



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |           |                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>(51) International Patent Classification <sup>5</sup> :</b><br><b>G01N 33/543, 33/577, 33/552</b><br><b>G01N 33/535, 33/78, 33/541</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 91/18291</b><br><b>(43) International Publication Date:</b> 28 November 1991 (28.11.91)                                                                                                                                                                                                                                                          |
| <b>(21) International Application Number:</b> PCT/US91/02092<br><b>(22) International Filing Date:</b> 27 March 1991 (27.03.91)<br><br><b>(30) Priority data:</b><br>526,580                      18 May 1990 (18.05.90)                      US<br><br><b>(71) Applicant:</b> PB DIAGNOSTIC SYSTEMS, INC. [US/US];<br>151 University Avenue, Westwood, MA 02090 (US).<br><br><b>(72) Inventor:</b> BAHAR, Izak ; 34 Bryon Road, Apt. 3, West<br>Roxbury, MA 02167 (US).<br><br><b>(74) Agent:</b> MACCARONE, Gaetano, D.; PB Diagnostic Sys-<br>tems, Inc., 151 University Avenue, Westwood, MA<br>02090-2399 (US).                      |           | <b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).<br><br><b>Published</b><br><i>With international search report.</i> |
| <b>(54) Title:</b> SOLID SUPPORT HAVING ANTIBODIES IMMOBILIZED THEREON<br><br><b>(57) Abstract</b><br><br>There is disclosed a solid support having antibodies immobilized thereon. The specific antibodies of interest are immobilized on the solid support as part of a complex which includes a nonspecific antibody and an antibody directed against the Fc fragments of the specific and nonspecific antibodies. In a preferred embodiment the solid support is used in solid phase competitive immunoassays and provides improved signal levels and desirable steeper standard curve slopes in the desired assay sensitivity range. |           |                                                                                                                                                                                                                                                                                                                                                                                                      |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                          |    |                          |
|----|--------------------------|----|------------------------------------------|----|--------------------------|
| AT | Austria                  | ES | Spain                                    | MG | Madagascar               |
| AU | Australia                | FI | Finland                                  | ML | Mali                     |
| BB | Barbados                 | FR | France                                   | MN | Mongolia                 |
| BE | Belgium                  | GA | Gabon                                    | MR | Mauritania               |
| BF | Burkina Faso             | GB | United Kingdom                           | MW | Malawi                   |
| BG | Bulgaria                 | GN | Guinea                                   | NL | Netherlands              |
| BJ | Benin                    | GR | Greece                                   | NO | Norway                   |
| BR | Brazil                   | HU | Hungary                                  | PL | Poland                   |
| CA | Canada                   | IT | Italy                                    | RO | Romania                  |
| CF | Central African Republic | JP | Japan                                    | SD | Sudan                    |
| CG | Congo                    | KP | Democratic People's Republic<br>of Korea | SE | Sweden                   |
| CH | Switzerland              | KR | Republic of Korea                        | SN | Senegal                  |
| CI | Côte d'Ivoire            | LI | Liechtenstein                            | SU | Soviet Union             |
| CM | Cameroon                 | LK | Sri Lanka                                | TD | Chad                     |
| CS | Czechoslovakia           | LU | Luxembourg                               | TG | Togo                     |
| DE | Germany                  | MC | Monaco                                   | US | United States of America |
| DK | Denmark                  |    |                                          |    |                          |

-1-

## SOLID SUPPORT HAVING ANTIBODIES IMMOBILIZED THEREON

BACKGROUND OF THE INVENTION

This invention relates to solid supports having antibodies immobilized thereon and, more particularly, to such solid supports which are useful in solid phase immunoassays.

5           Various types of assay elements are known for use in immunoassays to analyze for analytes present in biological fluids. In one type which is commonly referred to as a solid phase immunoassay, one member of the antigen-antibody interaction pair, e.g., the antibody, is bound to a solid carrier such as particulate materials, fibrous materials and the like. In these assays the solid support  
10 becomes the site at which the interaction between the bound material and a binding partner takes place.

