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(54) Title: HIV ANALYSIS METHOD AND DEVICE

(57) Abstract

A method and device for immunoassay analysis in general and in particular for immunoassay detection of HIV antibodies in whole blood, using a flexible plastic pouch that is permanently sealed around its perimeter, except for the top, and is divided into one or more reaction compartments and one or more waste compartments. The device is provided with apertures adapted for the insertion of straws or other channels for the introduction of reagents and specimens. In addition the device is provided with means, such as a filter, for retaining certain components of the reaction products within the reaction compartment and permitting the waste products of the reaction to pass into the waste compartment. After completion of the analysis, the device is sealed to contain the specimen and reactants so that the entire device may be disposed of safely.

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HIV ANALYSIS METHOD AND DEVICE

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates generally to the performance of medical diagnosis using transparent flexible envelopes and particularly to immunoassay analysis for HIV using transparent flexible envelopes.

Brief Description of the Prior Art

The prior art discloses a variety of devices for handling, processing, and testing of chemical, biological, or medical specimens within transparent, compartmented containers. Such devices include flexible, tubular containers having a series of individual compartments within which reagents may be contained and isolated until a specimen to be analyzed is added to the device.

It is also known to provide means for manipulating such devices to permit specimens and/or reagents to sequentially react within the same or successive compartments. Thus a specimen may be added to a first compartment containing a first reagent and allowed to react with such first reagent. The analyst may observe the reaction, and by color changes, precipitants, or other observable changes determine charactistics of the specimen.

After the initial reaction, the device may be manipulated to permit the specimen to pass from the first compartment to successive compartments and to react with successive reagents for subsequent observations.

The detection of HIV antibodies is commonly accomplished through immunoassay analysis. Immunoassay analysis consists of exposing samples to antigens which will bind to the antibodies and then detecting the presence of the antibodies by using enzyme immunoassay detection systems.

SUMMARY OF THE INVENTION

This invention provides an improved method and device for immunoassay analysis by means of a flexible plastic pouch that is permanently sealed around its perimeter and is divided into one or more reaction compartments and one or more waste compartments. In

particular, the invention provides a method and device for immunoassay analysis for HIV antibodies, that may be performed on whole blood.

The device is provided with apertures adapted for the insertion of straws or other channels (herein referred to generically as "the straw or straws") which act as passages for introducing reagents and specimens into the device. In addition the device is provided with means, such as a filter, for retaining certain components of the reaction products within the reaction compartment and permitting other components to pass into the waste compartment. Immunoassay tests are performed by opening the sealed pouch to insert a straw or straws and positioning said straw so that its lower end provides access to any reagents or filter within the reaction compartment. External reagents are then passed through the straw to permit reaction with the filter or reagent and prepare the filter and/or reagent for a subsequent reaction with the specimen. The specimen and, where appropriate, additional reagents are sequentially added to the reaction compartment through the straws until the reaction to be observed is complete.

The waste products of the reactions, such as wash solutions, excess reactants, and spent reactants are permitted to pass into the waste chamber through the filter.

After completion of the analysis, the device is sealed to contain the specimen and reactants so that the entire device may be disposed of safely.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a view of the preferred embodiment of a device in accordance with this invention with a flexible filter device within a single reaction compartment.

FIG. 2 is a side view of the device of FIG. 1 after insertion of slotted straws and a reagent tablet.

FIG. 3 is a view of an embodiment of the invention, containing two reaction compartments.

FIG. 4 is a view of an embodiment of the invention substituting a reagent for the filter device of the

preferred embodiment.

FIG. 5 is a view of an embodiment of the invention substituting a rigid filter for the flexible filter device of the preferred embodiment.

FIG. 6 is a plan view of an embodiment of the invention comprising two devices within a supporting rack with a flow control clamp affixed.

FIG. 7 is a view of an embodiment of the invention, containing two separate reaction compartments and a common waste compartment having a compressed absorbent sponge therein.

FIG. 8 shows front and side views respectively of a rigid channel for adding reactants and sample.

