to migrate through the carrier material when the material is moistened, for example, by the application of liquid sample containing the analyte to be determined. This mobile reagent zone is applied, for example, as follows:

A sheet of Schleicher and Schuell backed 8 μ nitrocellulose, 25 cm in length and 20 cm in width with zones of immobilised antibody at 5 cm intervals along its length, is prepared as described previously. Prior to the deposition of dye labelled antibody, a sublayer of, for example, 60% w/v of sucrose in distilled water is applied by airbrush on the microprocessor controlled system at 6 cm intervals along the length of the sheet. Then several passes (e.g. three) of dye labelled antibody prepared in 1% methacel KAM (Trademark for methylcellulose from Dow Chemical Company) and 0.6% (w/v) polyvinylalcohol are applied by airbrush or by microsyringe directly on top of the sublayer. Sheets are then allowed to dry, and cut into strips 5 cm in length and 1 cm in width, to be used in the completed device.

Gold sols, or coloured polystyrene particles can be deposited by a similar process.

In addition to the test zone various control zone options can be operated. For example a zone of anti-species IgG may be deposited after the test zone.

4. Sandwich Assays Using Strip Format

A sandwich-type reaction may be performed for the detection of human chorionic gonadotrophin (hCG) in a liquid sample. Preferably the label used is a direct label which is readily visible to the naked eye. Dye sols, gold sols or coloured latex particles may be linked to anti hCG antibody, as described above.

With direct labels, assays may be performed in which fresh urine samples are applied directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette to the absorbent wick of the test device. Each sample is allowed to run for five minutes in the device, and the colour generated at the reactive zone read either by eye, or using a light reflectometer.

Indirect labels such as enzymes e.g. alkaline phosphatase may also be used, but require the addition of substrate to generate a coloured endpoint.

Enzyme assays may be performed in which the anti-hCG antibody is conjugated to alkaline phosphatase, using conventional techniques, and diluted 1/100 in 0.01M phosphate buffered saline pH 7 containing 3% polyethylene glycol 6000, 1% (w/v) bovine serum albumin and 0.02% TRITON X305 (Trademark—obtainable from Rohm and Haas) before application to the sheet. Fresh urine samples are then applied, either directly from the urine stream, or by delivering an appropriate volume (e.g. 100 μl) from a container using a pipette, to the absorbent wick of the test device. Each sample is allowed to run for five minutes before a pad of liquid-swellable material soaked in BCIP substrate (at 1 mg/ml in 1M Tris/HCl pH 9.8) is placed in contact with the immobile antibody zone. After a further five minutes, the pad is removed, and colour generated read either by eye, or by using a light reflectometer.

A similar embodiment can be prepared using lutenising hormone (LH) instead of hCG.

5. Competitive Assays

A competitive type assay may be performed as exemplified by estrone-3-glucuronide, a urinary metabolite of estrone. Conjugates of estrone-3-glucuronide and bovine serum albumin are prepared as follows:

Preparation of BSA-Estrone-3-glucuronide

The conjugation of E-3-G and BSA may be achieved through the use of a mixed anhydride. All of the glassware, solvents and reagents employed in the preparation of the activated species must be thoroughly dried using an oven, dessicator or molecular sieves, as appropriate, for at least 24 hours.

Solutions of E-3-G (2 nM) in dry dimethylformamide (DMF) and tri-n-butylamine (TnB) (10 nM) in dry DMF were equilibrated separately at 4° C. Using pre-cooled glassware E-3-G in DMF (1.25 ml) and TnB in DMF (0.25 ml) were added to a pre-cooled 5 ml Reactivial containing a magnetic stirrer. A solution of isobutyl chloroformate in dry DMF (10 nM) was prepared and an aliquot (0.25 ml) was cooled to 4° C. and added to the Reactivial. The contents of the Reactivial were stirred for 20 minutes at 4° C. and a

solution of BSA (1 mg/ml) in bicarbonate buffer (0.5%) was prepared. When the mixed anhydride incubation was complete, the contents of the Reactivial were added to the BSA solution (2.5 ml) and stirred on a magnetic stirrer for 4 hours at 4° C. The conjugate preparation was purified by passage through a Tris buffer equilibrated Pharmacia PD-10 SEPHADEX G-25 column, transferred to an amber glass storage bottle and stored at 4° C.