Any of the known immunometric assays, e.g., sandwich or competitive assays, can be carried out in the solid phase format.  
15 Such assays are well known to those skilled in the art. Briefly, for example, in the competitive assay a sample fluid containing an analyte

-2-

of interest, e.g., an antigen, is applied to a solid support having bound thereto a binding partner of the analyte, e.g., an antibody. The amount of antibody bound to the support is that necessary to allow the requisite competition between the sample analyte and the labeled species. Subsequently, after a period of incubation, a labeled analyte or a labeled analogue thereof is added to the solid support and after another incubation period and a wash step to remove any unbound labeled material, the bound labeled material is detected to provide a signal which is a function of the amount of analyte in the patient sample fluid. The signal is inversely proportional to the amount of analyte in the sample.

The antibodies which are immobilized on the solid carrier can be bound thereto by various means including covalent binding, immunoprecipitation and the like. The immunoprecipitation technique can be carried out more conveniently as compared to the chemical reaction(s) required for covalent binding. However, in some assays such as the competitive immunoassay described previously, only a relatively limited amount of antibodies may be bound to the carrier because of various factors such as the assay concentration range, etc. If the binding technique employed is not efficient enough a low signal level may be obtained with a given amount of antibodies. Of course, the signal can be improved by adding more antibodies to the solid carrier but this alternative is not satisfactory if it results in the assay range being shifted outside of the range required for the analyte of interest.

Accordingly, it would be desirable to have new solid supports having antibodies immobilized thereon. It would also be

-3-

desirable to have such solid supports which could be used in competitive solid phase immunoassays to provide improved signal levels while at the same time exhibiting low nonspecific binding.

#### SUMMARY OF THE INVENTION

5                These and other objects and advantages are accomplished in accordance with the invention by providing a solid support having immobilized thereon a complex of a specific antibody of interest, a nonspecific antibody and an antibody directed against the Fc fragments of the specific and nonspecific antibodies. For example, consider a  
10    mouse monoclonal anti-digoxin antibody as the specific antibody of interest which is to be immobilized on a solid support for use in an immunoassay for digoxin. According to the invention the mouse monoclonal anti-digoxin antibodies are included in a complex together with an anti-mouse antibody which is raised against the Fc fragments  
15    of mouse antibodies and a mouse IgG antibody which is nonspecific. The complex is immobilized on the solid support by passive adsorption.

              The solid supports of the invention are prepared by initially mixing the antibody of interest, e.g., the anti-digoxin antibody, with the nonspecific antibody in a coating solution, typically a buffer  
20    solution, and then adding the specific antibody raised against the Fc fragments of the specific and nonspecific antibodies. The mixture is incubated under time and temperature conditions which allow the complex to form. The coating solution is applied to the solid support and the latter is dried. The antibody complex is immobilized to the  
25    solid support through passive adsorption.

              The solid supports of the invention have been found to be particularly advantageous when used in competitive solid phase

-4-

immunoassays for the analysis of analytes in a sample fluid. Higher signal levels can be obtained while at the same time maintaining the sensitivity of the assay in the desired assay range.

#### BRIEF DESCRIPTION OF THE DRAWING

5 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 the preferred embodiments thereof taken in conjunction with the accompanying drawing wherein the Figure is a simplified, isometric view of a preferred assay element in  
10 accordance with the invention.

#### DESCRIPTION OF THE PREFERRED EMBODIMENT

The solid supports of the invention may comprise any suitable solid carrier such as, for example, particulate materials, porous members possessing an intercommunicating network of openings  
15 throughout such that a fluid deposited on the member will propagate through the member, and the like. Suitable porous members include porous membranes, fibrous mesh materials and the like and may be of any suitable material such as glass, polymeric materials, paper, etc. In a preferred embodiment the solid carrier is a thin fibrous mesh member  
20 which will permit fluid deposited thereon to propagate throughout because of capillary action. The member may be any suitable fibrous material; a preferred material is a nonwoven glass fiber mesh, the fibers being very thin such as on the order of about 1 micrometer ( $\mu\text{m}$ ).

Any monoclonal antibodies of interest may be immobilized  
25 on the solid carrier such as, for example, antibodies to: haptens such as digoxin, triiodothyronine (T3) and the like; proteins such as myoglobin; hormones; etc. The nonspecific antibody may be any to

-5-

which the antibody directed against the Fc fragments of the specific and nonspecific antibodies will bind. Preferably the nonspecific antibody is of the same species as the specific antibody, i.e., they are both raised in the same type of host animal, e.g., mice. The antibody  
5 which is raised against the Fc fragments of the specific and nonspecific antibodies can be raised in any host animal.

