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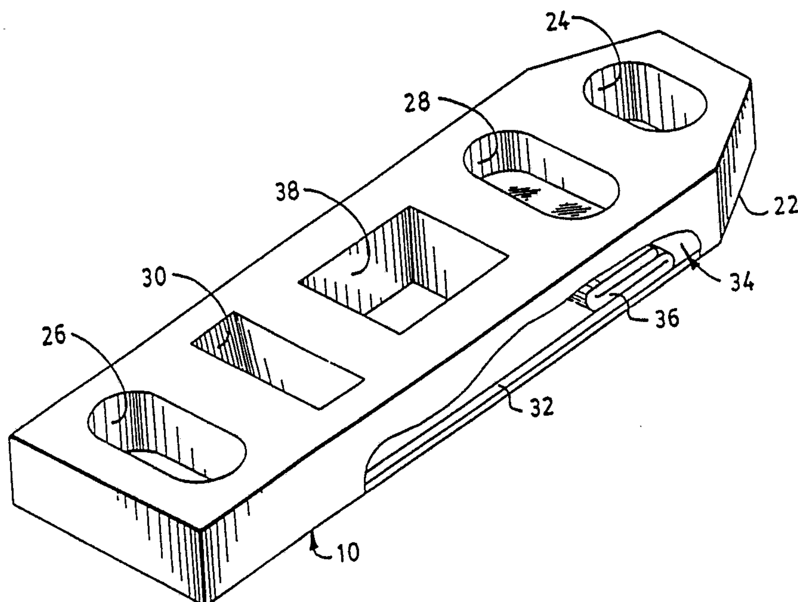
(54) Title: IMMUNOASSAY FOR ANTIBODIES TO INFECTIOUS DISEASE AGENTS



(57) Abstract

A rapid, sensitive and specific assay for the detection of antibodies to infectious diseases. The method utilizes a porous member to which there is immobilized a binding material capable of binding to the antibodies of a desired subclass or subclasses which are present in the test sample. According to the method, where the test sample includes antibodies to the infectious disease of interest, there is formed a ternary complex of the binding material, the antibody of interest and a labeled detector material, e.g., a labeled antigen, which is specific to the antibody of interest. After removal of the free labeled detector material from the reaction zone any bound labeled detector material is detected to provide an indication of the presence of the antibodies of interest.

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Immunoassay for antibodies to infectious disease agents

BACKGROUND OF THE INVENTION

The invention relates to a method for assaying for antibodies to infectious diseases and, more particularly, to a method which utilizes a porous solid phase.

5 Various methods for the detection of antibodies to infectious diseases, e.g., antibodies to the HIV I and II (AIDS) viruses, are known for confirmation of acute disease, exposure to a disease or immunity to a disease. For example, the known methods for the detection of antibodies to the AIDS virus 10 include enzyme-linked immunosorbant assays (ELISA), Western Blot and immunofluorescent techniques. An established method for blood donor screening is to first carry out an ELISA followed by confirmation of positive results by the Western Blot technique.

15 A typical ELISA technique involves reacting a test sample with an antigen reagent generally obtained from disrupted whole or density banded HIV I and II. Typically, the

antigen reagent is coated onto wells of a microtiter plate. After washing to remove unbound antibodies, anti-human IgG antiserum conjugated with an enzyme is added to the wells and incubated. After an appropriate incubation period, an enzyme
5 substrate is added to the mixture and a detectable, measurable product is formed in the presence of antibodies to HIV I and II.

The known assay procedures are not satisfactory in all respects. In some cases the time required to obtain a result is longer than is desirable. Also, the dependence on a second
10 antibody as the detector makes the assay subject to technique due to the required extensive wash steps. In other cases the assay technique may not provide a total test containment format. It would be desirable to have a rapid, sensitive and specific assay method for the detection of antibodies to
15 infectious diseases which could be carried out in a test format that allows for total test containment. Accordingly, it is an object of the invention to provide such an assay method.

