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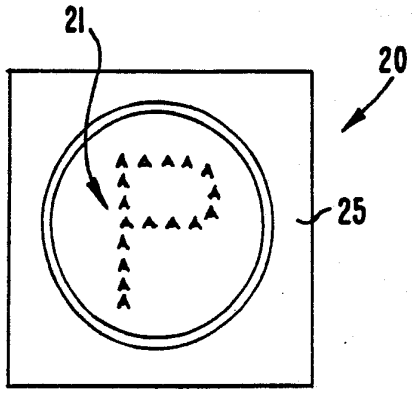
(57) Abstract



(57) Abstract

A simple method and apparatus for determining the presence of an antigen or antibody of interest in the liquid sample can include providing a first means (10) comprising a porous member (14) having associated therewith one of an antigen-antibody pair (A), applying to the porous membrane (14) a liquid sample to be tested for the other of the antigen-antibody pair, providing a second means (18) comprising a liquid suspension comprising a plurality of visually perceptible micro particles, preferably polystyrene particles, having attached thereto the one of the antigen-antibody pair associated with the porous membrane, and applying said liquid suspension to the porous membrane (14) after the application of said liquid sample to said membrane (14) to produce a visual indication if the other of the antigen-antibody pair is present in the liquid sample.

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"Immunoassay and Apparatus Having a Visual Readout"

Technical Field

This invention relates to assay methods and apparatus and, more particularly, to immunoassay methods and apparatus for developing a visual indication of the presence of an antigen or an antibody in a sample to be tested.

Background Art

A variety of methods and apparatus have been developed for determining the presence of antibodies or antigens of interest in a clinical sample. Such methods and apparatus have taken a number of forms, including kits for performing solid-phase immunoassays employing labels creating a visual endpoint signal. The method and apparatus for performing solid-phase assay with visual labels have been assembled in kit form so that they may be used conveniently inside as well as outside, the clinical laboratory. Such methods and apparatus have, generally, relied upon the affinity of one member of an antigen-antibody pair to bind with or to the other member of the antigen-antibody pair. In such prior methods for identifying one of the antigen-antibody pair, a man-made substrate, such as a nylon, nitrocellulose polyester or glass fiber membrane, carrying the other of the antigen-antibody pair was exposed to a liquid sample to be tested, possibly containing the member of the antigen-antibody pair of interest, to provide a solid-phase support area having adherent or bound thereto the member of the antigen-antibody pair of interest, if present in the liquid sample. A visual indication of the presence of the member of the antigen-antibody pair of interest was then developed by a number of subsequent steps including washing the solid-phase support, supplying to the solid-phase support a solution containing the other of

the antigen-antibody pair, which was labeled with, for example, an enzyme, and then supplying a solution of a chromogenic substance for the enzyme to generate a visible change of color resulting from the presence of the enzyme on the membrane surface to indicate the presence of the antigen or antibody of interest, if present in the sample.

In practice, such methods and kits have included generally a porous membrane in combination with a mesh and an absorbent pad. The porous membrane has had a portion of its surface treated with an antigen or antibody capable of bonding with an antibody or antigen of interest. Such kits may include solutions for preparing a liquid sample, for washing the membrane, for providing labeled antibody or antigen as appropriate, and for developing a visual indication of the label; in some kits, applicators to apply the liquid sample and solutions. Prior patents disclosing immunoassay methods, apparatus, and kits include U.S. Patents Nos. 3,645,687; 3,843,324; 3,966,897; 4,039,652; 4,061,468; 4,094,647; 4,125,372; 4,138,474; 4,153,675; 4,168,116; 4,180,383; 4,193,983; 4,235,601; 4,376,110; 4,424,279; 4,427,769; 4,486,530; 4,818,677; 4,632,901.

As set forth above and in said patents, the prior methods and kits for producing visual indications of the presence of an antigen or antibody of interest included a multiplicity of steps in which the membrane was washed with buffers, and various reagents were applied following application of the sample to the membrane to develop a visual indication of the presence of an antigen or antibody of interest. In addition to the steps of washing the membrane with buffers and reagents, the various reagents of these prior existing methods and apparatus were not as stable as desired. Prior kits, therefore, required in addition to the antigen-antibody-treated membrane a plurality of

reagents, some of which were susceptible to short shelf lives.