DETAILED DESCRIPTION OF THE INVENTION

Description of the Device

A preferred embodiment of this invention is shown in FIG 1. in which a transparent, flexible, rectangular shaped pouch 2 is shown. The device as shown is 7 inches long and 1 1/4 inches wide. Pouch 2 is formed by thermally sealing two pieces of a thin flexible material, such as clear polyester, approximately 0.00142 inches thick to each other at their sides and bottom by perimeter seal 4. This results in an internal space positioned between the opposing interior faces of the original flexible material which constitutes pouch 2.

Pouch 2 is divided into reaction compartment 8 and waste compartment 12 by filter 10 which extends horizontally across the middle of pouch 2 and is thermally sealed to the front and back of pouch 2. Filter 10 is a flexible transparent thermoplastic filter with approximately 12 micron pores and is approximately 0.00048 inches thick. As shown in FIG.2, filter 10 is V-shaped, with the apex of the V extending downward.

Interior seals 6 originate near the top of pouch 2 at perimeter seals 4 and extend downward and inward in a funnel shape to traverse filter 10, encompassing a portion of it within the seals and ending approximately 1 cm. below the filter. The portion of interior seals which extends below the filter forms a one-way valve 14, which is the

entry into waste compartment 12. Waste compartment 12 contains a partial partition 16, fabricated from a flexible film having multiple perforations. Partition 16 extends upward from the bottom of waste compartment 12, but is not connected to the top of said compartment. It is attached to the pouch at perimeter seals 4 and the lower end of interior seals 6.

Attached to the top of the exterior of and extending above each face of pouch 2 are adhesive backed waterproof labels 18 which extend approximately 7 mm above each face. The exposed adhesive surfaces of label 18 are covered with non-adhesive label liners 20, which extend 3 mm above the upper edge of each label, and the lower margins of which contact the upper margins of each face of the pouch. At the top and near each side of pouch 2 are apertures 22, for hanging pouch 2 on a rack, which pass through labels 18.

A cylindrical drinking straw 24, having a longitudinal slot 26 is provided. Slot 26 is a broad slot, about 1/4 of the circumference of the straw in width, and starts at a point below the upper end of straw 24 and except for such upper end extends the full length of the straw. The lower 1/2 inch portion of the straw is flattened and broadened.

FIG. 3 is a modification of the device in which a midline vertical seal 28 is added to form parallel testing areas. A straw is placed in each of the two triangular chambers thus formed. The filter is also bisected vertically. This permits the performance of simultaneous tests.

FIG. 4 is a variation of the invention, in which the filter is omitted and replaced by an antigen or antibody coated disc 30. Disc 30 may be constructed of rigid material such as polystyrene or of porous membrane, such as nitrocellulose. The antibody is not chemically bound, but when dry is permanently adsorbed into the surface or in the interstices. The disc may be placed at the time of manufacture or added by a technician at the time of use. It is known to use discs of various kinds for

the performance of an Enzyme Linked Immunosorbent Assay (ELISA), however in conventional applications, a small spot of antibody or antigen is placed on the disc. In contrast, the entire surface of the disc used in this embodiment is coated to bind a maximum amount of conjugate, which in turn presents a broad surace to react with the substrate.

FIG. 5 is another embodiment of the invention in which a cylindrical rigid filter 32 replaces the flexible filter of the preferred embodiment. The filter may be constructed of cellulose and must have longitudinal pores of a smaller diameter than the reagent beads, described below, which will rest on top of the rigid filter during the test.

FIG. 6 is a plan view of a double pouch embodiment of the device in which the pouches are attached to support 40 having a base 42. The pouches are held in place by clips 44. A clamp 46 is provided which is attached to the pouches and may be opened and closed to regulate the flow of reagents within the pouches. Straws 48 with broad slots 49 permit observation of the fluid passing down the straws. Pouches 50 may be separated by tearing them apart at perforations 52.

FIG. 7 is a single pouch embodiment in which the perforated membrane as described in FIG. 2 has been eliminated and a compressed sponge 54 is positioned within the waste compartment. Sponge 54 serves to absorb and contain waste material.

FIG. 8 illustrates a variation of the straw insertion device. The slotted straw is replaced by a V-shaped trough or channel which has the advantage of less surface contact with the pouch than a rounded straw. With less surface contact, there is likely to be less upward wicking of the liquid sample and reagents.

