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(57) Abstract

'Two-site' or 'sandwich' immunometric assay techniques for determination of the presence and/or concentration of antigenic substances in fluids using a combination of monoclonal and polyclonal antibodies. One antibody selected from a group consisting of monoclonal$_x$ and polyclonal$_{(1-x)}$ antibodies is presented in a soluble labeled form; a second antibody selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ is presented bound to a solid carrier; and x is 0 or 1. The present invention further encompasses a kit comprising in association: (a) a soluble first antibody to an antigenic substance present in a fluid to be assayed, the first antibody being labeled and being selected from a group consisting of monoclonal$_x$ and polyclonal$_{(1-x)}$ antibodies; and (b) a second antibody to the antigenic substance, the second antibody being bound to a solid carrier and being selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ antibodies; wherein x is 0 or 1.

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# IMMUNOMETRIC ASSAY USING POLYCLONAL AND MONOCLONAL ANTIBODIES AND A KIT FOR USE THEREIN

## Description of the Prior Art

### 1. Field of the Invention

This invention relates to methods for detecting and/or for determining the concentration of antigenic substances in fluids such as serum and to a kit for use therein. In another aspect it relates to immunometric assay techniques. In yet another aspect it relates to monoclonal and polyclonal antibodies.

### 2. Background of the Invention

The determination of the presence or concentration of antigenic substances, for example, those associated with a wide variety of physiological disorders, in serum or other body fluids relies increasingly upon immunoassay techniques. These techniques are based upon formation of a complex between the antigenic substance being assayed and an antibody or antibodies in which one or the other member of the complex may be labeled, for example, by a radioactive element such as $I^{125}$, which permits its detection and/or quantitative analysis after separation of the complexed labeled antigen or antibody from uncomplexed labeled antigen or antibody.

In the case of a competition immunoassay technique, the antigenic substance in a sample of fluid being tested for its presence competes with a known quantity of labeled antigen for a limited quantity of antibody binding sites. Thus, the amount of labeled antigen bound to the antibody is inversely proportional

to the amount of antigen in the sample. By contrast, immunometric assays employ a labeled antibody. In such an assay, the amount of labeled antibody associated with the complex is directly proportional to the amount of antigenic substance in the fluid sample.

Immunometric assays have been found to be particularly well suited for the detection of polyvalent antigens, i.e., antigenic substances that are able to complex with two or more antibodies at the same time. Such assays employ a quantity of unlabeled antibody bound to a solid support that is insoluble in the fluid being tested and a quantity of soluble antibody bearing a label such as a radioactive isotope that permits detection and/or a quantitative estimate of the amount of the ternary complex formed between solid phase antibody, antigen, and labeled antibody.

This kind of assay is frequently referred to as a "two-site" or "sandwich" assay since the antigen has two antibodies bonded to its surface at different locations. This and related techniques are described by Wide at pp. 199-206 of "Radioimmunoassay Methods", Edited by Kirkham and Hunter, E. & S. Livingstone, Edinburgh, 1970. An assay based on this technique for the detection of the antigen associated with serum hepatitis using an $I^{125}$ labeled antibody is described in U.S. Patent No. 3,867,517.

Unfortunately, however, large amounts of highly purified, active antibody specific to the antigen of interest for preparing a solid phase with sufficient antigen binding capacity is difficult to obtain from the "polyclonal" antibodies used in prior art sandwich assays. Methods for affinity purifying such antibodies have generally been time consuming and resulted in low

yields and loss of high affinity antibodies. When an immunogenic substance is introduced into a living body, the body's immune system reacts by generating antibodies to every site on the immunogen it recognizes. A large immunogenic protein molecule may have dozens of sites and a foreign cell may have hundreds. Thus, while each antibody producing cell produces antibody specific for a single antigenic site, the immune system has generated a specie of specific antibody producing cells for each immunogenic site recognized. In addition, the body has produced relatively large quantities of antibodies to antigens other than the one of interest such that most of the antibody in the polyclonal mixture is not specific for the antigen of interest. Accordingly, the antibodies used in prior immunometric assays are necessarily "polyclonal" in nature since the antibodies are derived from antisera raised in a conventional manner in animals and their purification is difficult.