The coating solution which is used to apply the antibody complex to the solid support is prepared by initially combining the specific antibody of interest and the nonspecific antibody in an  
10 appropriate solution, preferably a buffer solution. The solution may contain other additives such as, for example, proteins which are useful for reducing nonspecific binding, stabilizers such as sugars, surfactants which improve the wettability of the solid support and preservatives for preventing anti-microbial action. Subsequently, the antibody  
15 directed against the Fc fragments of the specific and nonspecific antibodies is added. The solution is then incubated under time and temperature conditions which are required to allow the complex formation to occur. These conditions will vary depending upon the specific materials, the amounts involved, etc. In a preferred  
20 embodiment the coating solution is incubated at 37°C for about one hour.

The solution is then deposited on the solid support and allowed to become immobilized thereon through passive adsorption. The solid support is dried to provide a member which is useful in solid  
25 phase immunoassays.

In a preferred embodiment the solid supports of the invention are utilized in the known competitive solid phase

-6-

immunometric assays for an analyte of interest. Such assays are well known to those skilled in the art and extensive discussion thereof is not required here. To further describe the advantages of the solid supports of the invention their use in a preferred fibrous solid phase competitive immunoassay will be described. Referring now to the Figure there is seen an assay element 10 which includes a plurality of chambers in a housing 12 wherein a first chamber 14 serves as a front reservoir for the storage of a fluid reagent to be utilized in the assay, a second of the chambers serves as a back reservoir 16 for the storage of another fluid reagent for the assay, a third chamber 18 serves a mixing well for mixing reagents and fourth chamber 20 is adapted to dispense a fluid to one end of a porous solid support 22. There is also shown a chamber 24 within the housing 12 wherein there is arranged an absorbing material for absorbing fluid removed from solid support 22 such as by a wash fluid as the latter propagates through the fibrous solid support. A frangible as puncturable foil (not shown) is disposed over the front and back reservoirs, 14 and 16, respectively, for containing the fluid reagents within these reservoirs thus providing a self contained assay element.

The solid support 22 in this preferred embodiment comprises a thin fibrous mesh pad to which there has been immobilized an antibody of interest in accordance with the invention. A preferred solid support is a nonwoven glass fiber mesh having very thin fibers such as on the order of about 1 micrometer ( $\mu\text{m}$ ). This preferred solid support is from about 0.30 mm to about 0.60 mm in thickness, preferably about 0.40 mm.

-7-

The solid support 22 extends from the dispensing chamber 20 to the chamber 24 which holds the absorbing material. The fluid absorbing material 26, which may be any suitable material, is located within chamber 24 and forms a part of the chamber 24 for taking up fluid expelled from the solid support and the guide area (not shown) in which the support is arranged. The absorbing material 26 is located contiguous the solid support 22 and in a preferred embodiment, as illustrated, is formed conveniently as an extension of the solid support material folded back and forth on itself.

10 The housing 12 also includes a chamber 28 which is positioned immediately above the area of the solid support where the immunometric interactions take place. It will be apparent to those skilled in the art that the immobilized antibody complex bound to the solid support need only be present on that part of the support where the immunometric interactions will take place. The housing 12 also preferably includes a transparent window (not shown) or an opening in the housing, positioned immediately below the bottom surface of the solid support 22 to provide access for the readout illumination used to measure the detectable change effected in the support as a result of the assay method.

20 In the assay method a volume of sample fluid, typically 30-45  $\mu$ l is deposited on the solid support 22 through chamber 28. The sample fluid is allowed to incubate for a suitable period at the appropriate temperature to allow the sample antigen to interact with the immobilized antibodies in the reaction site on the solid support. Subsequently, a conjugate of an enzyme-labeled antigen (the same as the sample antigen) or an analogue thereof is withdrawn from

-8-

chamber 14 and deposited on the solid support through chamber 28. The conjugate will bind to any specific antibodies on the solid support which are not bound to the sample antigen. The assay element 10 is again allowed to incubate.

