COMMONWEALTH OF AUSTRALIA

APPLICATION FOR A PATENT

23195/77

We, Nippon Chemipharm Co., Ltd., a company organized and existing under the laws of Japan, of No. 2-3, Iwamoto-cho 2-chome, Chiyoda-ku, Tokyo, Japan,

hereby apply for the grant of a Patent for an invention entitled

"Improvements in or relating to antigen membrane for use in Syphilis Diagnosis and Syphilis Diagnosis Apparatus using such membrane"

which is described in the accompanying specification.

This application is made under the provisions of Part XVI of the Patents Act 1952-76 and is based on an application for a patent or similar protection made

in Japan on 18th March, 1976 (51-29632)
in " on 18th March, 1976 (51-29633)
in " on 18th March, 1976 (51-29634)
in " (Utility Model Appln.) on 21st June, 1976 (51-81408)

Our address for service is: F. B. Rice & Co., 101 Mort St., Balmain, NSW 2041

Dated this 10th day of March, 1977

Nippon Chemipharm Co., Ltd.

by Patent Attorney

To: The Commissioner of Patents,
Commonwealth of Australia.
DECLARATION IN SUPPORT OF
A CONVENTION APPLICATION FOR A
PATENT OR PATENT OF ADDITION

In support of the Convention Application made by

NIPPON CHEMI PHAR Co., Ltd.

23195/77

for a patent for an invention entitled
"Improvements in or relating to antigen membrane for use in Syphilis Diagnosis and Syphilis Diagnosis Apparatus using such membrane".

Akira Yamaguchi, President, of and care of the applicant company, do solemnly and sincerely declare as follows:

1. I am authorised by Nippon Chemiphar Co., Ltd. the applicant on its behalf.

2. The basic application made in Japan on 13th March, 1976.
   as defined by Section 142 of the Act make the application.

3. Shuichi Suzuki of No. 1-40-6, Sugamo, Toshima-ku, Tokyo, Japan;
   Masuo Aizawa of No. 2-19-14, Amanuma, Suginami-ku, Tokyo, Japan;
   Isao Ishiguro of 7149, Asamiya-cho, Kasugai-shi, Aichi-ken, Japan;
   Rikio Shinohara of 6530, Unuma, Kagamihara-shi, Gifu-ken, Japan;
   Yoichi Nagamura of 153, Marunouchi, Shinden, Toyoka-shi, Aichi-ken, Japan.

which Nippon Chemiphar Co., Ltd. is entitled to make the application are as follows: The applicant company is the assignee of the invention from the said actual inventors.

The basic applications referred to in paragraph 2 of this Declaration are the first applications made in a Convention country in respect of the invention the subject of the application.

Declared at Tokyo this 1st day of March, 1977

Akira Yamaguchi, President
Nippon Chemiphar Co., Ltd.

F. B. RICE & CO.,
Patent Attorneys,
Sydney.
ANTIGEN MEMBRANE

NIPPON CHEMI-PHAR CO., LTD.

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11.3.77
51-29632 18.3.76
14.9.78

G01N 27/40 G01N 27/56 G01N 33/16
Suzuki, S., Rikio Shinohara, R., Aizawa, M., Nagamura, Y., and Ishiguro, I.

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63 567/73 483 805 87.41
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Claim 1. An antigen membrane for syphilis diagnosis comprising cardiolipin immobilized on a polymer matrix.

Claim 3. A method for diagnosing syphilis comprising bringing one surface of the antigen membrane of Claim 1 into contact with a solution to be tested; bringing the other surface of said antigen membrane into contact with a normal serum solution; and measuring electrochemically the membrane potential generated across, said antigen membrane.

Claim 7. An apparatus for use in diagnosing syphilis comprising an electrolytic cell partitioned into two compartments by the antigen membrane of claim 1; electrodes disposed in said compartments; and a detector connected to said electrodes for measuring the membrane potential across said antigen membrane.
Application Number : Lodged :
Complete Specification Lodged :
Accepted :
Published :
Priorities :
18th March, 1976.
18th March, 1976.
18th March, 1976
26th June, 1976.
Related Art :

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The Forth and Clyde, 101 Mort Street,
BALMAIN. 2041.
Complete Specification for the invention entitled:
"Improvements in or relating to antigen membrane for use in Syphilis Diagnosis and Syphilis Diagnosis Apparatus using such membrane".