Preparation of BSA-E-3-G dye Sol

A dispersion of dye (5% w/v) in distilled water was prepared with thorough mixing and aliquots were centrifuged at 3850 rpm (1500 g) for 10 minutes in a bench top centrifuge. The pellet was discarded and the supernatant was retained and centrifuged in aliquots at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge. The supernatant was discarded and the pellet was resuspended in half of its original volume in distilled water. This step was repeated four times to wash the pellet. The pellet was finally resuspended in distilled water and the absorbance at lambda max was determined.

Solutions of dye sol in distilled water and E-3-G/BSA conjugate diluted in phosphate buffer were mixed to give final concentrations of 10 µg/ml conjugate (based on BSA content) and an extrapolated dye sol optical density of 20 at the absorbance maximum. The reaction mixture was incubated for 15 minutes at room temperature and blocked for 15 minutes at room temperature with BSA in a NaCl solution (5 mM, pH 7.4 to yield a final BSA concentration of 25 mg/ml. The reaction mixture was centrifuged at 4850 rpm (3000 g) for 10 minutes in a bench top centrifuge, the supernatant was discarded and the pellet was resuspended in half of its original volume in Dextran (0.25% w/v)/Lactose (0.5% w/v) phosphate (0.04M pH 5.8 buffer.

Preparation of E-3-G Test Strips

Antibodies to E-3-G were deposited as described in example 3. BSA-E-3-G dye sol was deposited on the strips as described in 3.

Determination of E-3-G

Using reagents described above, a standard curve can be generated by running strips with samples with known concentrations of E-3-G. The colour at the immobile zone can be read, for example using a Minolta chromameter, and the concentration of E-3-G calculated by extrapolating from the reflectance value.

The invention described herein extends to all such modifications and variations as will be apparent to the reader skilled in the art, and also extends to combinations and subcombinations of the features of this description and the accompanying drawings.

We claim:

1. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device comprising:

a) a hollow casing having a liquid biological sample application aperture and means permitting observation of a test result;

b) a test strip comprising a dry porous carrier contained within said hollow casing, said carrier communicating directly or indirectly with the exterior of said hollow casing through said liquid biological sample application aperture to receive applied liquid biological sample, said carrier having a test result zone observable via said means permitting observation, said test strip, in the dry unused state, containing a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said label being a particulate direct label, wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said applied liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test result zone in the presence of, a material comprising a sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

migration of said applied liquid biological sample through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable via said means permitting observation, thereby to indicate the presence of said analyte in said liquid biological sample.

2. The device according to claim 1, wherein said label is selected from the group consisting of dye sols and metallic sols.

3. The device according to claim 1, wherein said labelled reagent comprises an anti-hCG antibody and said liquid biological sample is urine.

4. The device according to claim 1, wherein said labelled reagent comprises an anti-LH antibody and said liquid biological sample is urine.

5. The test device according to claim 1, wherein said dry porous carrier material is nitrocellulose.

6. The test device according to claim 5, wherein said nitrocellulose has a pore size greater than about 5 microns.

7. The test device according to claim 5, wherein said nitrocellulose has a pore size of about 8–12 microns.

8. The test device according to claim 1 which further comprises a control zone downstream from said test result zone in said dry porous carrier to indicate that said liquid biological sample is conveyed by capillarity beyond said test result zone, and a control zone observation aperture in said casing, said control zone also being observable from outside said hollow casing through said control zone observation aperture.

9. The test device according to claim 8, wherein said control zone contains a means for binding said labelled reagent which comprises specific binding means and is immobilized in said control zone.

10. The test device according to claim 1, further comprising an absorbent sink at a distal end of said dry porous carrier, said sink having sufficient absorptive capacity to absorb any labelled reagent not bound to said analyte washed out of said test zone.

11. The test device according to claim 1, wherein said means for binding is impregnated throughout said dry porous carrier in said test zone.

12. The device according to claim 1, wherein said means for binding binds directly with said first complex.

13. The test device as in claim 1, wherein said means permitting observation comprises a test result observation aperture defined through a wall of said casing.