In addition, a potential for cross-reaction with other materials in serum or other fluid than the antigen for which the test is intended exists in these prior art sandwich assays. This occurrence of cross-reactivity with other antigens also reduces the sensitivity of these prior art sandwich assays for the suspect antigen and increases the prospect of a "false-positive" assay.

In an attempt to alleviate the shortcomings present in the prior art, David et al. in U.S. Patent 4,376,110 disclose a methodology wherein the polyclonal antibody used as in an immunometric assay as the unlabeled antibody bound to a solid support and the polyclonal antibody used as the soluble labeled antibody are both replaced by at least one and usually two or more different monocloncal antibodies, i.e., each antibody

specific to a single antigenic site and separately produced by clones derived from unique cell lines. In a preferred embodiment, David et al. suggest that the monoclonal antibody used as the antibody bound to the solid support be the product of a different cell line than is the monoclonal antibody used for the labeled antibody and the two monoclonal antibodies be selected to bind the antigenic substance at sites remote from each other so as to not interfere with the others binding to the antigen.

By employing the modifications suggested by David et al., one is able to (a) eliminate the cross reactivity problem inherent in the prior art sandwich assay as well as (b) secure large quantities of relatively inexpensive monoclonal antibodies. However, the modifications suggested by David et al. yield a procedure which requires longer reaction periods due to the relatively low avidities possessed by currently available monoclonal antibodies in comparison to the avidities of polyclonal antibodies.

Accordingly, it would be very desirable to have an immunometric assay methodology and kit wherein the advantages of the monoclonal system of David et al. (high specificity, low cost, and ready availability of antibodies in high quantity) as well as the advantages of the polyclonal system of the prior art (high avidity, relatively fast reaction times, and high sensitivity) are both present.

## Summary of the Invention

In accordance with the present invention, there is provided an immunometric assay methodology and kit for use therein with the advantages of the monoclonal antibody system of David et al. (high specificity, low

cost, and ready availability of antibodies high quantity) as well as the advantages of the polyclonal antibody system of the prior art (high avidity, relatively fast reaction times, and high sensitivity) both present. More particularly, the present invention encompasses a process for asaying the concentrations of an antigenic substance in a fluid comprising the steps:

(a) contacting a sample of the fluid with a measured amount of a soluble first antibody to the antigenic substance in order to form a soluble complex of the antibody and antigenic substance present in the sample, the first antibody being labeled and being selected from a group consisting of $monoclonal_x$ and $polyclonal_{(1-x)}$ antibodies:

(b) contacting the soluble complex with a second antibody to the antigenic substance, the second antibody being bound to a solid carrier and being selected from a group consisting of $monoclonal_{(1-x)}$ and $polyclonal_x$ antibodies, the solid carrier being insoluble in the fluid, in order to form an insoluble complex of the first antibody, the antigenic substance and the second antibody bound to the solid carrier·

(c) separating the solid carrier from the fluid sample and unreacted labeled antibody:

(d) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody· and

(e) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a blank sample prepared in accordance with steps (a)-(d), the blank sample being known to be free of the antigenic substance, to determine the presence of antigenic substance in the fluid sample, or

relating the amount of labeled antibody measured with the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(d) to determine the concentration of antigenic substance in the fluid sample.

wherein x is 0 or 1.

In addition, the present invention also encompasses a process for the determination of the presence of an antigenic substance in a fluid comprising the steps:

(a) simultaneously contacting a sample of the fluid with first and second antibodies to the antigenic substance, the first antibody being labeled and soluble in the fluid, being provided for in a measured amount, and being selected from a group consisting of monoclonal$_x$ and polyclonal$_{(1-x)}$ antibodies, and the second antibody being bound to a solid carrier insoluble in the fluid and being selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ antibodies, in order to form an insoluble complex of the first antibody, the antigenic substance and the second antibody;

(b) separating the solid carrier from the fluid sample and unreacted labeled antibody;

(c) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody;

(d) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a blank sample prepared in accordance with steps (a)-(c), the blank sample being known to be free of the antigenic

substance, to determine the presence of antigenic substance in the fluid sample, or relating the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(c) to determine the concentration of antigenic substance in the fluid sample,

wherein x is 0 or 1.