The following statement is a full description of this invention including the best method of performing it known to us :-
Field of the Invention:

This invention relates to an antigen membrane for syphilis diagnosis, a syphilis diagnosis method and a syphilis diagnosis apparatus.

Description of the Prior Art:

Recently, with rapid progress of immunology, importance is attached to the application of immunology, and an immunochemical method is introduced into clinical analysis. Usefulness of immunology has been confirmed. In most of immunoassays, superior specificity of antigen-antibody reaction is utilized, and a trace amount of specific substance can be selectively detected.

Syphilis diagnosis is given as a typical example for the application to such immuno-chemical clinical analysis. However, in the conventional syphilis diagnosis method, the antigen-antibody complex formation (completion of reaction) is observed with naked eye. Accordingly, excellent selectivity and sensitivity of the immuno-chemical specificity are not sufficiently utilized in the final diagnosis step.

Recently, a technical method has been developed by which biologically active substance, such as enzymes, are immobilized to insoluble matrices without losing their function. And biophysiological active substances can be used in solid state.

SUMMARY OF THE INVENTION

In consideration of the above described facts, this invention is characterized in that an insoluble polymer material attaching antigen is used, whereby syphilis antibody of the patient of syphilis can be exactly and reproducibly detected.

Accordingly, an object of this invention is to provide an immobilized antigen membrane for syphilis diagnosis.
Another object of this invention is to provide a syphilis diagnosis method using the immobilized antigen membrane.

A further object of this invention is to provide a syphilis diagnosis apparatus used for the syphilis diagnosis method.

According to this invention, the above first object is attained by the fact that cardiolipin is immobilized to a polymer matrix.

One milliliter of Ogata antigen, which consists of 0.01% cardiolipin, 0.04% lecithin, 0.20% cholesterin in ethanol and is used for syphilis Wassermann reaction, is mixed with 6 milliliters of acetone solution containing 250 milligrams of acetyl cellulose. The mixed solution is cast on a glass plate (18 x 10 cm²), and dried at room temperature under reduced pressure. And then, the cast membrane is peeled off the glass plate. Thus, an immobilized antigen membrane is obtained.

Any one of a covalent, entrapment, and absorption methods can be used for immobilizing antigen while retaining the activity. Advantageously, when the entrapment method is employed, little lowering of the activity can be observed.

Cellulose derivatives such as triacetyl cellulose and cellulose acetate as well as different natural or synthetic polymers can be used for membrane matrices.

According to this invention, the above second object is attained by any one of the following three diagnosis methods.

The first diagnosis method is characterized in that one surface of the antigen membrane for syphilis diagnosis contacts the solution to be tested, and the other surface of the antigen membrane for syphilis diagnosis contacts normal blood serum, whereby the membrane potential of the antigen membrane is electrochemically measured to detect the syphilis antibody.
The second diagnosis method is characterized in that the antigen membrane for syphilis diagnosis and antigen-free membrane are piled one after another, and the solution to be tested contacts both surfaces of the piled membrane, whereby the membrane potential of the piled membrane is electrochemically measured to detect the syphilis antibody.

The third diagnosis method is characterized in that one surface of the antigen membrane for syphilis diagnosis and the antigen-free membrane contact the solution to be tested, and the other surface of the antigen membrane for syphilis diagnosis and the antigen-free membrane contact electrolyte, whereby the membrane potential of the antigen membrane for syphilis diagnosis is electrochemically measured to detect the syphilis antibody.

The antigen-free membrane is a polymer membrane containing no antigen. For example, the antigen-free membrane is prepared in such a manner that 6 milliliters of acetone solution containing 250 milligrams of acetyl cellulose is cast on a glass plate (18 x 10 cm²) and dried at room temperature under reduced pressure, and then the cast membrane is peeled off the glass plate.