Description of the Method

The practice of this invention uses reagents which are commonly available to perform immunoassay tests, such as Enzyme Immunoassay (EIA) or Enzyme Linked Immunosorbent Assay (ELISA). ELISA tests are commonly performed using plastic microwell plates in which each well

is coated with antigen or antibody. A sample solution containing either antigen or antibody is added to the well. After an appropriate interval of time the sample is washed out, and conjugate and substrate are sequentially added followed by washes. The resultant color intensity is analyzed by a spectrophotometer. Other conventional methods employ various types of thin porous material such as nitrocellulose membrane filters for the adsorbtion of the antigen or antibody, with the test generally performed by passing the usual reagents through the filter. Microbeads, such as used in the present invention may also be used in various other configurations to obtain similar results.

Reagents

In this method the reagents are provided in the form of 5 dry tablets which contain the following ingredients:

TABLET 1(Wash)

Bovine serum albumin, azide 3-[N-morpholino] propanesulfonic acid (MOPS), and Triton X67

This tablet is used for the preparation of Wash Solution 1 and serves the purposes of filling the non-specific attachment areas of the beads, provided in Tablet 2, leaving free the areas designed for attaching antibodies. When reconstituted with distilled water the specific gravity of the solution is 1.014. This tablet also aids in maintaining a pH of approximately 7.4

TABLET 2(Beads)

Latex microspheres coated with HIV antigen, such as peptide chain gp 41 and p 24, plus mannose, glycine, and azide.

This tablet contains the microspheres, generally referred to as "beads", with antigen attachment points. The specific gravity of the beads is 1.014.

TABLET 3(Conjugate)

Alkaline phosphatase conjugate plus albumin, sodium nitrate, magnesium

chloride, azide, safranine, and
methylene blue.

This tablet contains the conjugate with anti-human antibodies, which attaches to the human antibodies, supplied by the blood samples, which have previously attached themselves to the antigens coating the beads in Tablet 2.

TABLET 4(Substrate)

3-indoxyl phosphate, sodium chloride, and magnesium chloride.

This tablet is the source of the determinitive color change, when reacted upon by phosphatase in the conjugate splitting off the phosphate and causing a blue precipitate.

TABLET 5(Stop Solution)

Citric acid.

This tablet ends the reaction.

The reagents used in the usual above tests all can be purchased as commodity items. They consist of wash solutions composed primarily of salts and buffers, a conjugate composed of antihuman antibodies (if the test is to be performed on human serum or tissue) conjugated to an enzyme, and a substrate (developer) which can be acted upon by the enzyme, which then turns color. The intensity of the color is related to the amount of conjugate attached to the antigen or antibody as well as to the concentration of the substrate.

Pretesting preparations

The various tablets are placed in dropper bottles and water is added to form solutions or suspensions.

Conduct of the Test

Referring to FIGS. 1 and 2, Pouch 2 is hung on a suitable support, such as support 46 shown in FIG. 6. Slotted drinking straw 24 is inserted into pouch 2 through the top of the pouch so that its lower end lies at the top of the upper part of the V-shaped end of Filter 10. The upper end of straw 24 protrudes about 1 inch above pouch 2.

A flow obstructing clamp 46, shown in FIG. 6 is placed over one way valve 14. One drop of a blood sample to

be tested is placed into straw 24 and then washed down with 5 drops of the Tablet 2 beads suspension which then remain within reaction compartment 10. The now diluted blood sample is permitted to remain in contact with the beads for two minutes. After 2 minutes of incubation, two drops of the conjugate solution are added to the diluted blood and beads mixture and allowed to react for 1 minute. Clamp 46 is removed to allow the liquid to drain into waste compartment 12. Two ml. of wash water are added to wash out all remaining blood. All visible traces of dye or blood must be eliminated.

Clamp 46 is then reapplied and three drops of the Tablet 4 substrate mixture are added. The sample is then observed for color changes. A blue color of the beads or in the liquid indicates the presence of HIV antibody in the blood sample. If no antibody is present, the fluid remains colorless. The test is stopped by the addition of two drops of the Tablet 5 stop solution.

If instead of whole blood, serum is used as the sample, then the dye alone, present in the conjugate, colors the solution. Thus, all visible traces of the dye must be removed by washing prior to adding the substrate mixture.