- 5                    Since the enzyme label must be detected indirectly, a substrate solution, typically 50-100  $\mu$ l is applied to the porous solid support 22 through chamber 20. The substrate solution is utilized both as a wash fluid to remove from the solid support any unbound sample antigen or conjugate and to render the enzyme label detectable.
- 10    The substrate solution is caused to enter an end portion of the solid support and as the solution propagates through the solid support 22 it forces any unbound sample antigen and enzyme conjugate together with the fluid out of the solid support and into the absorber chamber 24 where it is taken up by the absorber material 26. The signal
- 15    provided by the fluorescent species liberated by the reaction of the enzyme with the substrate material is read out by means of suitable optical apparatus.

- It has been found that such competitive assays carried out with the solid supports of the invention provide higher signal levels and
- 20    desirably steeper standard curve slopes in the assay range.

                    Although the preferred competitive solid phase assay has been described with respect to a sequential format it should be recognized that it can also be carried out in the simultaneous mode.

- The invention will now be described further in detail with
- 25    respect to specific preferred embodiments by way of examples it being understood that these are intended to be illustrative only and the

-9-

invention is not limited to the materials, conditions, process parameters, etc. which are recited therein.

#### EXAMPLE I

An antibody solution was prepared containing 0.1  $\mu\text{g/ml}$  of anti-digoxin antibodies and 10  $\mu\text{g/ml}$  of mouse IgG antibodies in a pH 7.2 phosphate buffered saline solution which also contained other proteins, stabilizers, surfactants and anti-microbial agents. The solution was filtered through a 0.2  $\mu\text{m}$  filter and then 2 ml of a solution of Goat Anti-Mouse IgG, Fc Fragment Specific (Jackson ImmunoResearch) was diluted 1:100 with the antibody solution to form a coating solution.

The coating solution was incubated for one hour at 37°C and about 35  $\mu\text{l}$  added to an approximately 7 x 8 mm area of a Whatman GF/F fibrous glass filter material. The filter material was dried for about 10 minutes at 75°C and incorporated into an assay element as described previously.

The assay elements were then used to carry out the competitive immunometric assay method previously described with sample fluids containing known digoxin concentrations. The assays were carried out on an automated laboratory instrument. The enzyme label used in the conjugate was methyl umbelliferyl phosphate and the readout signal was obtained by directed 360 nm radiation upon the bottom surface of the solid support and measuring the reflected 450 nm radiation.

The signals obtained are shown in Table I. The assays were read kinetically and the signals obtained are the slopes of the

-10-

kinetic measurements. Each value shown in Table I is the average of three replicates.

Table I

|   | <u>Digoxin (ng/ml)</u> | <u>Signal</u> |
|---|------------------------|---------------|
| 5 | 0.00                   | 2372          |
|   | 0.50                   | 1911          |
|   | 1.00                   | 1275          |
|   | 2.00                   | 672           |
|   | 4.00                   | 251           |

10 It can be seen that the assay method of the invention provided high signal levels and a desirable steep standard curve slope in the assay range (about 0.5 - 4.0 ng/ml).

EXAMPLE II

15 A coating solution was prepared which was the same as that described in Example I except that it contained 0.0625  $\mu\text{g/ml}$  of a monoclonal antibody to T3 instead of the anti-digoxin antibodies. Assay elements were prepared as described in Example I and used to carry out the competitive solid phase assay method previously described. The results obtained are shown in Table II.

20 Table II

|    | <u>T3 (ng/ml)</u> | <u>Signal</u> |
|----|-------------------|---------------|
|    | 0.00              | 5973          |
|    | 0.50              | 5279          |
|    | 1.00              | 4487          |
| 25 | 2.00              | 3072          |
|    | 4.00              | 1981          |
|    | 8.00              | 1220          |

-11-

Again the assay method of the invention provided high signal levels and a desirable steep standard curve slope in the assay range (about 0.50 - 8.00 ng/ml).

- 5      Although the invention has been described in detail with respect to various preferred embodiments, 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.