According to this invention, the above third object is attained by the following four apparatuses. They will be described with reference to the drawings. At the same time, the above described diagnosis methods will be described in more detail.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 2 shows a schematic cross-sectional view of a syphilis diagnosis apparatus used for the first syphilis diagnosis method.
Referring to FIG. 2, an electrolytic cell 1 is partitioned into two compartments 3, 4 by a vertical wall 2. One compartment 3 partitioned by the wall is filled with a solution to be tested, while the other compartment 4 is filled with normal blood serum. An opening 2a made at the center of the vertical wall 2 is closed by an immobilized antigen membrane 5. Electrodes 6 and 7, for example, made of calomel or Ag-AgCl, are disposed in the compartment 3 filled with the solution to be tested and in the other compartment 4 filled with the normal blood serum, respectively. The electrodes 6 and 7 are connected to input terminals of an amplifier 8 and an output terminal of the amplifier 8 is connected to a detector 9 for measuring the potential difference across the immobilized antigen membrane.

In the syphilis diagnosis, a requisite amount of serum solution 10 to be tested, diluted with physiological saline, is poured into the compartment 3, while a requisite amount of normal serum solution 11, diluted with physiological saline, is poured into the compartment 4. When the serum concentrations of the solutions 10 and 11 are substantially equal to each other, and no syphilis antibody exist in the solution 10 to be tested, the charge density is substantially the same at both sides of the immobilized antigen membrane, and so the membrane potential is nearly zero.

When syphilis antibody exists in the serum solution 10 to be tested, the antibody reacts with the antigen exposed on the surface of the antigen membrane 5 in contact with the serum solution 10. An antigen-antibody complex is formed on the surface of the membrane 5. The membrane 5 becomes an asymmetric membrane. Accordingly, the one surface of the antigen membrane 5 in contact with the normal serum solution 11
is electrically charged due to the property of the antigen membrane 5 itself, while the other surface of the antigen membrane 5 in contact with the serum solution 10 to be tested is electrically charged due to the property of the antigen-antibody complex. The charge-densities of the both surfaces are different from each other. The difference of the charge densities is remarkably exhibited as membrane potential. Accordingly, the antigen-antibody reaction is followed by measuring the membrane potential. The membrane potential is electrochemically led to the amplifier 8 through the electrodes 6 and 7, and amplified thereby. The output of the amplifier 8 is applied to the detector 9. The existence of the syphilis antibody is detected with the output of the detector 9.

The membrane potential $\Delta \psi$ is expressed by the following equation:

$$\Delta \psi = \frac{RT}{F} \ln \left( \frac{\varrho_2}{\varrho_1} + \frac{\varrho_2^2}{\varrho_1^2 + \Delta c^2} \right)^{\frac{1}{2}}$$

where $\varrho_1$ represents the charge density of one surface of the antigen membrane in contact with the normal serum solution; $\varrho_2$, the charge density of the other surface of the antigen membrane on which the antigen-antibody complex is formed, and which contacts the serum solution to be tested; $c$, the concentration of the electrolyte; $R$, gas constant; $T$, absolute temperature (°K); and $F$, Faraday constant.

FIG. 3 shows a schematic cross-sectional view of a syphilis diagnosis apparatus used for the second syphilis diagnosis method.

Referring to FIG. 3, an electrolytic cell 21 is partitioned into two parts by a vertical wall 22. Both parts, namely compartments 23 and 24, are filled with the solution
to be tested. An opening 22a made at the center of the vertical wall 22 is closed by a piled membrane body 27 of an immobilized antigen membrane 25 and an antigen-free membrane 26. Electrodes 28 and 29, for example, made of calomel or Ag-AgCl, are disposed in the compartments 23 and 24 filled with the solution to be tested, respectively. The electrodes 28 and 29 are connected to input terminals of an amplifier 30, and an output terminal of the amplifier 30 is connected to a detector 31 for measuring the membrane potential of the piled membrane body 27.

In the syphilis diagnosis, a requisite amount of blood serum solution 32 to be tested, diluted with physiological saline, is poured into the compartments 23 and 24. When no syphilis antibody exists in the serum solution to be tested, no antigen-antibody reaction occurs on the surface of the antigen membrane 25 in contact with the serum solution. The charge density is substantially the same on both surfaces of the piled membrane body 27, and so the membrane potential is nearly zero.