Disclosure of the Invention

This invention provides a method and apparatus for determining the presence of one of an antigen-antibody pair with one or two simple steps and a single, extremely stable reagent. The invention can comprise a kit having only two active parts and simple instructions for use.

An assay kit of the invention can comprise a first means for providing an indication of the presence of an antigen or antibody of interest and a second means for developing the indication of the presence of the antigen-antibody pair in a sample. The first means can include a cartridge forming one or more openings and a porous material adjacent to the one or more openings. The porous material adjacent to at least one of the one or more openings has associated therewith one of the antigen-antibody pair. The second means provides colored microparticles having attached thereto said one of the antigen-antibody pair. The second means permits the retention of the colored micro particles by the first means if the porous material of the first means has been exposed to the other of the antigen-antibody pair and provides a visual indication of the presence of the antigen or antibody of interest.

A preferred apparatus of the invention is an assay kit comprising an enclosure for a porous membrane having one or more openings. The porous membrane within the enclosure has one of an antigen-antibody pair associated therewith at least immediately adjacent to one of the enclosure openings. The kit also comprises a liquid suspension comprising a plurality of colored latex particles having attached said one of the antigen-antibody pair, which is associated with the porous membrane. The kit can thus provide a visual

indication of the presence of the other of the antigen-antibody pair in a liquid sample by the application to the porous membrane at the one or more openings of said liquid suspension after a liquid sample containing said other of the antigen-antibody pair has been applied at the one or more openings of the enclosure.

The invention thus provides a simple method for determining the presence of an antigen or antibody of interest and the concentration of an antigen or antibody of interest in the liquid sample by providing a porous member having associated therewith one of an antigen-antibody pair, applying to the porous membrane a liquid sample to be tested for the other of the antigen-antibody pair, providing a liquid suspension comprising a plurality of colored micro particles, preferably polystyrene particles, having attached one of the antigen-antibody pair associated with the porous membrane, and applying said liquid suspension to the porous membrane after the application of said liquid sample to said membrane to produce a visual indication if the other of the antigen-antibody pair is present in the liquid sample. The concentration of the antigen or antibody of interest may be quantified by measuring the reflectance of the visual indication and determining the concentration from the measured reflectance.

In addition, with some antigens and some antibodies, the invention can provide a visual indication of the presence of an antigen or antibody of interest by directly adding the sample to be tested into the liquid suspension which includes the colored micro particles, preferably latex particles, having attached thereto the antibody or antigen for the antigen or antibody of interest, and then applying to the porous membrane having associated therewith the antibody or antigen for the antigen or antibody of interest, thus eliminating the preparation of a separate liquid sample and the

application of the separate liquid sample to the membrane.

Brief Description of Drawings

Fig. 1 is a plan view of one embodiment of a first means for providing an indication of the presence of an antigen or antibody of interest;

Fig. 2 is a cross-sectional view of the means of Fig. 1 at a plane through line 2-2 of Fig. 1;

Fig. 2A is a larger scale view of a portion of the means of Figs. 1 and 2 taken adjacent opening 11 of Fig. 1 to show membrane 14 and its backing;

Fig. 3 is a perspective view of a kit of this invention;

Fig. 4 is a plan view of another embodiment of first means for providing the indication of an antigen or antigen of interest; and

Fig. 5 is a graph showing the quantification of concentration by reflectance.

Best Mode for Carrying out the Invention

Fig. 1 is a plan view of one embodiment of first means for providing an indication of the presence of an antigen or antibody of interest comprising this invention, and Fig. 2 is a cross-sectional view of the means. As shown in Fig. 1, such means can comprise a cartridge 10 forming one or more openings, preferably the three openings 11, 12, and 13. Cartridge 10 carries a porous membrane 14. (See Fig. 2A.) Porous membrane 14 has associated therewith one of an antigen-antibody pair adjacent at least one of the one or more openings and, preferably, as shown in Fig. 1, openings 11 and 13. The one of the anti en-antibody pair is indicated in Figs. 1 and 2A by the letters A shown adjacent openings 11.