SUMMARY OF THE INVENTION

These and other objects and advantages are
20 accomplished in accordance with the invention by providing an assay technique for the detection of antibodies to infectious diseases which utilizes as the solid phase a porous member to which there is immobilized a binding material capable of binding to the antibodies of a desired subclass or subclasses, including
25 the antibodies to the infectious disease of interest, which are present in the fluid sample. According to the assay method, when the test sample includes antibodies to the infectious disease of interest, there is formed a ternary complex of the

immobilized binding material, the antibody of interest and a labeled detector material, e.g., a labeled antigen, which is specific to the antibody of interest. After any free labeled detector material is removed from the reaction zone such as by washing with a wash solution, the porous member is evaluated for the presence of any bound labeled detector material to provide an indication of the presence of the antibodies of interest.

The assay method of the invention may be used for any serology test, that is, any test for antibodies to an infectious disease. Typical infectious diseases for which screening may be carried out according to this assay technique include rubella, cytomegalovirus, toxoplasma, lyme disease, herpes I and II, Epstein-Barr virus, HTLV, HIV, chlamydia and hepatitis.

The assay method of the invention is rapid, sensitive and specific and, in a preferred embodiment, can be carried out in a single test module format which allows for total test containment. By utilizing a capture of all specificities of antibody of the desired subclass or subclasses in the sample and specific detection of the antibodies of interest, the method provides rapid capture through a relatively large amount of binding material and also allows a less vigorous and less technique-dependent wash due to a relatively low concentration of the labeled detector material. Thus, there is provided a reliable and convenient method for screening for infectious diseases.

BRIEF DESCRIPTION OF THE DRAWING

For a better understanding of the invention as well as other objects and further features thereof reference is made to the following detailed description of various preferred embodiments thereof taken in conjunction with the accompanying drawing wherein Fig. 1 is a simplified isometric view of a single test module which can be utilized for the assay method.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As described previously, a porous member is utilized as the solid phase for the assay method and to this member there is immobilized a binding material which is capable of binding to the antibodies of a desired subclass or subclasses, including the antibodies of interest, which are present in the test sample. It will be understood by those skilled in the art that, depending upon the particular stage of infectious disease for which screening is being carried out, it is necessary to capture antibodies of one or more subclasses, for example IgM, IgG, etc. Typically, assays which screen for exposure or immunity to infectious diseases detect antibodies of the IgG subclass. It is required therefore that the porous member have a relatively large surface area to allow for the capture of antibodies of the desired subclass or subclasses because of the large concentration of IgG antibodies found in patient samples. The porous member may be a porous membrane, a fibrous mesh pad or the like and may be of any suitable material such as glass, polymeric materials, paper, etc.

The binding material which is immobilized by the porous member may be any suitable material which is capable of binding antibodies of a desired subclass or subclasses in the test sample. Typical suitable binding materials include proteins such
5 as protein A and protein G, both naturally occurring and genetically engineered, polyclonal antibodies such as goat anti-human IgG and monoclonal antibodies such as mouse anti-human IgG. Mixtures of suitable binding materials may be used also. The amount of binding material necessary for any
10 particular assay varies with the assay and can be optimized by conventional experimental scoping techniques. It is preferred to calculate the amount of binding material necessary to bind all, or substantially all, of the antibodies of a desired subclass or subclasses in the patient sample and apply an excess of that
15 amount to the porous member.

The binding material may be applied to the porous member and immobilized thereto by any of various known techniques including physical entrapment and chemical bonding. For example, a solution of the binding material can be applied to
20 the porous member and the member subsequently dried to provide a porous member having the binding material distributed throughout and held therein by the structure of the member. In another embodiment, particularly where the porous member comprises a fibrous mesh material, the binding material can be
25 chemically bound to or adsorbed on polymeric particles and the fibrous mesh pad impregnated with the particulate matter. In this manner the binding material is immobilized to the porous member and remains there throughout the assay method. A

preferred technique is to apply a solution of the binding material to the porous member and subsequently heat the member to fix the binding material thereto.

As described previously, the assay method of the invention also involves the use of a labeled detector material which will bind specifically to the antibody of interest and not substantially to any of the other antibodies present in the test sample. The detector material of the labeled conjugate may be of any type which is capable of binding specifically to the antibody of interest including recombinant or purified cultured antigens, analogues thereof or synthetically prepared peptide sequences. Synthetically prepared peptide sequences are preferred because of their binding specificity. For HIV assays it is preferred to utilize labeled HIV I and II peptide sequences because of their lack of cross-reactive material and safety in handling. Any of the labels known for use in immunometric assays may be utilized including, for example, fluorescent moieties, enzymes, chemiluminescent moieties and radioactive materials. Any change in fluorescence, chemiluminescence, radioactivity or other change in visible or near visible radiation can be exploited. Thus, the label may be directly or indirectly detectable. Where the label is an enzyme it can be one which interacts with a substrate to cause a change in absorption where the substrate is a chromogen, in fluorescence if the substrate is a fluorophore, in chemiluminescence where the substrate is a chemiluminescent precursor or in phosphorescence where the substrate is a phosphor. It is preferred to utilize enzyme labels because of the amplification of the signal which is obtained.