To dispose of the used pouch a few drops of calcium hypochlorite are added. The calcium hypochlorite kills any HIV virus particles that may be present. The liners 20 of labels 18 are removed and the labels are pressed together, forming a water-tight seal. Pouch 2 may then be disposed of safely and without leakage.

In the event it is desired to obtain a sample and delay the test, a sample may be taken 1 hour or more before performing a test. The blood sample may be placed in the pouch using a strip of nylon mesh. The pouch is then closed and temporarily sealed by removing the liner from one of the labels and pressing that label against the opposite liner. The blood sample clots in the mesh. The serum then separates from the clot. When the test is to be performed, the pouch is opened, the slotted straw is inserted, and five drops of the beads mixture is dropped into the filter

through the straw. A clamp is applied and Tablet 1 Wash Mixture dropped in the pouch external to the straw to wash the serum down and bathe the beads, The test is then continued as described above.

The invention has been described in its preferred embodiment both with respect to apparatus and method. However, to those skilled in the art, modifications may be made which do not depart from the scope of the invention. The dimensions of the pouch may vary, as may the chemical formulation of the plastic film and filter. The film and filter may vary in their physical characteristrics, such as thickness, flexibility, strength and pore size. The reagents used may vary in chemical composition within the parameters of an EIA procedure.

The design of the pouch may be modified to accommodate more than 1 test simultaneously. For example the pouch may be provided with a vertical center line seal and two straws used to perform two tests simultaneously as shown in FIG.3. Further, the waste compartment may be modified by removal of the midline vertical seal to form a common waste compartment as shown in FIG. 7. Also shown in FIG. 7 is compressed adsorbent sponge within the waste compartment to absorb and contain the waste material.

Another embodiment of the invention includes a filter, which instead of being sealed to the pouch on each face at the same level, is sealed to one face at a higher level than to the opposite face, producing a planar rather than a V-shape as in the preferred embodiment.

As shown in FIG. 5, a clindrical rigid filter positioned in the pouch may be substituted for a flexible filter.

If it is deemed desirable to provide a less expensive system, or if a less sensitive system is satisfactory, such as in a confirmatory test, the filter may be eliminated and polystyrene or other absorbent membranes, or another type of disc containing absorbed antigen may be substituted for antigen coated beads. In such an embodiment as shown in FIG. 4 the disc is placed in the pouch and comes to rest at the location of the filter

in the preferred embodiment. The test is then performed in the same manner as described above for the filter version. Also the perforated partition may be eliminated as shown in FIGS. 3,4,and 5, or it may be replaced by a compressed sponge as shown in FIG. 7.

It is within the skill of the art to replace the dyes included in the conjugate tablet by other suitable dye or dyes, such as phenol red or methyl orange.

The invention described herein has many advantages, but is specifically addressed to a low cost device which may be used in the field with minimal training by lay persons. The test kit, including pouches, straws and reagents can be produced at very low cost, enabling the product to be distributed at low cost. With very minimal modification, the kit can be used to perform a multiplicity of tests for infectious or other agents.

Another important advantage of the invention is the ability of the test to use whole blood, thus eliminating the need to start the test with serum or other processed blood derivitives. The test will work with fresh whole blood, anticoagulated or clotted whole blood, serum, or plasma. The Triton X67, a soluble surface-active agent destroys the red blood cells releasing hemoglobin into the solution, imparting a red color to the mixture of blood and beads during the blood/bead contact phase. The hemoglobin does not interfere with the attachment of HIV antibody to the antigen on the surface of the beads. Since it is important to wash out excess antibody prior to adding conjugate, the loss of red color indicates that washing has been adequate. The construction and function of the pouch permits the blood or serum specimen to be processed immediately after drawing, or to be placed in the pouch and processed up to several hours or 1-2 days after adding the specimen. The inventors know of no other HIV diagnostic test that permits such a broad range of choices in handling the blood specimen. The filter and the film are both constructed of thermoplastic polyester which allows a flexible filter to be incorporated in a flexible pouch by means of heat sealing, eliminating all rigid components in

the pouch construction and use. This feature contributes to low cost manufacture and shipping, as each finished pouch weighs slightly more than three grams. The slit straw allows both blood and reagents to be delivered directly to the filter, largely eliminating reagent seeping along the creases of the device. Further the slit provides an escape for air as fluid is added, preventing air lock. The slit effectively prevents the possibly contaminated straw from being used for purposes (such as drinking) other than for which it is intended.

