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(71) Applicant: MUREX CORPORATION [US/US]; Post Office Box 2003, Norcross, GA 30091 (US).

(72) Inventor: HOSSOM, Miles, Gerald ; 3585 Schilling Ridge, Duluth, GA 30136 (US).

(74) Agent: BERNSTEIN, Jason, A.; Post Office Box 2003, Norcross, GA 30091 (US).

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(54) Title: IDIOTYPIC-ANTIGENIC CONJUNCTION BINDING ASSAY

(57) Abstract

A method for detection or quantitation of an analyte of interest suspected of being in a specimen comprising (a) contacting a first antibody with a sample suspected of containing the analyte in such a manner that said first antibody reacts with any of the analyte present in the specimen to form a first complex; (b) contacting with any of the first complex a second antibody which recognizes and binds preferentially to the juncture of the idiotypic determinant of the first antibody and the antigenic determinant of the analyte of the first complex to form a second complex; and (c) determining the presence or amount of the second complex as an indication of the presence or amount of the analyte of interest in the specimen.

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IDIOTYPIC-ANTIGENIC CONJUNCTION BINDING ASSAY

BACKGROUND OF THE INVENTION

The method of the present invention relates to the performance of an assay for the determination of the presence or quantity of a biological substance. Commonly, the substance of interest is an antigen but can also be an antibody or other substance. Numerous techniques have been developed to measure the presence and/or amount of antigen present in a sample of biological fluid. Certain of these involve the use of a solid phase system whereby a substance which will form an immunocomplex with the analyte is bound to an insolublized carrier or material, such as latex, cellulose, and the like. In this manner the immunocomplex when formed can be separated in its solid phase from the rest of the solution containing extraneous uncomplexed material. Thereafter, a material which has been labeled or "tagged" with a signal generating molecule is added to the immunocomplex so that the material binds to the analyte. After separating the bound label from the unbound, the sample is read and the amount of signal measured is related to the amount of analyte present to which it is bound.

Variations on the basic immunoassay technique have been developed to try to overcome some of the problems associated with immunoassays:sensitivity, reliability and cost effectiveness being major concerns. Although immunometric assay techniques have been found to be particularly useful in analyzing for antigens and antibodies, there has been difficulty in the past in establishing an

optimum level of sensitivity for the assay to be helpful in the detection or monitoring of disease states or maladies in the body. Some of the original immunoassays developed used radioisotope labelled substances as a signal generating material. While effective to a large degree, radioimmunoassays suffer from the handling problems and high costs of radioactive materials. To avoid these problems other techniques were developed to increase the sensitivity and reduce the cost and hazards of the immunoassay. Enzyme labels were developed along with fluorescent and luminescent labels to maintain sensitivity and reliability of the tests while reducing the cost per test, an expense that at bottom line is born by the patient.

Along similar lines new binding materials have been researched and developed to better the binding specificity and sensitivity to the analyte. In theory, the more specific the binding material is for the analyte the more sensitive the assay. To that end, multiple binding material assays have been developed so that less analyte is required to produce a reliably measurable signal. For instance, where an antigen is the analyte of interest several assays have been developed wherein two different antibodies are used: one is bound to the solid support and the other is labelled. The two antibodies bind to two different antigenic determinants on the antigen. More recently, monoclonal antibodies developed for this type of use can provide much greater selectivity and specificity for a given antigen. By being engineered to bind only to a given particular antigen monoclonals overcome the specificity problem associated with polyclonal

antibodies. An example of this use is in EPA No. 0044219, which describes a method of immunoanalysis using monoclonal antibodies specific to two distinct antigenic binding sites.