14. The test device according to claim 1, wherein said material further comprises a protein.

15. The test device according to claim 14, wherein said protein is bovine serum albumin.

16. The test device according to claim 1, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

17. The test device according to claim 1, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

18. The test device according to claim 1, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

19. The test device according to claim 18, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

20. An analytical method comprising:

contacting an analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device including:

a) a hollow casing having a liquid biological sample application aperture and means permitting observation of a test result;

b) a test strip comprising a dry porous carrier contained within said hollow casing, said carrier communicating directly or indirectly with the exterior of said hollow casing through said liquid biological sample application aperture to receive applied liquid biological sample, said carrier having a test result zone observable via said means permitting observation, said test strip, in the dry unused state, containing a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said label being a particulate direct label, wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said applied liquid biological sample,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test result zone in the presence of, a material comprising a sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

said carrier containing in said test result zone a means for binding said first complex, said means for binding comprising specific binding means and being immobilized in said test result zone;

migration of said applied liquid biological sample through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said binding means binds said first complex thereby to form a second complex;

said second complex being observable via said means permitting observation, thereby to indicate the presence of said analyte in said liquid biological sample, with said liquid biological sample suspected of containing said analyte;

allowing said liquid biological sample to convey by capillary action through said dry porous carrier into said test result zone and said labelled reagent is conveyed therewith;

and detecting the presence of said analyte in said liquid biological sample by observing the presence of any labelled reagent-analyte complex bound in said test result zone.

21. The method according to claim 20, wherein said dry porous carrier is a strip of dry porous material, and has a control zone downstream from said test result zone, to indicate to a user that the test has been completed; and said hollow casing has a second observation aperture through which said control zone is visible and wherein said liquid biological sample is a urine sample whereby urine is conveyed by capillarity through said dry porous carrier into said test result zone and said control zone, and said test result is obtained by observing the extent to which said labelled reagent-analyte complex becomes bound in said test result zone.

22. The method according to claim 21, wherein said control zone contains a means for binding said labelled reagent which comprises specific binding means and is immobilized in said control zone.

23. The method according to claim 20, wherein said material further comprises a protein.

24. The method according to claim 23, wherein said protein is bovine serum albumin.

25. The method according to claim 20, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

26. The method according to claim 20, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

27. The method according to claim 20, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

28. The method according to claim 27, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

29. An analytical test device for detecting an analyte suspected of being present in a liquid biological sample, said device comprising:

a) a hollow casing having a liquid biological sample aperture, a test result observation aperture, and a control zone observation aperture;

b) a test strip comprising a dry porous carrier contained within said hollow casing, said carrier communicating directly or indirectly with the exterior of said hollow casing through said liquid biological sample aperture to receive applied liquid biological sample, said carrier having a test result zone observable through said test result observation aperture and a control zone observable through said control zone observation aperture, said test strip, in the dry unused state, containing a labelled reagent capable of specifically binding with said analyte to form a first complex of said labelled reagent and said analyte, said label being a particulate direct label, wherein said labelled reagent is dry on said test strip prior to use and is released into mobile form by said applied liquid biological sample;

c) an absorbent sink at a distal end of said dry porous carrier, said sink having sufficient absorptive capacity to allow any labelled reagent not bound to said analyte to wash out of said test zone;

said carrier containing in said test result zone a means for binding said first complex, said means for binding said first complex comprising specific binding means and being immobilized in said test result zone, said carrier containing in said control zone a means for binding said labelled reagent, said means for binding said labelled reagent being immobilized in said control zone,

wherein mobility of said labelled reagent within said test strip is facilitated by at least one of 1) coating at least

a portion of said test strip upstream from said test result zone with, or 2) drying said labelled reagent onto a portion of said test strip upstream from said test result zone in the presence of, a material comprising a sugar, in an amount effective to reduce interaction between said test strip and said labelled reagent;

migration of said applied liquid biological sample through said dry porous carrier conveying by capillarity said first complex to said test result zone of said dry porous carrier whereat said means for binding said first complex binds said first complex, thereby to form a second complex;

said second complex being observable through said test result observation aperture, thereby to indicate the presence of said analyte in said liquid biological sample.

30. The test device according to claim 29, wherein said control zone contains a means for binding said labelled reagent which comprises specific binding means and is immobilized in said control zone.

31. The method according to claim 29, wherein said material further comprises a protein.

32. The method according to claim 31, wherein said protein is bovine serum albumin.

33. The test device according to claim 29, wherein said sugar is selected from the group consisting of sucrose, lactose and dextran.

34. The test device according to claim 29, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with polyvinyl alcohol.

35. The test device according to claim 29, wherein mobility of said labelled reagent within said test strip is further facilitated by blocking excess binding sites within said test strip with a protein.

36. The test device according to claim 35, wherein said protein is selected from the group consisting of bovine serum albumin and milk protein.

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