-12-

What is claimed is:

1. A solid support having an antibody of interest  
5 immobilized thereon comprising a solid support to which there is attached a complex of: (1) a specific monoclonal antibody of interest; (2) a nonspecific antibody; and (3) an antibody directed against the Fc fragments of said specific and nonspecific antibodies.
2. The solid support as defined in claim 1 wherein said  
10 solid support is a fibrous glass material.
3. The solid support as defined in claim 2 wherein said specific monoclonal antibody is a mouse anti-digoxin antibody or a mouse anti-triiodothyronine (T3) antibody, said nonspecific antibody is a mouse IgG antibody and said antibody directed against the Fc  
15 fragments of said specific and nonspecific antibodies is a goat anti-mouse antibody.
4. The solid support as defined in claim 1 wherein the specific antibody and the nonspecific antibody are of the same species.
5. A method for preparing a solid support having an  
20 antibody of interest immobilized thereon comprising
  - (a) adding to a solution containing a specific monoclonal antibody of interest and a nonspecific antibody, an antibody directed against the Fc fragments of said specific and nonspecific antibodies;
  - (b) incubating said solution at a temperature and for a  
25 period of time sufficient to allow said antibodies to form a complex;
  - (c) applying said solution to a solid support and

-13-

(d) drying said solid support whereby said antibody complex is immobilized on the solid support.

6. The method as defined in claim 5 wherein said solid support is a fibrous glass material.

5           7. The method as defined in claim claim 5 wherein step (b) is carried out at about 37°C for about one hour.

8. The method as defined in claim 5 wherein said specific and nonspecific antibodies are of the same species.

10           9. The method as defined in claim 8 wherein said specific antibody is a mouse monoclonal antibody and said nonspecific antibody is a mouse IgG antibody.

10. The method as defined in claim 9 wherein said specific antibody is a mouse anti-digoxin antibody or a mouse anti-triiodothyronine (T3) antibody.

15           11. A competitive solid phase immunoassay method comprising

20           (a) contacting a solid support as defined in claim 1 with (i) a sample fluid containing an analyte which is the binding partner of the specific monoclonal antibody immobilized on said solid support and (ii) a conjugate comprising a label bound to said analyte or an analogue thereof;

(b) separating the solid and fluid phases; and

(c) detecting the amount of the label present in either the solid or fluid phase.

25           12. The method as defined in claim 11 wherein said label is an enzyme and step (b) is carried out by applying a substrate solution to said solid support.

-14-

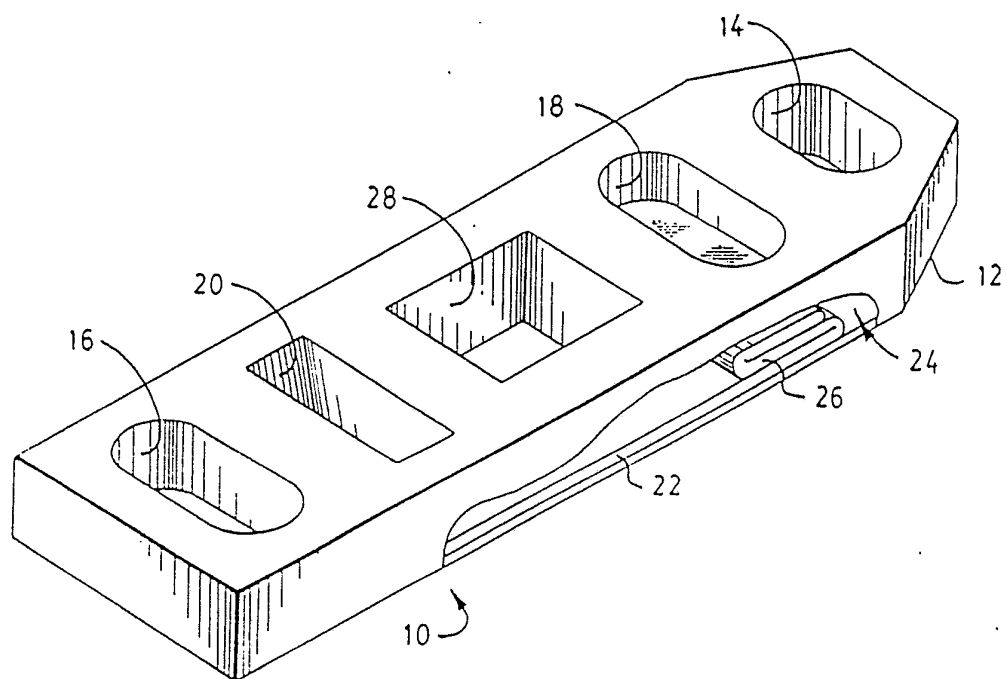
13. The method as defined in claim 12 wherein the reaction of said substrate and said enzyme provides a fluorescent species.