The provision of dry reagents to be reconstituted just prior to use offers greatly enhanced shelf-life and significantly reduced costs when compared to devices in which liquid reagent is prepackaged in the device.

In contrast to an EIA method known as "flow through," in which beads are placed on top of a small cylindrical rigid filter with the various reagents flowing through the column of beads and then the filter in a rigid device, the present invention can be characterized as "bathe and drain," the reagents first bathing the beads and then being drained, partly through the beads as in the flow-through design, and partly above the bead level. This system allows for rapid removal of used reagents and wash solution, thus contributing to rapid completion of the test. It does not require the use of mechanical means, such as air pressure, which are often used to provide rapid passage of waste liquids through the flow through type bead column and cylindrical filter devices.

The beads while lying on the filter may be manipulated by hand or instrument through the face of the pouch to increase contact of the beads with the blood or reagents.

The test can be used with commercially available non-carbonated bottled water, presently available in most areas of the world.

The conjugate tablet contains two dyes: safrinine, a red azine dye, and methylene blue, a triarylmethane dye. The dyes have the following actions: together they impart a purplish tint to the tablet, serving

as a color code to avoid confusion with other tablets used. The polyester filter is safraninophilic, absorbing the red dye and giving the filter a red tint. This tint serves to form a useful contrasting background to the beads so as to better distinguish their color in determining the results of the test. The methylene blue dye does not stain the filter but provides a blue color to the liquid from the dissolved conjugate tablet. Since it is important to remove all conjugate from the test area of the pouch before adding the developer, absence of blue color in the liquid indicates that adequate washing has occurred. Neither dye stains the beads nor interferes with the chemical reactions occurring in the test.

The labels not only provide a mechanism for patient identification and dates, but also both a temporary and permanent closure of the pouch. The temporary closure is used when the test is to be performed several hours after drawing blood. One drop of whole blood is placed within the pouch either directly, or on a strip of non-absorbable paper or mesh, such as nylon. One label liner is removed and that label pressed against the opposite liner. Such action allows the blood to clot within the pouch but not to dry.

Disposal of the test device provides for user safety, by a permanent closure being accomplished by the removal of both liners of the labels and the pressing of the adhesive surfaces of the labels together.

A modification permits the performance of a multiplicity of tests, for example, for various antibodies or antigens within a single type of pouch.

The perforated partition in the waste compartment essentially changes its surface charactistics as the perforations eliminate flow resistance due to the static attraction of the inner surfaces of the device faces. This enhances the rate of flow from the filter into the bottom of the waste compartment, thus helping to eliminate upward wicking of the waste fluid, which could cause false positive tests if not prevented. The absorptive sponge in the waste compartment serves the same purpose by first

absorbing fluid entering the waste compartment and then expanding in thickness to separate the leaves of the pouch and eliminate static attraction of the leaves to each other.

In the course of developing this device, it has been found that it is desirable to control the specific gravity of the dissolved components of the bead mixture so that it's specific gravity is substantially the same as that of the undissolved beads. Such control assures a more homogeneous mixture in contrast to a mixture of differing densities in which the beads would either float or fall to the bottom and thus the resultant mixture would not be consistent.

The preferred embodiment and variations disclosed herein are not intended to limit the scope of the invention, as it will be apparent to those skilled in the art that the embodiments described may be modified without departing from the spirit and scope of the invention, as defined in the following claims:

CLAIMS

We claim:

1. A substantially transparent,compartmented, flexible pouch analytical device comprising:

(a) one or more reaction compartments having an access opening for the introduction of reactants and samples;

(b) one or more waste compartments;

(c) filter means positioned between said reaction compartments and said waste compartments;

(d) means for sequentially adding reactants and samples to said reaction compartments;

(e) means for sealing said device;

(f) means for controlling the flow of reactants and the products of reactants from said reaction compartments to said waste compartments;

(g) means for preventing the flow of reactants and the products of reactants from said waste compartment to said reaction compartment.