The method of the invention may be practiced in various embodiments. In one embodiment the test sample may be applied initially to the porous member followed by an incubation step to allow the desired subclass or subclasses of antibodies in the sample fluid to interact and bind to the binding material on the porous member. A solution of the conjugate is then applied to the porous member followed by an incubation step. In another embodiment, a volume of fluid sample, e.g. about 10 ul, is added to a solution of the labeled conjugate in a buffer, and the mixture incubated to allow the interactions between the labeled conjugate and antibody of interest to take place. An aliquot of this reaction mixture is then deposited on the porous member followed by another incubation step to allow the interactions between the binding material and the antibodies in the fluid sample to occur. This embodiment is preferred because of the rapid liquid phase kinetics of the first reaction.

After the interactants have been brought together in the reaction zone and allowed to interact under the appropriate conditions for the requisite period of time, any free labeled conjugate is removed from the reaction zone such as by a wash step wherein a wash solution is applied to the porous member. The wash solution may be applied to the porous member in any way including depositing the wash solution on the center of the porous member or by applying it to an outer periphery of the member.

Subsequently, any bound labeled conjugate is detected by appropriate means. As described previously, the label maybe directly or indirectly detectable. In the case of an

enzyme label the substrate solution which is applied to the porous member to render the label detectable may also be utilized as the wash solution to remove from the porous member any free labeled conjugate.

5 In a particularly preferred embodiment of the invention the method is practiced with a single test capillary assay module which is suitable for use in automated analytical test instruments. Referring now to Fig. 1 there is illustrated a self-contained assay module, or element, 10 which carries all of 10 the test reagents, except for the sample fluid, necessary for a particular assay. This preferred assay element includes a plurality of chambers in a housing 22 wherein a first chamber serves as a front reservoir 24 for the storage of the labeled conjugate solution. The solution is covered with a frangible or 15 puncturable foil layer (not shown). A second of the chambers serves as a back reservoir 26 for the storage of a substrate solution which is also covered with a similar foil layer (not shown). An optional third chamber serves as a mixing bowl 28 for the mixing of reagents and a fourth chamber forms part of a 20 dispenser 30 which is utilized to dispense the substrate solution to one end of the porous member 32. There is also shown a chamber 34 within the housing 22 wherein there is arranged an absorbing material for absorbing fluid removed from the porous member such as by a wash fluid as it propagates through the 25 porous member 32.

In this preferred embodiment the porous member 32 is a thin porous member possessing an intercommunicating network of openings throughout such that a fluid deposited on

the member will propagate throughout the member because of capillary action. The thin porous member 32 may be any suitable element such as a porous membrane, a fibrous mesh pad or the like and may be of any suitable material such as glass, polymeric materials, paper, etc. In a particularly preferred embodiment porous member 32 comprises a nonwoven glass fiber mesh having very thin fibers such as on the order of about 1 micrometer.

The porous member 32 is mounted within a guide (not shown) formed within the housing 22 and having top and bottom surfaces which are spaced apart a distance sufficient to support the member 32. By way of example, the spacing between the top and bottom surfaces of the guide may be in the range of from about 0.30 mm to about 0.60 mm; the preferred spacing is about 0.40 mm.

The porous member 32 extends from the dispenser 30 to the chamber 34 which holds the absorbing material. The dispenser chamber 30 is configured as a well for holding a fluid, the dispenser 30 including a port at the bottom of the well and means for allowing communication of fluid from the bottom of the well into the porous member 32. Liquid absorbing material 36, which may be any suitable material, is located within chamber 34 and forms a part of the chamber 34 for taking up fluid expelled from the porous member 32 and the guide area, or reaction zone. Absorbing material 36 is located contiguous porous member 32 and in a preferred embodiment (as illustrated) is formed conveniently as an extension of the porous material folded back and forth on itself.