US Patent No. 4,376,110, issued to David et al., discloses a sandwich immunoassay using two monoclonal antibodies which are site specific, yet may be a product of the same or different cell lines. A "sandwich," or "two-site," immunoassay relies upon the formation of an insolublized antibody:antigen:labelled antibody complex. The two monoclonal antibodies are designed to minimize cross-reactivity and interference with each other when binding to the antigen. However, there still remains the problem of nonspecific binding and incomplete washing away of unbound materials. Some small amount of labelled antibody can remain in the reaction area by binding nonspecifically to either the reaction support surface or extraneous material in the area other than the insolublized antibody. This nonspecific binding results in more label remaining in the area and generating a signal even though it is not bound to an antigen. Since a false high back-ground signal level is measured, the overall assay is less sensitive than if the nonspecific binding could be reduced or eliminated. Attempts have been made at increasing the sensivity of the assay by increasing the specificity of the antibody components, e.g., by using monoclonal antibodies and by improving the wash step to effect a more efficient separation of the bound from unbound label. Existing methods, however, still have yet to achieve the levels of sensitivity and consistencey that are desired by the medical diagnostic industry in detecting minute quantities of analytes.

Since there are few methods currently available for conveniently measuring the amount of antigen or antibody directly, labelled compounds have been developed which, when attached to the analyte, emit a signal of some kind which can be measured,thereby giving an indirect quantitation of analyte. To that end labels such as radioisotopes, such as 132I; enzymes, such as alkaline phosphatase; fluorescent, such as flourescein; and luminescent, such as luciferin have been developed for use in immunoassays. The primary concern with these labeled compound assay systems is the interference of background signal noise. Background noise occurs, in part, because of nonspecific binding of labeled compound to material in the sample other than the analyte. In a fluorescent assay system, naturally fluorescing substances often are present in the sample fluid which emit a signal that can partially "drown out" the signal being transmitted by the antigen bound tag. To reduce this noise a wash step is necessary to separate the insolubilized bound material from the unbound material in solution. The results is an improved signal, yet current techniques are still unable to reduce the background noise level to the point where a much more sensitive assay is obtainable.

US Patent No. 4,446,233, issued May 1, 1984 to Auditore-Hargreaves et al., discloses a homogeneous immunoassay using covalent hybrid antibodies. The antibody contains one site specific for an analyte and one binding site specific for an indicator. The unique feature of that invention is the use of a single antibody which has been designed so as to contain two distict binding sites on the same antibody, joined through

inter-heavy-chain disulfide bonding. The antibody is composed of two different heavy-chain light-chain half-molecules or two idiotypic determinants. The method is limited, however, by the difficulty and expense of designing and producing an antibody with two different chains.

Several techniques have been disclosed that involve the detection of an immune complex of antigen:antibody using various detection compounds to attach themselves to the complex. To date, however, none exist that use .pa an antibody as such, and none selectively seek to recognize the juncture at the binding site between analyte and the antibody.

US Patent No. 4,514,508, issued April 30, 1985 to Hirschfeld, describes an assay based on the recognition and binding of a labelled detection compound to the Fc portion of an antibody molecule that is complexed to an antigen. The detection compound is complement or the C1q component of complement. The distinguishing feature is that the complement molecule binds to the Fc region of the bound antibody structure, and not to the antibody:antigen juncture. The problem with complement is that it will bind to complexes other than just the one of interest; therefore, in assaying biological fluids, there is the likelihood that there will be antigen:antibody complexes present as a result of other infections or organisms in the body, giving a false reading.

Similarly, US Patent No. 4,332,783, issued June 1, 1982, to Pernice et al., describes a process for the determination of an immunocomplex using two reagents: an insolublized first reagent specific for the antigen in the immunocomplex, and a labelled second

antibody specific for the antibody in the immunocomplex. Each reagent recognizes a separate component of the complex and is thereby distinguishable from a reagent that selectively identifies the binding site between an analyte and an antibody directed against that analyte. However, the carrier-bound antibody may bind non-complexed antigen present in the sample solution; likewise, labelled antibody may bind to non-complexed antibody, resulting in a prozone effect that would reduce the sensitivity of the assay.

SUMMARY OF THE INVENTION

The present invention remedies the deficiencies of the prior art to provide a highly sensitive and reliable method for detection of analytes.

Briefly, the present invention relates to a method for detection or quantitation of an analyte of interest suspected of being in a specimen, comprising (a) contacting a first antibody with said specimen in such a manner that said first antibody reacts with any of the analyte present in said specimen to form a first complex; (b) contacting with any of the first complex a second antibody which recognizes and binds preferentially to the juncture of the idiotypic determinant of the first antibody and the antigenic determinant of the analyte of the first complex to form a second complex; and (c) determining the presence or amount of the second complex as an indication of the presence or amount of the analyte of interest in the specimen.