2. A device according to Claim 1, in which said device is fabricated by thermally sealing two or more pieces of a thin flexible material.

3. A device according to Claim 1, in which said filter means is a flexible, thermoplastic filter.

4. A device according to Claim 1, in which said filter means is v-shaped.

5. A device according to Claim 1, in which said reaction compartment is formed by thermally sealing two pieces of a thin flexible material, said thermal seals positioned to form a funnel between said two pieces and to traverse said filter means whereby the contents of said reaction compartment are directed toward said filter means.

6. A device according to Claim 1, in which said reaction compartment is formed by positioning two pieces of a thin flexible material in face-to-face engagement and permanently sealing adjacent side portions of said material to define said reaction compartment, and in which said access opening is provided with sealing means consisting of a pair of opposed adhesive labels covered with removable liners, one of said pair attached to the uppermost portion

of each of said faces.

7. A device according to Claim 1, in which said means for adding reagants is a slotted straw.

8. A device according to Claim 1, in which said flow control means is a flow obstructing clamp, which may be opened and closed.

9. A method for immunoassay analysis comprising:

(a) fabricating a substantially transparent, compartmented, flexible pouch analytical device having a reaction compartment, an access opening for the introduction of reactants and samples into said reaction compartment, a waste compartment, and a filter means positioned between said reaction compartment and said waste compartment;

(b) depositing a specimen within said reaction compartment;

(c) adding to said reaction compartment a wash solution and microspheres coated with HIV antigen, said wash solution and said microspheres having approximately the same specific gravity;

(d) maintaining said specimen, said wash solution, and said microspheres in contact with each other for an incubation period to allow human HIV antibodies contained within said specimen to attach themselves to the HIV antigens coating said microspheres;

(e) adding a conjugate mixture of anti-human antibodies which will attach to human HIV antibodies, if present, and a component having the characteristic of reacting with a selected substrate to cause a color change visible through said transparent device and permitting said conjugate mixture to remain in contact with said sample for a time sufficient to allow said anti-human antibodies to attach to said human HIV antibodies;

(f) removing all excess sample and all conjugate mixture which has not attached to human HIV antibodies by draining and washing the liquid within the reaction department into the waste department through said filter means;

(g) adding a substrate mixture selected to react

with any color change component which remains within the reaction chamber as a result of said anti-human antibodies attaching to human HIV antibodies;

(h) adding a reactant to stop the reaction;

whereby the presence of color within the reaction chamber caused by the addition of substrate indicates the presence of human HIV antibodies within the sample.

10. A method in accordance with Claim 9 in which a dye is added to said conjugate mixture whereby the operator may visually determine when all of the conjugate mixture which has not attached to human HIV antibodies has been moved from the reaction department into the waste compartment.

11. A method in accordance with Claim 9 in which an absorbent sponge is positioned within said waste compartment, whereby said sponge both absorbs waste products and keeps the faces of said compartment open to facilitate the receipt of said waste products.