The housing 22 also preferably includes a chamber 38 which is positioned immediately above the top horizontal surface of porous member 32 and has a port at the bottom periphery thereof to allow fluid to be delivered to the porous member 32. The housing 22 may include a transparent window area (not shown) positioned immediately below the bottom horizontal surface of porous member 32 to provide access for the illumination used to measure any detectable change effected in the porous member as a result of the assay method or preferably an opening in the housing to permit readout illumination to be directed onto the porous member without having to pass through the material of which the housing is comprised.

The sample fluid tested according to the assay method of the invention may be any including whole blood, plasma or serum. According to a preferred embodiment a small amount, e.g., about 10 ul of serum taken from a patient sample is added to an enzyme-labeled conjugate solution, e.g., about 190 ul, in chamber 24 via a pipette which perforates the foil layer over chamber 24 and the assay element is allowed to incubate for the necessary period of time. It will be understood that the amount of patient sample required can vary from assay to assay. Subsequently, an aliquot, e.g. about 15 ul, of the reaction mixture in chamber 24 is aspirated into a clean pipette tip and then deposited on the upper surface of porous member 32 through chamber 38,. The sample fluid is drawn throughout porous member 32 by capillary action and the assay module is again allowed to incubate for a suitable period to allow the

interactions to take place. Subsequently the foil layer covering chamber 26 to form a seal over the substrate solution in the chamber is perforated by a pipette carrying a clean tip and a desired volume of the substrate solution, typically about 75 ul, is aspirated into the pipette tip. The substrate solution is then deposited into chamber 30 from where it is allowed to come into contact with one end of porous member 32 and then drawn throughout the member by capillary action. The assay module is then allowed to incubate to permit the reaction between the substrate material and any bound enzyme label to take place. It is apparent that the substrate solution is also utilized as a wash fluid in this embodiment. As the substrate solution propagates through porous member 32 it forces any free enzyme-labeled conjugate together with the fluid out of the porous member and into absorber chamber 34 where they are taken up by absorber material 36. The signal provided by the species liberated by the reaction between the substrate material and the enzyme, for example, a fluorescent species, is then read by means of a suitable readout means, e.g., a fluorometer. Both qualitative and quantitative results can be obtained with this method.

The method of the invention as carried out with the preferred assay module illustrated can be practiced with an automated assay instrument thus providing a totally self-contained test which requires a minimum of operator involvement and which eliminates operator variability. A preferred assay module of the type illustrated in Fig. 1 is disclosed and claimed in copending, commonly assigned

application Serial No. 354,026 filed May 19, 1989, the entire disclosure of which is incorporated by reference herein.

The invention will now be described further in detail with respect to specific preferred embodiments by way of examples, it being understood that these are intended to be illustrative only and the invention is not limited to the materials, conditions, procedures, etc. recited therein.

EXAMPLE I

Assays for HIV antibodies in patient samples of serum or plasma were carried out according to the method of the invention in accordance with the following procedure. A solution of protein A/G, Omnibind [R] available from Pierce Chemical Co., at a concentration of 250 ug/ml in 50 mM of TRIS buffer, pH 7.5, with 0.1% Triton X 100, was prepared and 25 ul was applied to an approximately 1 cm^2 porous glass fibrous mesh pad, Whatman GF/F which was arranged in an assay module of the type illustrated in Fig. 1. The fibrous mesh has a thickness of approximately 0.42 mm. The amount of binding material applied to the mesh was about seven times the amount theoretically calculated to be necessary to bind all of the IgG antibodies in a typical patient sample. The assay module was dried at 75°C for ten minutes to fix the binding material to the fibrous pad.

A total of 10 ul of sample was added to 190 ul of HIV specific peptide which was previously covalently conjugated to alkaline phosphatase and diluted to the desired concentration in a buffer consisting of 50 mM tris [hydroxymethyl] - aminomethane, (TRIS) pH 7.6, 150 mM NaCl, 1mM $MgCl_2$, 0.1

mM $ZnCl_2$, 0.1% Triton X 100, 1% gelatin and 1% BSA. The reaction mixture was incubated for six minutes at 37°C.