As shown in Figs. 2 and 2A, porous membrane 14 is backed by a mesh 15, preferably made of polyester,

although other similar materials may be used. An absorbent material 16 may be carried by cartridge 10 immediately under mesh 15. As shown in Figs. 1 and 2, cartridge 10 is formed by a relatively thick, e.g., 1/4 inch (.63 cm.) plastic top portion 10a and a lower, box-shaped, plastic bottom portion 10b. Cartridge 10 may be formed with any inexpensive, easily molded, thermoplastic material such as polystyrene, polyethylene, polypropylene, nylon, and the like and its bottom is preferably closed by any liquid in porous backing 17. Membrane 14 may be any commercially available micro-porous membrane such as that sold under the IMMOBILON trademark by Millipore Corporation, Bedford, Massachusetts, that sold under the IMMUNODYNE trademark by Pall Biosupport Corporation, East Hills, New York, or that sold under the HPI name by Amicon Division of W. R. Grace & Co. of Danvers, Massachusetts. Such membranes preferably have pore sizes of 0.6 to 5 micrometers. Any inexpensive absorbent materials, such as cotton or other celluosic materials, may be used for element 16.

As shown in Figs. 1 and 2, top portion 102 of cartridge 10 forming openings 11, 12, and 13 preferably is formed with sloping walls 11a, 12a, and 13a as shown in Fig. 2 to provide "wells" for container-like retention of liquid materials, such as a liquid sample and developing solution that may be applied to membrane 14 at openings 11 and 13.

As set forth above, where it is desirable to test for an antibody of interest, an antigen for that antibody is associated with membrane 14 adjacent at least one of the one or more openings of first means 10; and where it is desirable to test for an antigen of interest, an antibody for that antigen is associated with membrane 14 adjacent one of the one or more openings of cartridge 10. As shown in Fig. 1, such an antigen and antibody is associated with membrane 14

adjacent openings 11 and 13 to permit cartridge 10 to be used for two tests. Membrane 14 is preferably provided at opening 12 with a sample of the visual indication of a positive test to permit comparison of the test results at openings 11 and 13 with the visual indication of a positive test at opening 12.

For example, when the invention is adapted to test for chlamydia, the following two methods can be used for coupling antibodies to membrane 13.

Examples I and II

Coupling of Antibody to Membrane

In the first example, antibodies are coupled covalently to membranes with active functional groups in the following manner: commercially available membrane 13, such as IMMOBILON (Millipore Corporation, Bedford, MA) or IMMUNODYNE (Pall Biosupport Corporation, East Hills, NY), with pore sizes of 0.65-3.0 um is cut into pieces of approximately 2.54 cm. x 17 cm. and immersed in a solution (potassium phosphate buffer (0.5M, pH=7.4)) of IgG antibodies raised against C, E and F serovars of chlamydia trachomatis, or against a specific surface antigen such as the major outer membrane protein (MOMP), or the 60K protein isolated from the F-serovar and incubated in the antibody solution with gentle shaking for one to twenty-four hours at room temperature. The concentration of the antibody solution is approximately 0.31-1.9 ugram per cm^2 of membrane surface area. Excess antibody solution is removed; and after one wash with deionized water, the membrane is immersed in a ten percent solution of 2-aminoethanol in carbonate buffer (0.01M, pH-9.5) and incubated with gentle shaking for one to sixteen hours. Excess 2-aminoethanol solution is removed, and the membrane is washed twice with deionized water and air dried. The membrane and associated antibodies are ready for incorporation into the first means for indicating the presence of a chlamydia antigen.

In the second example, antibodies are absorbed non-covalently on a nylon-type membrane having a pore size of 1.2 u to 5 u such as the HPI membrane of Amicon Division of W. R. Grace & Co. identified above in the following manner:

The same antibodies used for Example I are used. Approximately 1-100 uL of antibody solution in phosphate-buffered saline (0.04M, pH=7.4) is placed on a nylon-type membrane with no functional groups and allowed to air dry at room temperature. The concentration of antibody solution is about 0.79 to 44ug per cm^2 of the membrane surface area. After about sixteen to twenty-four hours of drying, the membrane is ready to be used in the assay.

A liquid suspension for detecting the presence of, or developing a visual indication of, an antigen or antibody of interest on the membrane comprises the second means of this invention. Such a liquid suspension includes a multiplicity of microscopically small (i.e., about 30 to about 200 nanometers in diameter) particles that are preferably visible with the unaided eye in the aggregate or, less preferably, may become visible with the use of instrumentation or be made visible with ultraviolet light. Such visually perceptible particles have associated with their surfaces a substance adapted to bond to the antigen or antibody of interest, preferably the other of the antigen-antibody pair.