Also encompassed in the present invention is an improved immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid. This assay is of the type comprising forming a ternary complex of a first labeled antibody, the antigenic substance, and a second antibody, the second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labeled antibody bound to the solid carrier or the amount of unreacted labeled antibody. The improvement in the immunometric assay of the present invention comprising employing a labeled antibody selected from a group consisting of $monoclonal_x$ and $polyclonal_{(1-x)}$ antibodies and an antibody bound to a solid carrier and selected from a group consisting of $monoclonal_{(1-x)}$ and $polyclonal_x$ antibodies, wherein x is 0 or 1.

The present invention further encompasses a kit comprising in association:

(a) a soluble first antibody to an antigenic substance present in a fluid to be assayed, the first antibody being labeled and being selected from a group consisting of $monoclonal_x$ and $polyclonal_{(1-x)}$ antibodies· and

(b) a second antibody to said antigenic substance, the second antibody being bound to a solid carrier and being selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ antibodies:

wherein x is 0 or 1.

By employing a monoclonal antibody as either the labeled antibody or the antibody bound to a solid carrier, one is able to obtain the high specificity possessed by the methodology of David et al. In addition, by employing a polyclonal antibody as the corresponding bound antibody or labeled antibody, respectively, one is able to obtain the high avidity, relatively fast reaction times, and high sensitivity of the prior art methodologies employing only polyclonal antibodies. Furthermore, when the monoclonal antibody is chosen as the antibody bound to the solid carrier, one can further obtain the cost benefit associated with the assay of David et al. This additional benefit results from the fact that (a) the amount of antibodies bound to the solid category are generally 1 to 2 orders of magnitude greater than the amount of antibodies required to be labeled and (b) monoclonal antibodies are cheaper than polyclonal antibodies.

## Detailed Description of the Invention

As indicated above, according to the present invention, one polyclonal antibody used in an immunometric assay for an antigenic substance is replaced by a monoclonal antibody.

The present invention is useful for the determination of the presence or concentration of a wide variety of polyvalent antigenic substances. Accordingly, as used herein, the term antigen or antigenic substance

refers broadly to substances to which antibodies can be produced. Among such substances may be mentioned hormones such as insulin and human thyroid stimulating hormone (HTSH), gamma globulins, allergens, viruses, virus subunits, bacteria, toxins such as those associated with tetanus and with animal venoms. and even some drugs. Among the specific antigens which may be assayed by the process of the present invention may be mentioned thyroid stimulating hormone (TSH), human IgE, human CG, carcinoembryonic antigen (CEA), hepatitis A and B, hepatitis Non A/Non B, IgE and alphafetoprotein.

The monoclonal antibodies useful in the present invention are obtained by the process discussed by Milstein and Kohler and reported in Nature, 256:495-497 (1975). The details of this process are well known and will not be repeated here.

Preferably, the monoclonal antibody selected will have an affinity of at least $10^8$ liters/mole and, more preferably, an affinity of at least about $10^9$ liters/mole. It is also preferred that the polyclonal antibody have an average affinity of at least $10^9$, more preferably $10^{10}$, liters/mole.

Preferably, the monoclonal antibody is selected to be the bound antibody and the polyclonal antibody is selected to be the soluble labeled antibody. The reason for this choice is that the amount of solid phase antibody is approximately 1 or 2 orders of magnitude greater than the amount of labeled antibody required in an immunometric assay. Accordingly, this choice enables one to rely on a relatively low cost, readily available monoclonal antibody for the bulk of one's antibody requirements.

The unlabeled monoclonal antibody or polyclonal used in the present invention to extract the antigenic substance from the sample being tested may be immobilized on any of the common supports used in immunometric assays. Among these may be mentioned filter paper, plastic beads or test tubes made from nylon, polyethylene. polystyrene, polypropylene or other suitable material. Also useful are particulate materials such as agarose, crosslinked dextran, and other polysaccharides. The techniques for such bonding are well known to those skilled in the art. For example, antibodies may be bound to polysaccharide polymers using the process described in U.S. Patent No. 3,645,852.