Subsequently 15 ul of the sample/labeled conjugate mixture was applied to the fibrous pad and the module
5 incubated at 37°C for six minutes following which 75 ul of a substrate/wash solution consisting of 1mM of methyl umbelliferyl phosphate and 1M diethanolamine with 0.1% Triton X 100 was added to the wash port of the module. The substrate/wash solution was allowed to enter the fibrous pad
10 and propagate through it by capillary action thereby washing the sample area. The module was then incubated at 37°C for four minutes.

Readings were taken of the reaction zone at regular intervals over a three minute period using a front surface
15 fluorometer by directing 360 nm radiation through an opening in the assay module beneath the reaction zone and collecting the reflected 450 nm radiation. The increase in fluorescence, a function of the amount of enzyme-labeled conjugate bound by the peptide-specific antibody in the sample, was calculated. The
20 result obtained was compared to the results obtained with defined negative and positive calibrators and was determined to be positive or negative on the basis of a determined cutoff value.

A total of 132 patient samples were determined to
25 be positive for HIV antibodies by conventional ELISA and Western Blot analyses and a total of 117 patient samples were found to be negative with a conventional ELISA assay. These serum or plasma samples were analyzed according to the

method of the invention as described above. The results are shown in Table I.

TABLE I

Result of reference methods	Result of test method	
	Positive	Negative
Positive	132	0
Negative	0	117

The results showed complete correlation with the reference methods thereby providing a sensitivity and specificity of 100%.

EXAMPLE II

Samples from patients with several potential interferences were tested by the method described in Example I. All were negative for HIV antibodies by the test method as well as by a reference method. The results are shown in Table II.

TABLE II

Samples	Range	No of samples	Result
Lipemic	Cholestrol 260-580 Triglycerides 840-2155	5	Neg
Lipemic Study 2	Triglycerides 830-2650	28	Neg
Lipemic Freeze/thaw	Triglycerides 830-2650	28	Neg
HTLV I	Positive	2	Neg
HTLV I & II	Positive	3	Neg
EBV	Positive	3	Neg
Hemoglobin	400-1,000 mg/dl	5	Neg
Multi-transfusion	n/a	7	Neg
ANA	Positive	4	Neg
Rheumatoid factor	Positive	4	Neg

TABLE II (cont.)

	Hyper Ig:			
	IgA	to 8,000 mg/dl	2	Neg
	IgM	to 10,000 mg/dl	2	Neg
5	IgG	to 8,000 mg/dl	7	Neg
	Total	16,000 mg/dl	1	Neg
	Plasma:			
	EDTA	to 5x	10	Neg
	Heparin	to 10x	10	Neg
10	Cirate	to 10x	9	Neg

EXAMPLE III

Specimens constituting panel "c" were obtained from Boston Biomedica, Inc. These specimens were serially obtained from one patient and are identified from day 1 in Table
15 III. The specimens were tested according to the method described in Example I. The results obtained, which are shown in Table III, show that the test method of the invention can detect a positive sample consistently on day 107 which is substantially earlier (from 28 to 35 days) than most conventional
20 tests according to published data.

TABLE III

Sample Day	Result
1	--
65	--
86	+/-
107	+
121	+
128	+
135	+
142	+
164	+

The examples show that the present invention provides a solid phase immunoassay for HIV antibodies which has the sensitivity and specificity of conventional ELISA methods. Moreover, the present assay method provides a more rapid analysis which is not as susceptible to specimen matrix or wash techniques and volumes as are conventional assay methods. Less specimen handling is required by this assay method than is the case in conventional ELISA methods thus resulting in less potential exposure to infectious material.

Although the invention has been described with respect to various specific preferred embodiments it is not intended to be limited thereto but rather those skilled in the art will recognize that variations and modifications may be made therein which are within the spirit of the invention and the scope of the appended claims.

What is claimed is:

1. A method for screening a fluid test sample for the presence of antibodies to an infectious disease comprising the steps of:

5 applying to a porous member (i) a fluid test sample which may include antibodies to an infectious disease and (ii) a labeled detector material which binds specifically to the antibodies of interest, said porous member having immobilized thereto a binding material which is capable of binding the
10 antibodies of a desired subclass or subclasses, including said antibodies of interest, which are present in said fluid sample;

incubating said porous member to allow, when said antibodies of interest are present in said fluid sample, the formation of a ternary complex of said immobilized binding
15 material, said antibody of interest and said labeled detector material;

removing free labeled detector material from said porous member; and

evaluating said porous member for the presence of
20 any bound labeled detector material.