Where the assay method is used to test for the presence of an antibody of interest, it is not necessary that the visually perceptible particles have associated with their surfaces the antigen for the antibody of interest. The particles can have associated with their surfaces a ligand capable of bonding with the antibody such as those present on a number of proteinaceous substances.

The preferred second means is based upon a suspension of micro particles, preferably colored polystyrene particles, having an antibody or antigen (for the antigen or antibody of interest) coupled to the particle surfaces. A preferred second means can be made as follows:

Example III

Coupling of Antibody to Particles

Approximately 10 to 13 percent suspension of blue polystyrene particles (60-100 nm in diameter), available from the Particle Technology Division of Seradyn, Inc., is first washed to remove surfactants. A preferably blue dye for the particles is Keyplast Blue B 200% made by Keystone Analine Corporation of Chicago, Illinois. The washed particles are diluted in phosphate-buffered saline (PBS, 0.04M, pH=7.4) to a final concentration of one percent. Monoclonal mouse IgG's raised against surface antigens, such as MOMP and 60-K protein, are added to obtain a final concentration of 5-50 ug/mL of IgG's. The mixture is mixed and kept continuously agitated for two to sixteen hours at room temperature. The optimum dilution of the antibody coupled particles to be used in a chlamydia assay is determined in the following manner: two positive control solutions of chlamydia trachomatis elementary bodies are prepared by first treating a suspension of chlamydia trachomatis elementary bodies of F-serovar with detergents such as sodium dodecyl sulfate (SDS) and Triton X-100. These detergents are believed to break the bacterial cell walls and expose the surface antigens so that they are more accessible to the antibodies. The treated suspension of elementary bodies is then diluted to give protein concentrations of 1 ug/mL and 5ug/mL, which seem to represent the protein concentration of elementary bodies in urethral or endocervical specimens from moderately infected and heavily infected chlamydia

patients, respectively. The detergent solution without any elementary bodies is used as a negative control.

Several dilutions of the stock solution of antibody-coupled particles are made in Tris-buffered saline (0.02M, pH=7.4) containing (0.05%) non-ionic detergents such as Tween-20.

Assays are performed according to the procedure described in Example IV. Dilutions that give the best color differentiation between the negative control and the positive controls are chosen for use in the assay tests.

Example IV

Assay Procedure for Chlamydia Antigen Detection

A test cartridge is prepared by assembling the antibody-coated membrane, prepared as set forth in Example I or Example II, the mesh spacer and absorbent pads together as shown in Figs. 1, 2 and 2A. Endocervical and urethral swab specimens are incubated in 0.75 mL of Tris buffer (0.02M, pH=7.4) containing 0.25 percent SDS and 0.05 percent Triton X-100 for ten minutes at room temperature and agitated on a vortex mixture at a moderate speed for 30 to 45 seconds. Swabs are then removed, and each extract is added to the sample well of the test cartridge. After the extract has completely drained through the membrane, a solution of antibody coupled blue particles, prepared by the method described in Example II is added to the sample well of the test cartridge. A blue color is observed on the membrane indicating the presence of chlamydia trachomatis. When the preferred cartridge of Figs. 1, 2 and 2A is used, the central opening 12 is provided with blue particles for reference purposes; and a positive test is indicated where the membrane showing at opening 11 or at opening 13 is a darker blue from the reference showing at opening 12.

The optimal dilution of antibody-coupled particles for developing a strong, contrasting blue color as a

visual indication of chlamydia trachomatis is thus preferred. Such a dilution can be packaged in vials or other containers for incorporation into an assay kit as a chlamydia antigen-detecting and -developing material. Such detecting and developing materials are stable at room temperature. In addition, they can tolerate temperature extremes, such as 45° C. for a number of weeks and can be frozen at -20° C. and still retain their functionality.

Fig. 3 shows a packaged assay kit of the invention with first means or cartridge 10 and a container 18 of the second means or the detecting or developing material. The two means of the invention can be packaged in any convenient manner, including a carrier 19 shown in Fig. 3.