DESCRIPTION OF THE INVENTION

Immune response reactions have been identified as an area of extreme interest by scientists searching for tools to detect and monitor disease states. The ability of an organism to react to exposure to a foreign entity provides the basis for the exploration of the relationships and interactions between host and pathogen. Two of the myriad of components involved are antibodies and antigens, and much effort has been expended in developing assays using antibodies to detect and quantify a given antigen. For the purposes of this application the use of an antigen as an analyte is merely by way of illustration but not limitation; many other kinds of analytes are detectable utilizing the method of this invention. Many types of materials can be assayed for using the method of this invention, including but not limited to bacteria (e.g. Chlamydia trachomatis), fungi (e.g. Candida albicans), parasites (e.g. Trichomonas vaginalis), mammalian cells (e.g. tumor cells), macromolecules (e.g. OMP MC gonorrhea protein), enzyme complexes (e.g. creatinine phosphokinase), IgM, viruses (e.g. hepatitis B), and the like.

Antigens are substances that are able to provoke a specific immune response by reacting with the components of the specific immune response, e.g., antibodies or specifically sensitized T-lymphocytes. There are exposed reactive areas on the surface of antigens containing surface groupings of a molecular nature, such as amino acids or carbohydrate side chains. These chemical structures when reactive with antibodies are called antigenic determinants, or epitopes, and can be

of two kinds: (1) sequential determinants, e.g., primary amino acid sequences; or (2) conformational determinants, i.e., determined by secondary, tertiary or quaternary structure. It is these antigenic determinants that enable a particular antigen to be recognized by a specific antibody.

Antibodies are proteins comprised of two pairs of polypeptide chains. These polypeptide chains consist of constant regions and variable regions. The constant regions vary little from one antibody to the next; however, the variable regions of every monoclone of an antibody has a unique amino acid sequence. These sequences are able to recognize a complementary sequence on an antigen against which the antibody is directed. The areas on the variable region are called idiotypes and specifically bind to the antigenic determinant present on the surface of the antigen. In a manner similar to the enzyme-substrate lock and key phenomenon, an antibody's variable region matches with a particular antigenic determinant, either via a sequential determinant or a conformational determinant.

When an antibody that can recognize a particular antigen comes in contact with the antigen, it will bind to it and form a unique juncture. The juncture formed is now part of a new molecular entity: an immunocomplex. This juncture can be thought of as a distinct conformational determinant, which itself is recognizable as an antigen. A second antibody can be introduced, now, that can discern the new juncture and can bind to it. This "conjunction" binding serves as the underlying novel concept enabling the method and variations thereon of this invention to function.

In order to achieve the specificity of the antibodies necessary for the method of the present invention, it is desirable to use monoclonal antibodies for this purpose. Monoclonal antibodies are prepared generally according to the process described by Milstein and Kohler in Nature 256:495-497, 1975. The process is well known in the art and need not be repeated here. Let it suffice to say that a monoclonal antibody can be designed to recognize and bind to the juncture created between the antibody:antigen immunocomplex described above. The procedures are set out in the Examples contained hereinbelow.

In accordance with general immunoassay techniques a system with a plurality of antibodies can be used to detect or quantify an analyte, such as an antigen, present in a patient specimen, such as urine, feces, sputum, semen, mucus, blood, and the like. For such specimens that are solid, they can be solublized by adding water or other appropriate fluid. Usually one antibody is labelled with a signal-generating substance, the emitted signal of which can be read and measured. A homogeneous assay is carried out in a single phase, commonly a liquid medium. A heterogeneous assay uses two phases: solid and liquid; whereby one antibody is insolublized by adsorbing it onto a solid support surface, such a plastic beads, cellulose, glass fiber filter paper, glass, polymers, and the like. Usually the antibody that is not insolublized is conjugated with a labelling compound. In a two monoclonal antibody heterogenous immunoassay a first antibody is insolublized on a solid support surface. An analyte of interest, such as an antigen, is then added, and, an immunocomplex of first antibody:antigen forms. At this point a new molecular entity

has formed which can be recognized by a second antibody. This second antibody has been labelled with a signal-generating substance and is added to the immunocomplex. As previously discussed, this novel second antibody will recognize the juncture at the first-antibody:antigen binding site and bind to it, creating a new immunocomplex of first antibody:antigen:second antibody. The second antibody is designed so as to distinguish and bind specifically to the first antibody:antigen complex rather than to the idiotypic determinant of the first antibody alone or the antigenic determinant on the antigen alone.