2. The method as defined in claim 1 wherein said applying step comprises combining said fluid test sample with a solution of said labeled detector material, incubating the mixture and then applying said mixture to said porous member.

25 3. The method as defined in claim 1 wherein said applying step comprises applying said fluid test sample to said porous member, incubating said member and then applying said labeled detector material to said porous member.

4. The method as defined in claim 1 wherein said binding material is a member of the group consisting of protein A, protein G, polyclonal anti-human antibodies, monoclonal anti-human antibodies and mixtures thereof.

5 5. The method as defined in claim 1 wherein the label of said labeled detector material is a member of the group consisting of fluorescent materials, radioactive materials and enzymes.

6. The method as defined in claim 1 wherein

10 said porous member comprises a nonwoven fibrous glass mesh.

7. The method as defined in claim 1 wherein said detector material is a member of the group consisting of antigens, analogues thereof and synthetically prepared peptide sequences.

15 8. The method as defined in claim 1 wherein said label is an enzyme and said step of removing free labeled detector material from said porous member comprises applying to the porous member a solution of a substrate material for said enzyme.

20 9. The method as defined in claim 1 wherein said binding material is capable of binding the IgG antibodies which are present in the fluid test sample.

10. The method as defined in claim 1 wherein said fluid test sample comprises human plasma or serum.

25 11. A method for screening a fluid test sample for the presence of antibodies to HIV comprising the steps of:

applying to a porous member (i) a fluid test sample and (ii) a labeled detector material which binds specifically to

HIV antibodies, said porous member having immobilized thereto a binding material which is capable of binding the antibodies of a desired subclass or subclasses, including the HIV antibodies of said subclass or subclasses, which are present in said fluid sample;

incubating said porous member to allow, when antibodies to HIV are present in said fluid sample, the formation of a ternary complex of said immobilized binding material, said HIV antibodies and said labeled detector material;

removing free labeled detector material from said porous member; and

evaluating said porous member for the presence of any bound labeled detector material.

12. The method as defined in claim 1 wherein said applying step comprises combining said fluid test sample with a solution of said labeled detector material, incubating the mixture and then applying said mixture to said porous member.

13. The method as defined in claim 11 wherein said binding material is a member of the group consisting of protein A, protein G, polyclonal anti-human antibodies, monoclonal anti-human antibodies and mixtures thereof.

14. The method as defined in claim 11 wherein said binding material is a mixture of protein A and protein G and said porous member comprises a thin nonwoven fibrous glass mesh.

15. The method as defined in claim 11 wherein said detector material is a member of the group consisting of

HIV antigens, analogues thereof and synthetically prepared peptide sequences.

16. The method as defined in claim 15 wherein said detector material is a synthetically prepared peptide sequence.

17. The method as defined in claim 11 wherein the label of said labeled detector material is a member of the group consisting of fluorescent materials, radioactive materials and enzymes.

18. The method as defined in claim 17 wherein said label is an enzyme and said step of removing free labeled detector material from said porous member comprises applying to the porous member a solution of a substrate material for said enzyme.

19. The method as defined in claim 18 wherein said substrate material interacts with said enzyme to provide a fluorescent material and said evaluating step comprises irradiating said porous member with electromagnetic radiation within the absorption range of said fluorescent material.

20. The method as defined in claim 11 wherein said binding material is capable of binding the IgG antibodies which are present in the fluid sample.

21. A self-contained assay module for screening a fluid test sample for the presence of antibodies to an infectious disease comprising a housing including:

a thin porous member arranged between top and bottom guide surfaces, said porous member having immobilized thereto a binding material capable of binding antibodies of a

desired subclass or subclasses which are present in a fluid test sample which is intended to be screened with said assay module;

a first reservoir for storing a solution of a labeled detector material which binds specifically to the antibodies of interest in a fluid test sample intended to be screened with said assay module;

a second reservoir for storing a wash solution;

an opening disposed above a central portion of said porous member;

a chamber for dispensing fluid to an end portion of said porous member and a chamber for receiving fluid which leaves said porous member, said fluid dispensing chamber and said fluid receiving chamber being disposed contiguous opposed ends of said porous member; and

wherein said fluid dispensing chamber includes means for entraining fluid in the chamber to propagate into and within said porous member.