The labeled monoclonal or polyclonal antibody used in the present invention may be provided with the same labels used in prior art immunometric assays. Among these may be mentioned fluorogenic labels for detection by fluorometry as described in U.S. Patent No. 3,940,475 and enzymatic markers as described in U.S. Patent No. 3,645,090. It is presently preferred to label the antibody with a radioisotope such as $I^{125}$ using, for example, the procedure of Hunter et al., Nature, 144:945 (1962), or that of David et al., Biochemistry, 13:1014-1021 (1974).

In a typical assay, the amount of labeled antibody associated with the insoluble sandwich complex is determined by examination of the insoluble carrier material by suitable means. However, it is also possible to relate the presence or absence of antigen in the fluid sample being assayed to the amount of labeled antibody which does not react during the assay and remains in a soluble form.

The kit of the present invention preferably further comprises a set of standards which will enable one to generate a standard curve. By comparing the value obtained from an unknown sample with the standard curve, one can quantitatively assay the sample. In addition, kits which contain an enzyme labeled antibody can further optionally comprise an enzyme marker developer for purposes of being able to detect the amount of enzyme present in the phase being assayed.

The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention.

## Example 1

### Reagent Preparation

A. Polyclonal antibodies - anti-human IgE serum was produced from goat and purified via affinity chromatography.

B. Monoclonal antibodies - monoclonal antibodies for IgE were obtained from a commercial source.

C. Labeled antibodies - polyclonal antibodies and monoclonal antibodies were each labeled with horse radish peroxidase via the technique of Nakane, et al., Journal of Histochemistry and Cytochemistry, 22 (12):1084-1091 (1974).

D. Bound antibodies - monoclonal antibodies for IgE were covalently bound to polystyrene beads via standard commercial techniques.

## Example 2

The following immunometric assay protocol was employed for assaying IgE:

Assay Method 2 (Room Temperature Plus Room Temperature 30 Minutes)

1. 12x75 mm borosilicate glass tubes.
2. 25 µL IgE standards or patient samples.
3. 250 µL Conjugate.
4. Add one bead to each tube. Mix gently.
5. Incubate at 37°C. (rotator 180 rpm) for 30 minutes.
6. Wash 3 times with $d^i$stilled $H_2O$.
7. Add 300 µL o-phenylenediamine (OPD) substrate solution. Mix gently.
8. Incubate at room temperature for 30 minutes ±1 minute.
9. Add 1.0 mL 1N HCl to stop the reaction.
10. Read $A_{492}$ vs. $d^i$stilled $H_2O$.

The same bound monoclonal antibody ($Bm_1$) was employed in all assays. Different labeled monoclonal antibodies ($Lm_1$ through $Lm_{11}$) and different labeled polyclonal antibodies ($Lp_1$ through $Lp_3$) were employed in each assay. The results of these assays are set forth in Table I.

Example 3

The immunometric assay protocol of Example 2 was employed with one modification. The sole modification was in step 5 in that the incubation period was increased to 150 minutes.

The results of these assays are also set forth in Table I.

TABLE I

Δ O.D.