5 14. The method as defined in claim 11 wherein step (a) is carried out by first applying said sample fluid to said solid support and then applying a solution of said conjugate to the solid support.

10 15. The method as defined in claim 11 wherein said specific monoclonal antibody immobilized on said solid support is a monoclonal anti-digoxin antibody or a monoclonal anti-triiodothyronine (T3) antibody.

16. The method as defined in claim 14 wherein said specific monoclonal antibody immobilized on said solid support is a monoclonal anti-digoxin antibody or a monoclonal anti-triiodothyronine (T3) antibody.

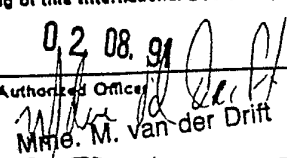
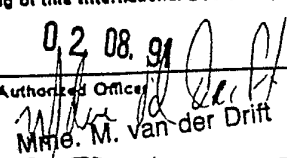
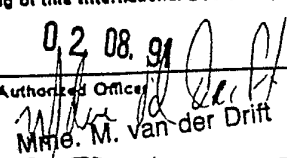
1/1



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 91/02092

| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>4</sup><br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC <sup>5</sup> : G 01 N 33/543, G 01 N 33/577, G 01 N 33/552,<br>G 01 N 33/535, G 01 N 33/78, G 01 N 33/541                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                     |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|--------------|---|-------------------------------------------------------------------------------------------------------------|--------------|---|--------------------------------------------------------------------------------------------------------|--------------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%; border-bottom: 1px solid black;">Classification System</td> <td style="border-bottom: 1px solid black;">Classification Symbols</td> </tr> <tr> <td style="padding: 10px;">IPC<sup>5</sup></td> <td style="padding: 10px;">G 01 N 33/00</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                     |                                     | Classification System                                                                                                | Classification Symbols                                                                                         | IPC <sup>5</sup>                                                                                       | G 01 N 33/00                                                                                                                                                                                                        |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| Classification System                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Classification Symbols                                                                                                                                                                                              |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| IPC <sup>5</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | G 01 N 33/00                                                                                                                                                                                                        |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category <sup>9</sup></th> <th style="width: 70%; padding: 5px;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; padding: 5px;">Relevant to Claim No. <sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">US, A, 3 799 742<br/>(C.M. COLEMAN) 26 March 1974<br/>(26.03.74), see fig. and abstract.<br/>--</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1, 2, 5<br/>6</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">WO, A1, 88/06 731<br/>(P B DIAGNOSTIC SYSTEMS, INC.)<br/>07 September 1988<br/>(07.09.88), see abstract.<br/>--</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1, 2, 5<br/>6</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">EP, A1, 0 296 366<br/>(BOEHRINGER MANNHEIM GMBH)<br/>28 December 1988 (28.12.88),<br/>see abstract.<br/>--</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1, 2, 5<br/>6</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">EP, A2, 0 101 912<br/>(GENETIC DIAGNOSTIC CORPOR-<br/>ATION) 07 March 1984<br/>(07.03.84), see totality,<br/>esp. abstract and claims<br/>1-3, 20.<br/>--</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-3,<br/>11, 15<br/>16</td> </tr> </tbody> </table> |                                                                                                                                                                                                                     |                                     | Category <sup>9</sup>                                                                                                | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup> | Relevant to Claim No. <sup>13</sup>                                                                    | A                                                                                                                                                                                                                   | US, A, 3 799 742<br>(C.M. COLEMAN) 26 March 1974<br>(26.03.74), see fig. and abstract.<br>-- | 1, 2, 5<br>6 | A | WO, A1, 88/06 731<br>(P B DIAGNOSTIC SYSTEMS, INC.)<br>07 September 1988<br>(07.09.88), see abstract.<br>-- | 1, 2, 5<br>6 | A | EP, A1, 0 296 366<br>(BOEHRINGER MANNHEIM GMBH)<br>28 December 1988 (28.12.88),<br>see abstract.<br>-- | 1, 2, 5<br>6 | A | EP, A2, 0 101 912<br>(GENETIC DIAGNOSTIC CORPOR-<br>ATION) 07 March 1984<br>(07.03.84), see totality,<br>esp. abstract and claims<br>1-3, 20.<br>-- | 1-3,<br>11, 15<br>16 |