22. The assay module as defined in claim 21 which is capable of screening a fluid test sample for the presence of HIV antibodies.

23. The assay module as defined in claim 22 wherein said thin porous member is a nonwoven fibrous glass mesh.

24. The assay module as defined in claim 22 wherein said binding material is a member of the group consisting of protein A, protein G, polyclonal anti-human

antibodies, monoclonal anti-human antibodies and mixtures thereof.

25. The assay module as defined in claim 24 wherein said binding material is a mixture of protein A and
5 protein G.

26. The assay module as defined in claim 21 wherein said detector material is a member of the group consisting of antigens, analogues thereof and synthetically prepared peptide sequences.

10 27. The assay module as defined in claim 21 wherein said label is an enzyme.

28. The assay module as defined in claim 21 and further including an opening disposed in said housing contiguous the bottom surface of said porous member.

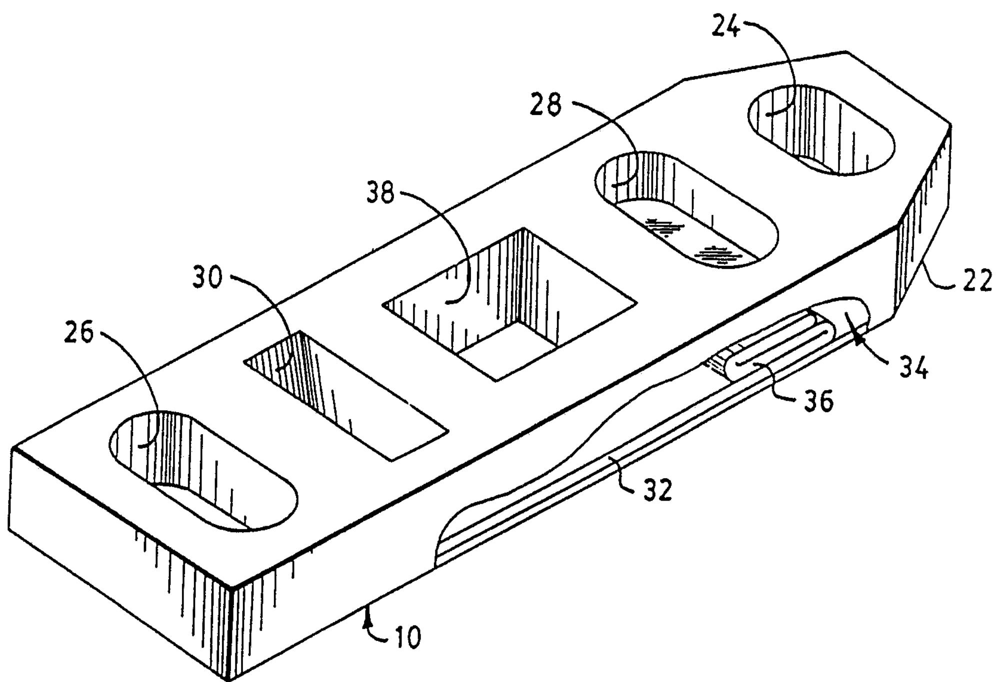
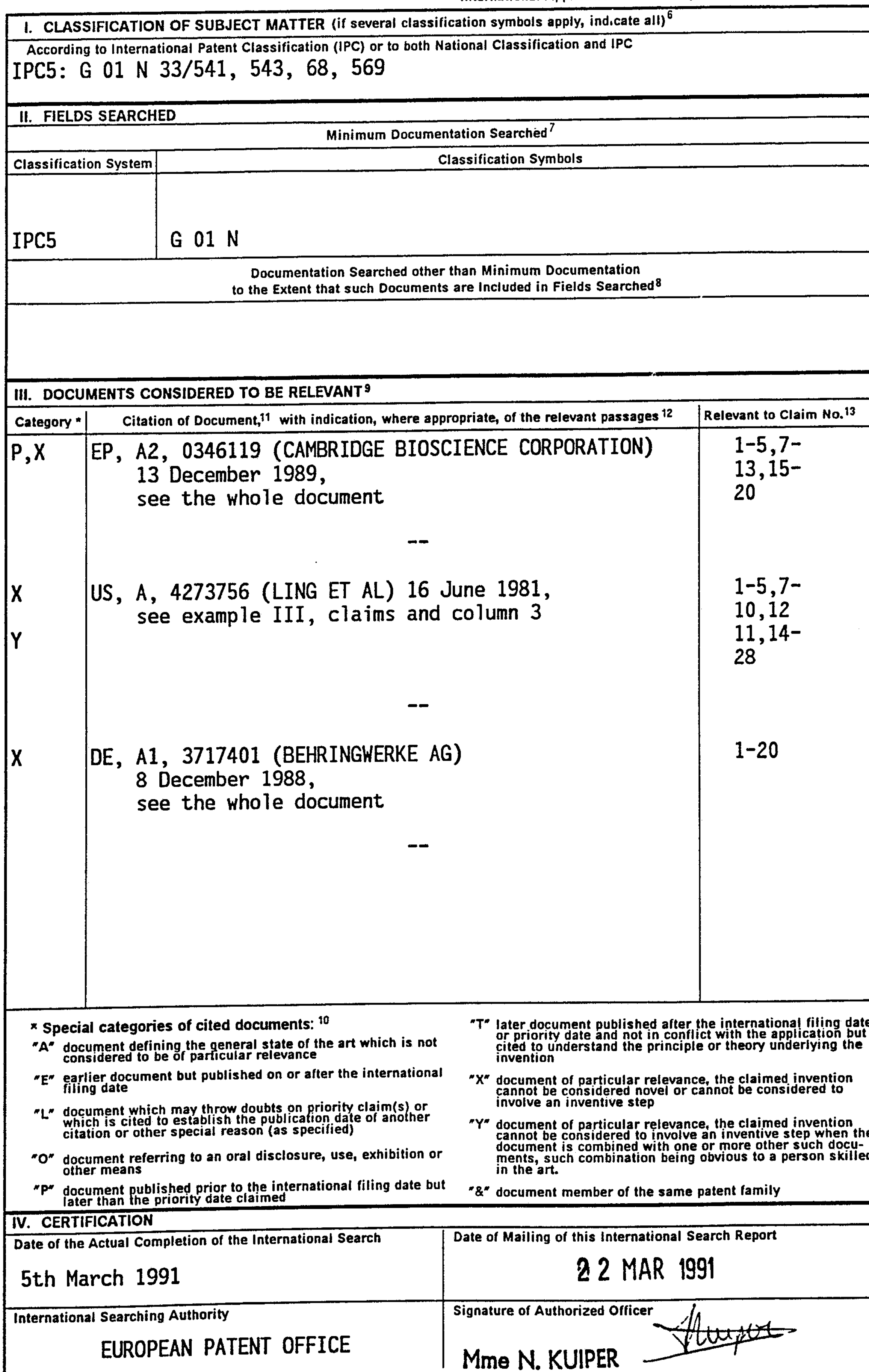