After second antibody has bound to the first antibody:antigen immunocomplex a wash solution, typically a buffer, is added to separate bound (insolublized) material from unbound material. The reaction area is then read and the amount of signal produced by the label is measured. .pa The amount of signal is correlative with the concentraton of antigen originally present in the patient sample.

By utilizing the distinctive sequential and/or comformational characteristics of a specific antibody:antigen binding junture a highly specific assay can be obtained. The novel use of a labelled antibody that recognizes only the complex formed when antigen is present in the sample and binds to the insoluble antibody permits a specific and unique method for measurement of said antigen.

In another embodiment of this invention a triple antibody system can be used, wherein an insolublized first antibody "captures" the analyte, a second antibody binds to an antigenic determinant different and distinct from that of the first antibody, and a labelled third antibody which recognizes only the junction of the

analyte and second antibody. Such an assay would be useful in the analysis of large analytes such as bacteria, fungi, parasites, mammalian cells (e.g., tumor cells), macromolecules (e.g., OMP MC gonorrhea protein), enzyme complexes, IgM, etc. These analytes are sufficiently large or bulky that when an insolublized antibody binds to it, that access to the junction between the two could be sterically hindered and the second antibody that binds to the juncture would be blocked. To circumvent this a second antibody is designed to bind to an antigenic determinant different and distinct from that of the insolublized first antibody. Now a third antibody can bind to the junction between the second antibody and the analyte because it would not be sterically hindered by the presence of a large antigen- bearing molecule or cell. In a triple antibody system the capture antibody can be either a monoclonal or a polyclonal antibody, while the second and third antibodies are preferably monoclonals.

Since second antibody and third antibody are in close proximity to each other when complexed with an analyte, their physical juxtaposition can serve as a basis for an assay based on the interaction of two labelling components, wherein the second antibody is conjugated with one constituent of a labelling scheme and the third antibody, which recognizes the juncture between second antibody and analyte, labelled with a second labelling constituent. An illustration of such a method would employ a second antibody conjugated with a coenzyme, and third antibody conjugated with a corresponding enzyme. When the enzyme and coenzyme are brought in proper physical and geometric proximity (i.e., when in a complexed

form as described above), they will react with an added substrate to produce a detectable signal. The method is highly specific because substrate will be acted upon only when second antibody and third antibody are very close to each other and in a particular geometric alignment, which can be created by designing the antibody- conjugate constituents properly. In this way, there is a selectivity and proximity interaction requirement which effectively minimizes nonspecific reaction between unbound second antibody or third antibody and their labelled conjugates.

Alternative labelling systems embodying this triple antibody conceptinclude, but are not limited to, a fluorescent label and a quencher molecule; an enzyme and an inhibitor molecule; a primary enzyme that acts on a substrate, which, in turn produces a product that is the substrate for a secondary enzyme that will act on the product-substrate to produce a signal.

A fluorescence quenching assay can be performed utilizing the selective binding of an antibody to the antigen:first antibody complex. In one embodiment of this assay antigen is labelled with a quencher molecule and second antibody is labelled with a chemiluminescent substance. When the second antibody recognizes the bound antigen:first antibody complex, it binds to the antibody:antigen junction. Quencher is now in proximity to chemiluminescer and by its interaction will reduce the amount of signal emmitted by the chemiluminescer. The reduction of signal is indirectly related to the amount of analyte present.

An alternative procedure is to add a labelled first antibody to an antigen to form an immunocomplex. Subsequently, an insolublized second antibody is added which binds to the juncture of the labelled first antibody and antigen. This method would be advantageous where steric problems between an insolublized first antibody and antigen might occur.

The Examples that follow further describe, define and illustrate a number of different embodiments of this invention. They are by way of illustration only and are not to be construed as a limitation.