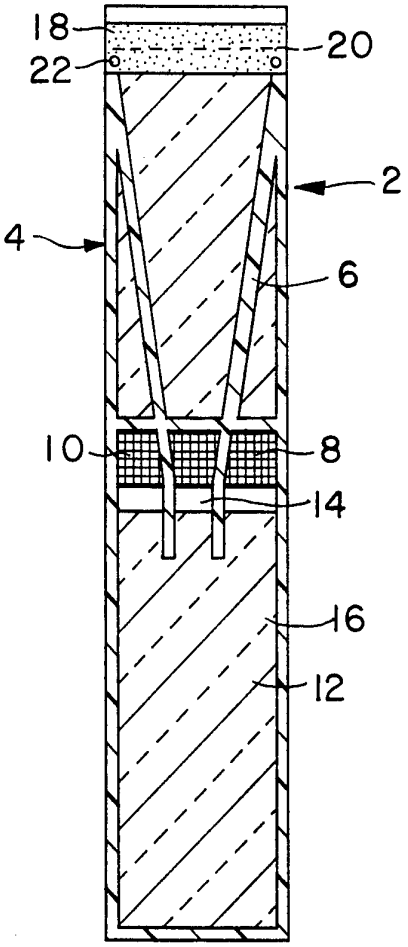


FIG. 1

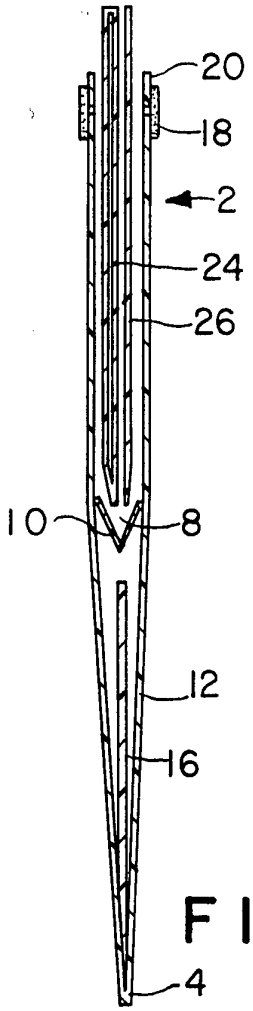


FIG. 2

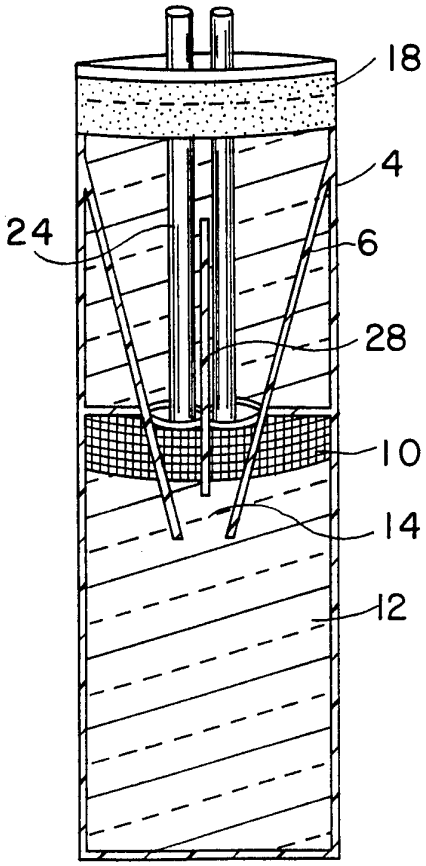


FIG. 3

FIG. 5

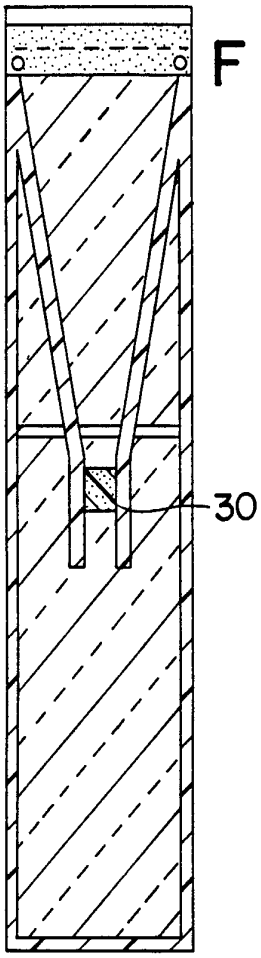
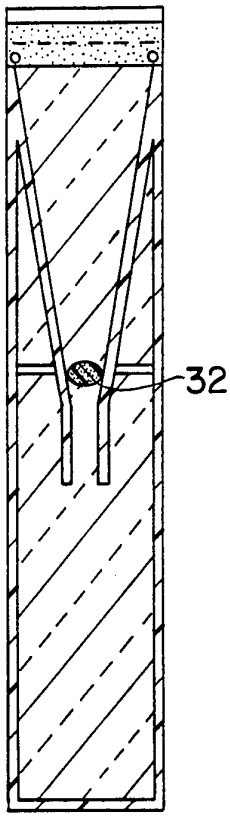