In testing for the chlamydia antigen using the invention, a sample can be prepared by taking endocervical or urethral swabs taken from a patient and incubating the swabs in a buffer solution such as 0.75 mL of Tris buffer (0.02 M, pH-7.4) containing 0.25 percent SDS and 0.05 percent Triton X-100 for ten minutes at room temperature and then agitating the buffered swab solution, for example, in a vortex mixture at moderate speed for 30 to 45 seconds. The resulting solution comprises the sample to be tested. In use of the kit form of the invention, the sample solution is added to at least the well formed by walls 11a of cartridge 10 and drains through membrane 14 at opening 11, exposing any chlamydia antigen in the sample to the antibodies coupled to membrane 14 at opening 11. Mesh 15 permits the sample to pass through the porous membrane 15 and subsequently blocks the sample material in the absorbent material 16 from the porous membrane 14. If chlamydia antigen is present in the sample, it will become bound to or associated with the antibodies on membrane 14 at opening 11. The detecting solution is next poured into the well formed

by walls 11a and drained through membrane 14 at opening 11. If chlamydia antigen is present in the sample, the antibody-coupled, blue, polystyrene particles in the detecting solution will become associated with the chlamydia antigen present on membrane 14 at opening 11 and will be retained at opening 11, providing a blue color at opening 11, indicating the presence of chlamydia antigen. Opening 12 permits comparison of the test results with a reference positive result.

The method and apparatus of this invention can also be used to test for gonorrhea and other bacteria, hormones, therapeutic drugs, cancer markers, drugs of abuse, and other such tests.

Fig. 4 shows another embodiment of first means. The Fig. 4 embodiment of the first means includes a cartridge 20 having an upper part 25 forming only one opening 21. A membrane 24 is made of the same IMMOBILON or IMMUNODYNE materials identified above and is carried by cartridge 20 adjacent opening 21. Cartridge 20 can be provided with a mesh and an absorbent material in a lower box-like part in the same manner as shown in Figs. 2 and 2A. Unlike the first means of Figs. 1 and 2, only a portion of the surface of membrane 24 of the Fig. 4 embodiment is provided with antibodies or antigens for the antigen or antibody of interest. As shown, for example, in Fig. 4, membrane 24 can be treated with antibodies for an antigen interest over only a portion of the membrane that can be seen through opening 21. As shown in Fig. 4, the antibodies are applied in the form of the capital letter "P". The antibodies can be added to a portion of membrane 24 using the steps set forth above in Examples I and II and, preferably, the steps set forth in Example II. The developing solution for use with the first means of Fig. 4 is the same developing solution described above that is made and selected as set forth in Examples III and IV. The Fig. 4 embodiment is used in the same

manner described above with the embodiments of Figs. 1-3, but instead of providing a blue indication of a positive test for chlamydia antigen over the entire opening, as was the case at openings 11 and 13 of the first means of Figs. 1 and 2, the embodiment of Fig. 4 provides a positive indication of the presence of chlamydia antigen in the sample by developing a blue letter P that stands out against the white background of membrane 24.

While the preferred embodiment provides a visual indication through the use of colored latex particles, the presence of which is visible to the unaided eye under room light, the invention may include micro particles in the developing solution which are not visible with the unaided eye in normal room light but become visible only with the use of artificial light or mechanical means such as particles that fluoresce (particles coupled with fluorescent dyes such as Fluorescein or Rhodamine) in ultraviolet light or by photometry, microscopy, or other such means of assistance. Furthermore, the invention may be used in a clinical laboratory with a vacuum system to urge the liquid sample and detecting-developing material through the substrate without the mesh and absorbent material.

Example V

Measurement of Concentration

The dose response of samples in the assay procedure described in Example IV can be measured by measuring percent reflectance of the blue color developed over the entire test area of a membrane, using a reflectance meter such as the Model 577, Model 575 or Model 670 Reflection Meters manufactured and sold by Seradyn Photovolts Division. Such reflection meters direct light at a sample from one side at an acute angle and measure the light reflected from the sample to the other side at an equal acute angle. The following table shows some typical dose response data

for the negative and the two positive controls described in Example III. The positive visual indication was provided by the detecting solutions of Example III with Keyplast Blue B 200% dye made by Keystone Analine Corporation, Chicago, Illinois. A Model 577 Reflection Meter was used with an amber filter (maximum transmission of light at 600 nm). The wavelength of the filter was chosen to provide optimal measurement of reflectance from the blue dye. The instrument was calibrated with a Tappi Control to read maximum reflectance of 90.4% as specified.