FIG. 1



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/06680

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

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

IPC5: G 01 N 33/541, 543, 68, 569

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	EP, A2, 0346119 (CAMBRIDGE BIOSCIENCE CORPORATION) 13 December 1989, see the whole document	1-5,7-13,15-20
	--	
X Y	US, A, 4273756 (LING ET AL) 16 June 1981, see example III, claims and column 3	1-5,7-10,12 11,14-28
	--	
X	DE, A1, 3717401 (BEHRINGWERKE AG) 8 December 1988, see the whole document	1-20
	--	

* Special categories of cited documents:[10]

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

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
5th March 1991	22 MAR 1991
International Searching Authority	**Signature of Authorized Officer**
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X Y	EP, A1, 0008473 (AKZO N.V.) 5 March 1980, see page 6, lines 12-17, examples I and III-IV and claims	1-5,7-10,12 6,11,13-28
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Y	WO, A1, 8706345 (MUREX CORPORATION) 22 October 1987, see page 3, page 4, lines 6-13, pages 10-11, page 12, line 14 and example 3	1,6,14
Y	Radioimmunoassay and related Procedures in Med.Proceed.1977, Vol. 1, 1978, S. Jonsson: "Protein-a-containing staphylococcus aureus as an immunoglobulin-binding reagent in radioimmunoassay and in a non-radioactive surface immunoassay ", see page 161 - page 175 see pages 161-2, table 1	1,4,13
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECCND SHEET)

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A	EP, A1, 0306772 (ABBOTT LABORATORIES) 15 March 1989, see fig 3 and col 5-7 -- --------	21-28

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/US 90/06680

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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