FIG. 4



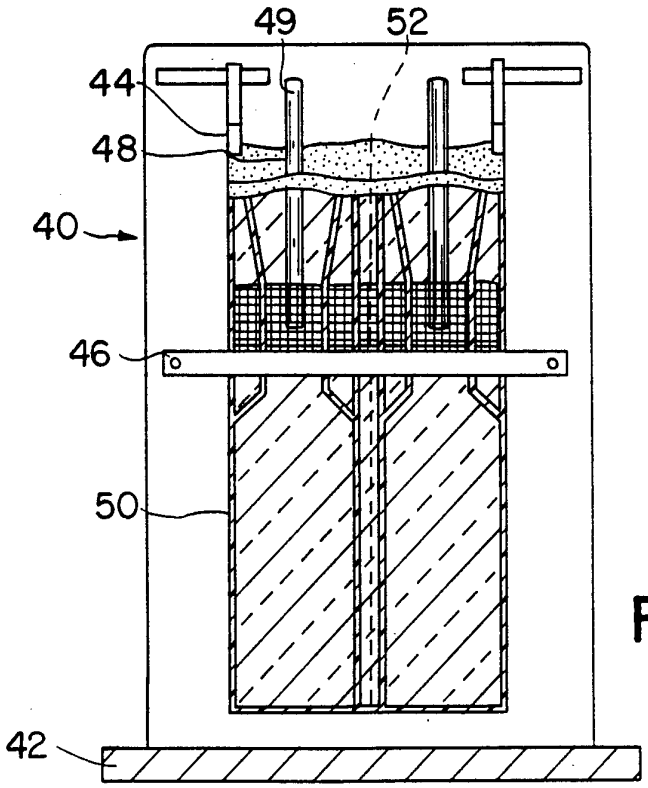


FIG. 6

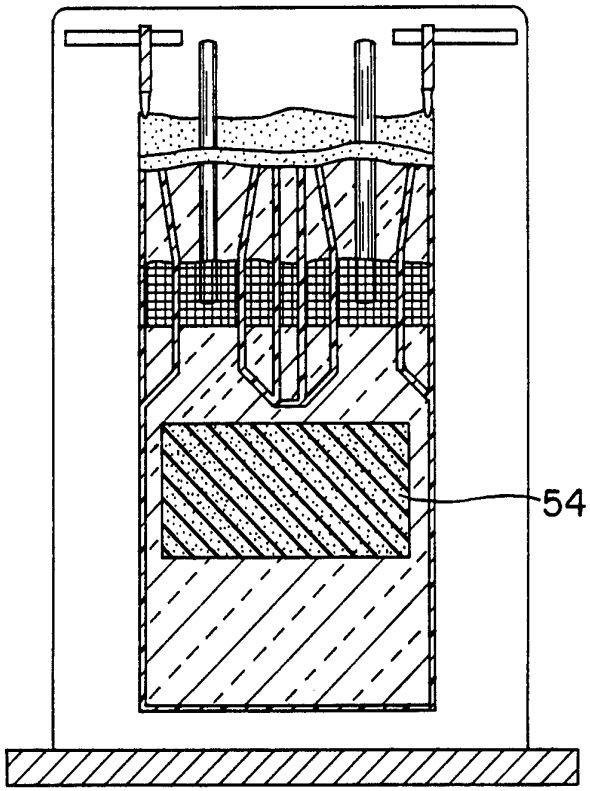


FIG. 7

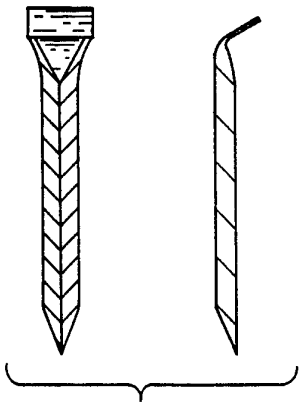


FIG. 8

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 93/12154

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/543 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 306 206 (COGENT LIMITED) 8 March 1989 ---	
A	EP,A,0 485 228 (ORTHO DIAGNOSTIC SYSTEMS) 13 May 1992 ---	
A	EP,A,0 480 497 (AKZO N.V.) 15 April 1992 ---	
A	EP,A,0 255 190 (REPLIGEN CORPORATION) 3 February 1988 see claims 21,24 -----	9-11

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 1994	29. 04. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cartagena y Abella,P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 93/12154

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		DE-A-	3881795	22-07-93
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		US-A-	5262301	16-11-93