EXAMPLE 1

A. ASSAY PROCEDURE FOR HUMAN CHORIONIC GONADOTROPIN (hCG)

This antibody is prepared according to the general procedure disclosed by Milstein & Kohler in NATURE 256:495-497, 1975. A complex is formed of human chorionic gonadotropin ("hCG") and an antibody directed against it. This complex serves as the new antigen to which the monoclonal antibodies are designed.

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against the hCG:antibody complex antigen, with an appropriate myeloma cell line. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be

described in connection with mice. The mouse is first immunized by injection of the antigen chosen generally for a period of eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the antigen, and then killed so that the immunized spleen may be removed. The fusion can then be carried out utilizing immunized spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificty for the particular antigen. The monoclonal antibody selected, which is specific for the antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labelling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labelled with a multitude of different labels, such enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. Some of the enzymes utilized as labels are alkaline phosphatase, glucose oxidase, galactosidase, peroxidase, urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

B. ASSAY STEPS--SEQUENTIAL METHOD

To insolublized first antibody directed against hCG (polyclonal or monoclonal) are added one to three drops of urine suspected of containing hCG. The mixture is incubated for one minute to permit formation of a first complex of the first antibody with any hCG present. To this one drop of alkaline phosphatase labelled second antibody, prepared in Step A above, is added. The mixture is allowed to incubate for two minutes to permit formation of a second complex of the first complex and second labelled antibody. Subsequently, the mixture is filtered. Any second complex will remain within or on the filter and is washed with Tris buffered saline to remove unbound reactants. To the insolublized second complex is then added indoxyl phosphate substrate to develop color. After one to three minutes a blue color will appear if a second complex has been formed indicating that hCG is present in the specimen.

EXAMPLE 2

A. ASSAY PROCEDURE FOR GROUP A STREPTOCOCCUS

Idiotypic-antigenic conjunction binding antibody to group A Streptococcus is prepared according to the procedure described in Example 1 part A above, except that group A Streptococcus antigen is substituted for hCG.

B. ASSAY STEPS--SIMULTANEOUS METHOD

To insolublized first antibody directed against group A Strep are simultaneously mixed one to three drops specimen suspected of containing group A Strep antigens and one drop of alkaline phosphatase labelled second antibody, prepared in Step A, EXAMPLE 1 above. The

mixture is allowed to incubate for one to five minutes to permit the formation of a complex of the first antibody with any group A Strep present. Then the mixture is filtered. Any complex will remain within or on the filter and is washed with 1 ml of Tris buffered saline to remove unbound reactants. To the insolublized complex is added one drop is indoxyl phosphate substrate to develop color. After one to three minutes a blue color will appear if a complex has been formed indicating that group A Strep is present in the specimen.

EXAMPLES 3-8

A. ASSAY PROCEDURES FOR OTHER ANALYTES

The procedure for idiotypic-antigenic conjunction binding antibody preparation of EXAMPLE 1 STEP 1 above is followed except that the hCG used is substituted each in turn with Chlamydia trachomitis, Candida albicans, Trichomonas vaginalis, OMP MC gonorrhea protein, creatinine phosphokinase, hepatitis B, and the like.

B. ASSAY PROCEDURE FOR DETECTION OR QUANTITATION OF ANALYTE

The procedure according to either Step B, EXAMPLE 1 or Step B, EXAMPLE 2, above is followed, substituting for the hCG antigen an antigen such as one in the list described above. An appropriate first antibody is selected as being directed against the analyte suspected of being in a given specimen.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth, but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as

defined by the appended claims. This list is not exclusive, merely illustrative; the procedure can be used with any material capable of forming an antigen:antibody complex.

WHAT IS CLAIMED IS:

1. A method for detection or quantitation of an analyte of interest suspected of being in a specimen comprising:

(a) contacting a first antibody with said specimen suspected of containing said analyte in such a manner that said first antibody reacts with any of said analyte present to form a first complex;

(b) contacting with any of said first complex a second antibody which recognizes and binds preferentially to the juncture of the idiotypic determinant of said first antibody and the antigenic determinant of said analyte of said first complex to form a second complex;

(c) determining the presence or amount of said second complex as an indication of the presence or amount of said analyte of interest in said specimen.