| Bound antibody/- Labeled antibody | IgE Concentration, IU/ml: / Incubation Periods, Min. | 0 | | 75 | | 200 | | 400 | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30/30 | 150/30 | 30/30 | 150/30 | 30/30 | 150/30 | 30/30 | 150/30 |
| $Bm_1/Lm_1$ | | 0.078 | 0.042 | 0.097 | 0.048 | 0.093 | 0.050 | 0.096 | 0.081 |
| $Bm_1/Lm_2$ | | 0.087 | 0.056 | 0.114 | 0.112 | 0.095 | 0.145 | 0.156 | 0.108 |
| $Bm_1/Lm_3$ | | 0.040 | 0.053 | 0.080 | 0.187 | 0.108 | 0.322 | 0.127 | 0.476 |
| $Bm_1/Lm_4$ | | 0.140 | 0.100 | 0.114 | 0.159 | 0.108 | 0.367 | 0.099 | 0.268 |
| $Bm_1/Lm_5$ | | 0.085 | 0.081 | 0.077 | 0.084 | 0.078 | 0.210 | 0.057 | 0.162 |
| $Bm_1/Lm_6$ | | 0.060 | 0.090 | 0.050 | 0.156 | 0.069 | 0.256 | 0.101 | 0.377 |
| $Bm_1/Lm_7$ | | 0.057 | 0.078 | 0.082 | 0.336 | 0.078 | 0.416 | 0.114 | 0.363 |
| $Bm_1/Lm_8$ | | 0.174 | A* | 0.193 | A | 0.139 | A | 0.235 | A |
| $Bm_1/Lm_9$ | | 0.074 | 0.131 | 0.071 | 0.124 | 0.069 | 0.235 | 0.080 | 0.253 |
| $Bm_1/Lm_{10}$ | | 0.113 | 0.075 | 0.079 | 0.209 | 0.108 | 0.202 | 0.097 | 0.290 |
| $Bm_1/Lm_{11}$ | | 0.077 | 0.071 | 0.065 | 0.126 | 0.125 | 0.145 | 0.060 | 0.289 |
| $Bm_1/Lp_3$ | | 0.084 | 0.144 | 0.462 | 1.233 | 1.009 | N/A** | 1.260 | >2 |
| $Bm_1/Lp_2$ | | 0.130 | 0.210 | 0.436 | 1.063 | 0.977 | >2 | 1.508 | >2 |
| $Bm_1/Lp_3$ | | 0.142 | 0.188 | 0.879 | >2 | 1.485 | >2 | 1.785> | >2 |

* "A" denotes artifact
** N/A denotes not available.

The data set forth in Table I indicates that an assay employing monoclonal antibodies as both the labeled antibody and the bound antibody is unable to produce a detectable signal even after a long incubation period of 150 minutes. In sharp contrast, the data in Table I shows that in an assay employing a monoclonal antibody and a polyclonal antibody one is able to obtain excellent results after a short incubation period of only 30 minutes.

Based upon this disclosure, many other modifications and ramifications will naturally suggest themselves to those skilled in the art. These are intended to be comprehended as within the scope of this invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for assaying the concentration of an antigenic substance in a fluid comprising the steps:
   (a) contacting a sample of the fluid with a measured amount of a soluble first antibody to the antigenic substance in order to form a soluble complex of the antibody and antigenic substance present in said sample, said first antibody being labeled and being selected from a group consisting of $monoclonal_x$ and $polyclonal_{(1-x)}$ antibodies;
   (b) contacting the soluble complex with a second antibody to the antigenic substance, said second antibody being bound to a solid carrier and being selected from a group consisting of $monoclonal_{(1-x)}$ and $polyclonal_x$ antibodies, said solid carrier being insoluble in said fluid, in order to form an insoluble complex of said first antibody, said antigenic substance and said second antibody bound to said solid carrier;
   (c) separating said solid carrier from the fluid sample and unreacted labeled antibody;
   (d) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody; and
   (e) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a blank sample prepared in accordance with steps (a)-(d), said blank sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labeled antibody

measured with the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(d) to determine the concentration of antigenic substance in said fluid sample,

wherein x is 0 or 1.

2. The process of claim 1 wherein said solid carrier resulting from step (d) is washed to separate the fluid from the carrier.

3. The process of claim 1 wherein the labeled antibody is labeled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material and said examination is by means selected from the group consisting of radiometric means, enzymatic means and fluorometric means.

4. The process of claim 1 wherein x is 0.

5. A process for the determination of the presence of an antigenic substance in a fluid comprising the steps:

(a) simultaneously contacting a sample of the fluid with first and second antibodies to said antigenic substance, said first antibody being labeled and soluble in said fluid, being provided for in a measured amount, and being selected from a group consisting of monoclonal$_x$ and polyclonal$_{(1-x)}$ antibodies, and said second antibody being bound to a solid carrier insoluble in said fluid and being selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ antibodies, in order to form an

insoluble complex of said first antibody, said antigenic substance and said second antibody·

(b) separating said solid carrier from the fluid sample and unreacted labeled antibody·

(c) measuring either the amount of labeled antibody associated with the solid carrier or the amount of unreacted labeled antibody·

(d) relating the amount of labeled antibody measured with the amount of labeled antibody measured for a blank sample prepared in accordance with steps (a)-(c), said blank sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labeled antibody measured with the amount of labeled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(c) to determine the concentration of antigenic substance in said fluid sample,

wherein x is 0 or 1.