Sample	Average Percent Reflections
Negative Control	65.4
Low Positive Control (1 ug/ml)	61.3
High Positive Control (5 ug/ml)	50.2

Thus, the correlation between reflectance and antibody or antigen concentration may be plotted as shown in Fig. 5 and used to quantify the concentration in a sample.

While the description above presents presently preferred embodiments of the invention, other embodiments may be apparent within the scope of the following claims.

Claims

1. An assay method for determining the presence of an antigen or antibody in a liquid sample, comprising:

providing a porous membrane having associated therewith one or an antigen-antibody pair;

providing a flow of liquid sample to be tested for the other of the antigen-antibody pair through said porous membrane;

providing a liquid suspension comprising a plurality of visually perceptible particles having attached to their surfaces a substance capable of bonding to said other of the antigen-antibody pair; and

providing a flow of said liquid suspension through said porous membrane after the flow of said liquid sample through said membrane to produce a visual signal if said liquid sample includes the other of the antigen-antibody pair.

2. The assay method of claim 1 wherein said visually perceptible particles have attached to their surfaces said one of the antigen-antibody pair.

3. The assay method of claim 2 wherein the visually perceptible particles are colored styrene particles having average diameters in the range of about 60 nanometers to about 100 nanometers.

4. An assay kit, comprising:

an enclosure for a porous membrane, said enclosure having a plurality of openings;

a porous membrane within said enclosure, said porous membrane having one of an antigen-antibody pair associated therewith, at least immediately adjacent to one of said plurality of enclosure openings; and

a liquid suspension comprising a plurality of colored styrene particles having attached thereto said one of the antigen-antibody pair,

said assay kit being functional to provide a visual indication of the presence of the other of the antigen-antibody pair in a liquid sample by applying to the porous membrane at the plurality of openings said liquid suspension after a liquid sample containing said other of the antigen-antibody pair.

5. The assay kit of claim 2 wherein said enclosure has three openings and said porous membrane has associated therewith said one of the antigen-antibody pair adjacent to two of the three openings.

6. The assay kit of claim 2 wherein said enclosure encloses a mesh and an absorbent material under said porous membrane.

7. The assay kit of claim 2 wherein said styrene particles have average diameters in the range of about 30 nanometers to about 200 nanometers.

8. The assay kit of claim 2 wherein said porous membrane is capable of covalently binding to or physically absorbing or adsorbing antibodies or antigens on its surface.

9. The assay kit of claim 5 adapted to test for the chlamydia antigen wherein said porous membrane has associated therewith an antibody of the chlamydia antigen and said colored styrene particles have attached an antibody for the chlamydia antigen.

10. An assay kit, comprising:

first means for providing a visual indication of the presence of an antigen or antibody of an antigen-antibody pair,

said first means including a carrier forming one or more openings and a porous material adjacent the one or more openings having one of an antigen-antibody pair associated therewith adjacent at least one of said one or more openings; and

second means for developing said indication of the presence of an antigen-antibody pair, said second means providing visually perceptible micro particles having attached thereto said one of the antigen-antibody pair, said second means permitting the retention of visually perceptible particles by said first means after its exposure to the other of the antigen-antibody pair to provide said visual indication.

11. The assay kit of claim 10 wherein said carrier forms a plurality of openings and said porous material has said one of the antigen-antibody pair associated with the porous material adjacent only one of the plurality of openings.

12. The assay kit of claim 10 wherein said porous material is capable of covalently binding to or physically absorbing or adsorbing antibodies or antigens on its surface.

13. The assay kit of claim 10 wherein said visually perceptible micro particles are colored polystyrene.

14. The assay kit of claim 10 adapted to test for chlamydia or gonorrhea antigen.

15. The assay kit of claim 10 wherein said visually perceptible micro particles are fluorescent.

16. The assay kit of claim 10 wherein the second means is stable at room temperature and above.

17. An assay method for an antigen, comprising:

providing a porous membrane having associated therewith one of the antibody pairs where one or both of the antibody pairs can be monoclonal or polyclonal;

providing a flow of a liquid sample to be tested for the antigen of interest through said porous membrane;

providing a liquid suspension comprising a plurality of colored particles having attached thereto the other of the antigen-antibody pair; and

providing a flow of said liquid suspension through said porous membrane after the flow of said liquid sample through said membrane to produce a visual signal of the antigen.