2. A heterogeneous method for conducting an assay for the detection or quantitation of an analyte suspected of being in a specimen comprising:

(a) providing a first antibody bound to an insolubilized carrier;

(b) contacting with said first antibody said specimen in such a manner that said first insolublized antibody reacts with any of said analyte present to form an insolublized first complex;

(c) washing said complex to separate bound analyte from unbound analyte;

(d) contacting with said first complex a second antibody which recognizes the and binds preferentially to the juncture of the idiotypic determinant of said first antibody and the antigenic determinant of said analyte to form a second complex;

(e) washing said second complex to separate bound material from unbound material; and

(f) determining the presence or amount of said second complex as an indication of the presence or amount of said analyte of interest in said specimen.

3. The method of claim 2 wherein said first antibody and said second antibody are contacted with said analyte simultaneously.

4. The method of claim 2 wherein said first antibody and said second antibody are contacted with said analyte sequentially.

5. The method of claim 4 wherein said first antibody is contacted with said analyte prior to contacting with said second antibody.

6. The method of claim 4 wherein said second antibody is contacted with said analyte prior to contacting said first antibody.

7. The method of claims 1 or 2 wherein at least one of said antibodies is labelled with a signal generating material.

8. The method of claim 7 wherein the labelled antibody is labelled with a substance selected from a radioisotope, an enzyme, a fluorescent material, a luminescent material, a bioluminescent material, a chromogenic material or a DNA or RNA probe material.

9. The method of claim 8 wherein the labelling material is an enzyme and the reagents for generating a signal include a substrate reactive with said enzyme.

10. The method in claim 8 wherein the labelling material is a fluorescent molecule and the reagents for modulating a signal include a fluorescence quenching molecule.

11. The method of claims 1 or 2 wherein the analyte is selected from an antigen, antibody, protein, hormone, enzyme, drug, vitamin, polysaccharide, bacteria, protozoa, virus or fungus.

12. The method of claims 1 or 2 wherein said first antibody is a polyclonal or monoclonal antibody.

13. The method of claims 1 or 2 wherein said second antibody is a polyclonal or monoclonal antibody.

14. The method of claims 1 or 2 wherein said first antibody and said second antibody are both monoclonal antibodies which are the product of different cell lines.

15. The method of claims 1 or 2 wherein said first antibody and said second antibody are both monoclonal antibodies which are the product of the same cell line.

16. The method of claim 7 wherein said second antibody is designed to recognize and bind preferentially when immunocomplexed analyte is present.

17. The method of claim 7 wherein said first antibody is labelled with a signal generating substance rather than said second antibody.

18. The method of claim 14 wherein said second antibody is insolublized.

19. A method for detection or quantitation of an analyte suspected of being in a specimen comprising:

(a) providing a first antibody, bound to an insolublized carrier, that is specific for an analyte of interest;

(b) contacting with said first antibody said specimen under conditions permitting the binding of first antibody to a distinct antigenic determinant on said analyte, resulting in the formation of an insolublized first complex of first antibody and any analyte in said specimen;

(c) separating bound analyte from unbound analyte;

(d) contacting with said first complex a second antibody which will bind to said analyte at a second and distinct antigenic determinant on said analyte so as to form a second complex;

(e) washing said second complex to separate bound from unbound second antibody;

(f) contacting with said second complex a labelled third antibody which will recognize and bind preferentially to the juncture of the idiotypic determinant of said second antibody and the antigenic determinant of said analyte to form a third complex;

(g) separating bound from unbound labelled third antibody; and

(h) measuring the amount of signal generated which is correlative with the presence or quantity of any analyte originally present in said specimen.

20. A heterogeneous assay for the detection or quantitation of an antigen suspected of being in a specimen comprising:

(a) contacting a first monoclonal antibody which has been bound to an insolublized solid surface with said antigen in such a manner that said first monoclonal antibody reacts with any of said antigen present in said solution to form a first complex;

(b) washing said first complex to separate bound from unbound first monoclonal antibody;

(c) contacting with any of said first complex a second monoclonal antibody conjugated with an enzyme indicator label such that said second monoclonal antibody recognizes and binds preferentially to the juncture of the idiotypic determinant of said first monoclonal antibody and the antigenic determinant of said antigen to form a second complex;

(d) washing said second complex separate bound from unbound second monoclonal antibody;

(e) contacting with said second complex a substrate capable of reacting with said enzyme label such that a signal is generated; and

(f) reading the amount of signal present, if any, as being related to the amount of antigen present in said specimen.