| Category <sup>9</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>                                                                                                      | Relevant to Claim No. <sup>13</sup> |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | US, A, 3 799 742<br>(C.M. COLEMAN) 26 March 1974<br>(26.03.74), see fig. and abstract.<br>--                                                                                                                        | 1, 2, 5<br>6                        |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | WO, A1, 88/06 731<br>(P B DIAGNOSTIC SYSTEMS, INC.)<br>07 September 1988<br>(07.09.88), see abstract.<br>--                                                                                                         | 1, 2, 5<br>6                        |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | EP, A1, 0 296 366<br>(BOEHRINGER MANNHEIM GMBH)<br>28 December 1988 (28.12.88),<br>see abstract.<br>--                                                                                                              | 1, 2, 5<br>6                        |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | EP, A2, 0 101 912<br>(GENETIC DIAGNOSTIC CORPOR-<br>ATION) 07 March 1984<br>(07.03.84), see totality,<br>esp. abstract and claims<br>1-3, 20.<br>--                                                                 | 1-3,<br>11, 15<br>16                |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>*</sup> Special categories of cited documents: <sup>14</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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</p> <p>"Z" document member of the same patent family</p> </div> </div>                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                     |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| <b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of the Actual Completion of the International Search<br/><br/> <div style="text-align: center;">10 July 1991</div> </td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">           Date of Mailing of this International Search Report<br/><br/> <div style="text-align: center;">02.08.91</div> </td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">           International Searching Authority<br/><br/> <div style="text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="border-bottom: 1px solid black; padding: 5px;">           Signature of Authorized Officer<br/><br/> <div style="text-align: center;"> <br/>             M. M. van der Drift           </div> </td> </tr> </table>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                     |                                     | Date of the Actual Completion of the International Search<br><br><div style="text-align: center;">10 July 1991</div> | Date of Mailing of this International Search Report<br><br><div style="text-align: center;">02.08.91</div>     | International Searching Authority<br><br><div style="text-align: center;">EUROPEAN PATENT OFFICE</div> | Signature of Authorized Officer<br><br><div style="text-align: center;"> <br/>             M. M. van der Drift           </div> |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| Date of the Actual Completion of the International Search<br><br><div style="text-align: center;">10 July 1991</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Date of Mailing of this International Search Report<br><br><div style="text-align: center;">02.08.91</div>                                                                                                          |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |
| International Searching Authority<br><br><div style="text-align: center;">EUROPEAN PATENT OFFICE</div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Signature of Authorized Officer<br><br><div style="text-align: center;"> <br/>             M. M. van der Drift           </div> |                                     |                                                                                                                      |                                                                                                                |                                                                                                        |                                                                                                                                                                                                                     |                                                                                              |              |   |                                                                                                             |              |   |                                                                                                        |              |   |                                                                                                                                                     |                      |

| 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.     |
| A                                                                          | EP, A2, 0 310 413<br>(NIHON CHEMICAL RESEARCH<br>KABUSHIKI KAISHA also known<br>as JAPAN CHEMICAL RESEARCH<br>CO., LTD.) 05 April 1989<br>(05.04.89), see abstract.<br>--                                                                                                                                            | 1, 2, 5,<br>6             |
| A                                                                          | FR, A1, 2 556 840<br>(IMMUNOTECH.) 21 June 1985<br>(21.06.85), see abstract.<br>--                                                                                                                                                                                                                                   | 1, 11                     |
| A                                                                          | US, A, 4 803 167<br>(E. HABER et al.)<br>07 February 1989 (07.02.89),<br>see abstract and column 5,<br>lines 15-21.<br>--                                                                                                                                                                                            | 1, 3                      |
| A                                                                          | Chemical Abstracts, Volume<br>107, No. 21, November 23,<br>1987 (23.11.87), Columbus,<br>Ohio, USA, J. Kovar et al.<br>"Mouse lymphocyte hybridoma<br>IMG CZAS T3-01 producing<br>antibodies against triiodo-<br>thyronine", pages 599 and<br>600, see the abstract-No.<br>196 396d & Czech. CS 239<br>761.<br>----- | 1, 3, 5,<br>10, 15,<br>16 |

ANHANG  
zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

ANNEX  
to the International Search  
Report to the International Patent  
Application No.