18. An assay method for an antibody, comprising:

providing a porous membrane having associated therewith an antigen specific for the antibody to be detected;

providing a flow of a liquid sample to be tested for the antibody of interest through said porous membrane;

providing a liquid suspension comprising a plurality of colored latex particles having attached thereto a ligand capable of binding to the antibody to be tested; and

providing a flow of said liquid suspension through said porous membrane after the flow of said liquid sample through said membrane to produce a visual signal of the antibody.

19. An immunoassay kit for testing for the chlamydia antigen, comprising:

first means for providing a visual indication of the presence of chlamydia antigen,

said first means including a carrier forming one or more openings and a porous material adjacent the one or more openings having antibodies for the chlamydia antigen associated therewith adjacent to at least one of said one or more openings; and

second means for developing said indication of the presence of the chlamydia antigen,

said second means providing visually perceptible micro particles having attached thereto antibodies for the chlamydia antigen, said second means permitting the retention of visually perceptible particles by said first means after its exposure to the chlamydia antigen to provide said visual indication.

20. An assay method for chlamydia antigen, comprising:

providing a porous membrane having associated therewith antibodies for the chlamydia antigen;

applying to said porous membrane liquid sample to be tested for the chlamydia antigen;

providing a liquid suspension comprising a plurality of colored polystyrene particles having an average diameter of about 60 to about 100 nanometers and having attached to their surfaces antibodies for the chlamydia antigen; and

applying said liquid suspension to said porous membrane after the application of said liquid sample to said membrane to produce a visual signal of the chlamydia antigen.

21. An assay method for determining the concentration of an antigen or antibody in a liquid sample, comprising:

providing a porous membrane having associated therewith one or an antigen-antibody pair;

applying to said porous membrane a liquid sample to be tested for the other of the antigen-antibody pair;

providing a liquid suspension comprising a plurality of visually perceptible particles having attached to their surfaces a substance capable of bonding to said other of the antigen-antibody pair;

applying said liquid suspension to said porous membrane after the application of said liquid sample through said membrane to produce a visual signal if said liquid sample includes the other of the antigen-antibody pair; and

measuring the reflectance of said visual signal and determining the concentration of antigen or antibody in said liquid sample.

22. The assay method of claim 21 wherein said visually perceptible particles are colored and said measurement of reflectance is by directing a light of selected wavelength and known intensity at the membrane surface and colored particles and measuring the intensity of the light of selected wavelength reflected from the membrane surface and colored particles.