6. The process of claim 5 wherein said solid carrier resulting from step (b) is washed to separate the fluid sammple from the carrier.

7. The process of claim 5 wherein the labeled antibody is labeled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material and said examination is by means selected from the group consisting of radiometric means, enzymatic means and fluorometric means.

8. The process of claim 5 wherein x is 0.

9. In an immunometric assay to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of a first labeled antibody, said antigenic substance, and a second antibody, said second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labeled antibody bound to the solid carrier or the amount of unreacted labeled antibody, the improvement comprising employing an antibody selected from a group consisting of $\text{monoclonal}_x$ and $\text{polyclonal}_{(1-x)}$ antibodies as said labeled antibody and an antibody selected from a group consisting of $\text{monoclonal}_{(1-x)}$ and $\text{polyclonal}_x$ antibodies as said antibody bound to said solid carrier,

wherein x is 0 or 1.

10. The process of claim 9 wherein the fluid sample is first contacted with the second antibody to form a binary complex of the antigenic substance and said second antibody insoluble in the fluid and then contacted with said first labeled antibody to form the ternary complex.

11. The process of claim 9 wherein the fluid sample is first contacted with the second antibody to form a binary complex of the antigenic substance and said second antibody insoluble in the fluid, the sample separated from the solid carrier and the solid carrier contacted with a solution of said first labeled antibody to form said ternary complex.

12. The process of claim 11 wherein said solid carrier after formation of the ternary complex is washed to separate the fluid sample from the carrier.

13. The process of claim 9 wherein the labeled antibody is labeled with a member selected from the group consisting of a radioactive isotope, an enzyme and a fluorogenic material.

14. The process of claim 9 wherein x is 0.

15. A kit comprising in association:
(a) soluble first antibody to an antigenic substance present in a fluid to be assayed, said first antibody being labeled and being selected from a group consisting of monoclonal$_x$ and polyclonal$_{(1-x)}$ antibodies: and
(b) a second antibody to said antigenic substance, said second antibody being bound to a solid carrier and being selected from a group consisting of monoclonal$_{(1-x)}$ and polyclonal$_x$ antibodies:
wherein x is 0 or 1.

16. The kit of claim 15 wherein x is 0.

17. The kit of claim 15 further comprising a set of standards.

18. The kit of claim 17 wherein x is 0.

19. The kit of claim 15 further comprising a set of standards and an enzyme marker developer.

20. The kit of claim 19 wherein x is 0.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 83/01147

**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[3]: G 01 N 33/54

**II. FIELDS SEARCHED**

Minimum Documentation Searched [4]

| Classification System | Classification Symbols |
|---|---|
| IPC[3] | G 01 N |

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

**III. DOCUMENTS CONSIDERED TO BE RELEVANT** [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| X | EP, A, 0062892 (AMERICAN HOECHST CORP.) 20 October 1982 see the entire document | 1-9,11-20 |
| X | WO, A, 82/01773 (CELLTECH LIMKED) 27 May 1982 see the entire document | 1-3,5-7,9-13 15,17,19 |
| X | Clinical Chemistry, vol. 29, no. 1, published in January 1983 (Washington, US) G. De Groote et al. "Use of monoclonal antibodies to detect human placental alkaline phosphatase", see pages 115-119 | 1-3,5-7,9, 10,12,13 |
| X | US, A, 4361647 (J. REMINGTON et al.) 30 November 1982 see columns 2-4; example 2; claims 1,3,5 & 6 | 1-10,12-14 |
| | | ./. |

* Special categories of cited documents: [15]

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**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| 20th March 1984 | 19 AVR. 1984 |
| **International Searching Authority** [1] | **Signature of Authorized Officer** [20] |
| EUROPEAN PATENT OFFICE | G.L.M. Kruydenberg |

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, 16 with indication, where appropriate, of the relevant passages 17 | Relevant to Claim No 18 |
|---|---|---|
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| Y | EP, A, 0048357 (LA JOLLA CANCER RESEARCH FOUNDATION) 31 March 1982<br>see the entire document | 1-14 |
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