ANNEXE  
au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/US 91/02092 SAE 46332

In diesem Anhang sind die Mitglieder  
der Patentfamilien der im obenge-  
nannten internationalen Recherchenbericht  
angeführten Patentdokumente angegeben.  
Diese Angaben dienen nur zur Unter-  
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family  
members relating to the patent documents  
cited in the above-mentioned inter-  
national search report. The Office is  
in no way liable for these particulars  
which are given merely for the purpose  
of information.

La présente annexe indique les  
membres de la famille de brevets  
relatifs aux documents de brevets cités  
dans le rapport de recherche inter-  
national visé ci-dessus. Les renseigne-  
ments fournis sont donnés à titre indica-  
tif et n'engagent pas la responsabilité  
de l'Office.

| Im Recherchenbericht<br>angeführtes Patentdokument<br>Patent document cited<br>in search report<br>Document de brevet cité<br>dans le rapport de recherche | Datum der<br>Veröffentlichung<br>Publication<br>date<br>Date de<br>publication | Mitglied(er) der<br>Patentfamilie<br>Patent family<br>member(s)<br>Membre(s) de la<br>famille de brevets                                                                                                                        | Datum der<br>Veröffentlichung<br>Publication<br>date<br>Date de<br>publication                                                                           |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| US-A - 3799742                                                                                                                                             | 26-03-74                                                                       | Keine - None - Rien                                                                                                                                                                                                             |                                                                                                                                                          |
| WO-A1- 8806731                                                                                                                                             | 07-09-88                                                                       | AU-A1-12949/88<br>AU-B2- 605257<br>DK-A - 6094/88<br>DK-A0- 6094/88<br>EP-A1- 281201<br>FI-A0- 883599<br>FI-A - 883599<br>JP-T2- 1502526<br>NO-A - 884658<br>NO-A0- 884658<br>NZ-A - 223637<br>US-A - 4918025<br>ZA-A - 8800550 | 26-09-88<br>10-01-91<br>02-11-88<br>02-11-88<br>07-09-88<br>01-08-88<br>04-09-88<br>31-08-89<br>19-10-88<br>19-10-88<br>28-05-90<br>17-04-90<br>26-10-88 |
| EP-A1- 296366                                                                                                                                              | 28-12-88                                                                       | DE-A1- 3718140<br>JP-A2-63311171                                                                                                                                                                                                | 08-12-88<br>19-12-88                                                                                                                                     |
| EP-A2- 101912                                                                                                                                              | 07-03-84                                                                       | US-A - 4606855<br>EP-A3- 101912<br>AU-A1-17288/83<br>IL-A0- 69339<br>IL-A1- 69339<br>JP-A2-59072059<br>SE-A0- 8304145<br>SE-A - 8304145<br>US-A - 4477576<br>ZA-A - 8305409                                                     | 19-08-86<br>30-05-84<br>02-02-84<br>30-11-83<br>27-02-87<br>23-04-84<br>26-07-83<br>27-01-84<br>16-10-84<br>28-03-84                                     |
| EP-A2- 310413                                                                                                                                              | 05-04-89                                                                       | EP-A3- 310413<br>JP-A2- 1088367                                                                                                                                                                                                 | 13-12-89<br>03-04-89                                                                                                                                     |
| FR-A1- 2556840                                                                                                                                             | 21-06-85                                                                       | FR-B1- 2556840                                                                                                                                                                                                                  | 24-12-87                                                                                                                                                 |
| US-A - 4803167                                                                                                                                             | 07-02-89                                                                       | Keine - None - Rien                                                                                                                                                                                                             |                                                                                                                                                          |