21. A method for detection or quantitation of an analyte suspected of being in a specimen comprising:

(a) pre-immobilizing a first antibody on or within a solid porous matrix material prior to contacting said first antibody with another reaction material;

(b) contacting with said immobilized first antibody said specimen in such a manner that said first antibody reacts with any of said analyte present to form a first complex;

(c) contacting with any of said first complex a second antibody which recognizes and binds preferentially to the juncture of the idiotypic determinant of said first antibody and the antigenic determinant of said analyte to form a second complex;

(d) washing said second complex so as to remove unbound material from said reaction zone; and

(e) determining the presence or amount of said second complex as an indication of the presence or amount of analyte of interest in said specimen.

22. The method of claim 22 wherein said second antibody is preimmobilized in said solid porous matrix rather than said first antibody.

23. The method of claim 22 wherein at least one of said antibodies is labelled with a signal generating material.

24. The method of claim 24 wherein the labelled antibody is labelled with a substance selected from a radioisotope, an enzyme, a fluorescent material, a luminescent material, a bioluminescent material, a chromogenic material or a DNA or RNA probe material.

25. The method of claim 25 wherein the labelling material is an enzyme and the reagents for generating a signal include a substrate reactive with said enzyme.

26. The method of claim 22 wherein the analyte is selected from an antigen, antibody, protein, hormone, enzyme, drug, vitamin, polysaccharide, bacteria, protozoa, virus or fungus.

27. The method of claim 22 wherein said first antibody is a polyclonal or monoclonal antibody.

28. The method of claim 22 wherein said second antibody is a polyclonal or monoclonal antibody.

29. The method of claim 22 wherein said first antibody and said second antibody are both monoclonal antibodies which are the product of different cell lines.

30. The method of claim 22 wherein said first antibody and said second antibody are both monoclonal antibodies which are the product of the same cell line.

31. The method of claim 22 wherein said second antibody is designed to recognize and bind preferentially when immunocomplexed analyte is present.

32. A monoclonal antibody specific for the juncture of an antigen:antibody complex whereby said monoclonal antibody recognizes and binds preferentially to the juncture of the idiotypic determinant of said complexed antibody and the antigenic determinant of said complexed antigen.

33. The monoclonal antibody of claim 33, wherein said antigen is any substance capable of forming a complex with a particular antibody.

34. The monoclonal antibody of claim 34, wherein said antigen is selected from human chorionic gonadotropin, streptococcus, Neisseria gonorrhea, chlamydia, herpes, Escherichia coli, cytomegalovirus, rubella, rotavirus, Candida, Trichomonas vaginalis or HTLV.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/02214

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(4): G01N 33/543; G01N 33/564; A61K 39/395
U.S. CL: 435/7; 436/507; 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/5,6,7,172.2,240 935/110 436/507,518,540,548,819 530/387,808

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts Online; Biosis

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]
Y	US,A, 4,544,640 (SOMA) 01 October 1985, see column 1, lines 46-58 and column 4, lines 53-56.	1-34
Y	US,A, 4,514,508 (HIRSCHFELD) 30 April 1985, see column 4, lines 12-45.	1-31
Y	US,A, 4,279,884 (BRADWELL) 21 July 1981, see column 1, lines 18-36.	32-34
Y	Chemical Abstracts, Volume 88, No. 23, issued 05 June 1978 (Columbus, Ohio, USA), Kyoichi Kano, "Detection of Circulating Immune Complexes By The Inhibition of Anti-Antibody," see page 437, column 2, the abstract no. 168279p, Clin. Immunol. Immunopathol., 1978, 9(4), 425-35 (Eng).	32-34

* 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]
29 December 1986	05 JAN 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Esther M. Kepplinger Esther M. Kepplinger

Form PCT/ISA/210 (second sheet) (October 1981)