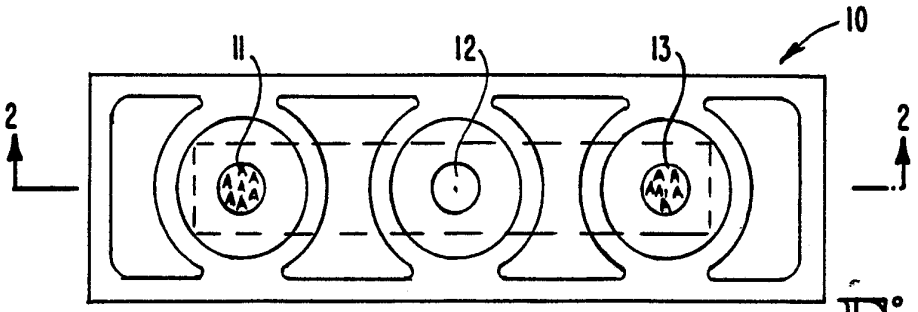

Fig.1

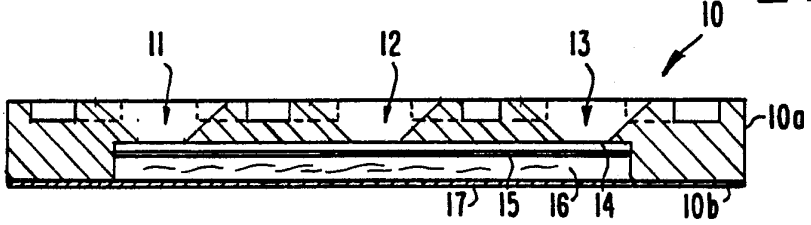

Fig.2

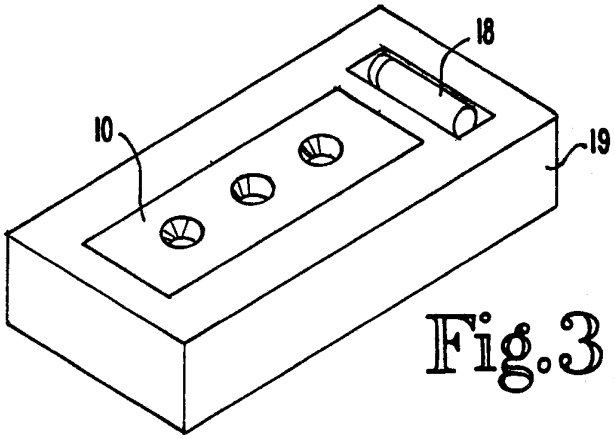

Fig.3

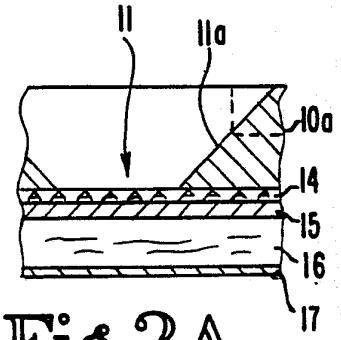

Fig.2A

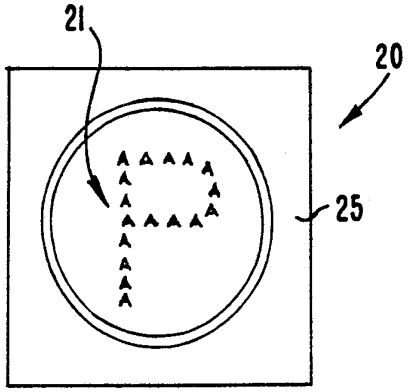

Fig.4

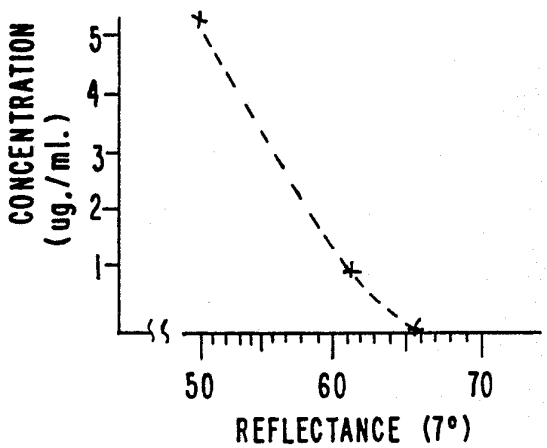

Fig.5

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/03025

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : G01N 33/543,544,545,546,553,53

U.S. Cl : 435/7; 436/518,523,525,528,531,533-535; 422/61

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/7,805,810; 422/55,58,61; 436/518,523,525,528,531,533-535, 807-810

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

CAS, APS DATABASES

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US,A 4,727,019 (VALKIRS ET AL) 23 February 1988 Note column 3, line 23- column 5, line 23; column 6, lines 32-60; claims 103,111-114.	1,2,17,18 3,4-16,19, 20-22
X,P Y,P	US,A 4,920,046 (MCFARLAND ET AL) 24 April 1990 Note Abstract; column 1, line 46 - column 2, line 9; column 4, lines 7-54; column 7, line 41 - column 8, line 9; column 8, line 62 - column 9, line 11; column 9 lines 31-53.	1-5,7,8,10-13,15,18 6,9,14,16,17, 19-22
Y,P	US,A 4,859,612 (COLE ET AL) 22 August 1989 Note column 6, lines 37-57 and 61-64; column 7, lines 3-29; column 8, lines 12-16 and 66-67; column 16, lines 28-55; column 17, lines 11-38.	1-22
Y	EP,A 0,227,173 (JANSSEN PHARMACEUTICA N.V.) 01 July 1987, Note page 2, line 48 - page 3, line 11; page 8, lines 20-21; page 10, lines 15-30 and 55-58. (CON'T)	1-22

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
06 July 1990	19 NOV 1990
International Searching Authority [1]	**Signature of Authorized Officer**
ISA/US	NGUYEN NGOC-HO INTERNATIONAL DIVISION by Carol A. Spiegel Ngoc-Ho Nguyen

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	EP	0,293,779 (DAIICHI PURE CHEMICALS CO. LTD) 07 December 1988, Note page 3, lines 47-52; page 5, lines 17-36.	3,7,10, 20-22

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...., because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.