When some syphilis antibody exists in the serum solution 32 to be tested, the antibody reacts with the antigen exposed on the surface of the antigen membrane 25 in contact with the serum solution 32. An antigen-antibody complex is formed on one surface of the piled membrane body 27. The piled membrane body 27 becomes asymmetric. Accordingly, the surface of the antigen-free membrane 26 of the piled membrane body 27 in contact with the serum solution 32 to be tested is electrically charged due to the property of antigen-free membrane 26 itself, while the surface of the antigen membrane 25 of the piled membrane body 27 in contact with the serum solution 32 to be tested is electrically charged due to the property of the antigen-antibody complex. The charge-densities
of the both surfaces of the piled membrane body 27 are different from each other. The difference of the charge densities is remarkably exhibited as membrane potential. Accordingly, the antigen-antibody reaction is followed by measuring the membrane potential. The membrane potential is electrochemically led through the electrodes 28 and 29 to the amplifier 30, and amplified thereby. The output of the amplifier 30 is applied to the detector 31. The existence of the syphilis antibody is detected with the output of the detector 31. The membrane potential $\Delta \psi$ is expressed by the following equation:

$$\Delta \psi = \frac{RT}{F} \ln \frac{Q_1 + (Q_2^2 + \Delta C^2)^{1/2}}{Q_1 + (Q_1^2 + \Delta C^2)^{1/2}}$$

where $Q_1$ represents the charge density of the surface of the antigen-free membrane in contact with the serum solution 32 to be tested; $Q_2$, the charge density of the surface of the antigen membrane on which the antigen-antibody complex is formed, and which contacts with the serum solution 32 to be tested; $C$, the concentration of the electrolyte; $R$, gas constant; $T$, absolute temperature ($^\circ$K), and $F$, Faraday constant.

FIG. 4 shows a schematic cross-sectional view of a syphilis diagnosis apparatus used for the third syphilis diagnosis method.

Referring to FIG. 4, an electrolytic cell 41 is partitioned into three parts by vertical walls 42 and 43. The central compartment 44 is filled with the solution 53 to be tested. The left and right compartments 45 and 46 are filled with the electrolyte 54. Openings 47 and 48 made on the vertical walls 42 and 43 are closed by an immobilized
antigen membrane 47 and an antigen-free membrane 48. Electrodes 49 and 50, for example, made of calomel or Ag-AgCl, are disposed in the left and right compartments 45 and 46. The electrodes 49 and 50 are connected to input terminals of an amplifier 51, and an output terminal of the amplifier 51 is connected to a detector 52 for measuring the membrane potential of the immobilized antigen membrane.

In the syphilis diagnosis, a requisite amount of physiological saline containing anti-serum is poured into the compartment 44, while a requisite amount of physiological saline is poured into the compartment 45 and 46. The antibody reacts with the antigen exposed on the surface of the immobilized antigen membrane 47 in contact with the physiological saline 53 containing the anti-serum. The antigen-antibody complex is formed on the surface of the antigen membrane 47. The antigen membrane 47 becomes asymmetric. Accordingly, the one surface of the antigen membrane 47 in contact with the physiological saline 54 is electrically charged due to the property of the antigen membrane itself, while the other surface of the antigen membrane 47 in contact with the physiological saline 53 containing the anti-serum is electrically charged due to the property of the antigen-antibody complex. The charge densities of the both surfaces are different from each other. The difference of the charge densities is remarkably exhibited as membrane potential. The antigen-antibody reaction is followed by the measurement of the membrane potential. The membrane potential is led to the amplifier 51 through the electrodes 49 and 50, and amplified thereby. The output of the amplifier 51 is applied to the detector 52. The existence of the syphilis antibody is detected with the output of the detector 52. The membrane
potential Δψ is expressed by the following equation:

\[ Δψ = \frac{RT}{F} \ln \frac{Ω_2 + (Ω_2^2 + 4Ω^2)^{1/2}}{Ω_2 - (Ω_2^2 + 4Ω^2)^{1/2}} \]

where \( Ω \) represents the charge density of the surface of the antigen membrane in contact with the physiological saline, \( Ω_2 \) the charge density of the surface of the antigen membrane on which the antigen-antibody complex is formed; \( C \), the concentration of the electrolyte; \( R \), gas constant; \( T \), absolute temperature (°K); and \( F \), Faraday constant.

When the concentration of the electrolyte, for example, physiological saline, contained in the compartment 44, is equal to the concentration of the electrolyte contained in the compartments 45 and 46, the membrane potential of the antigen membrane is zero. No antigen-antibody reaction occurs on the antigen-free membrane 48.

The reason why the electrodes 49 and 50 are separated from the solution to be tested is that insulating films are otherwise formed on the electrodes 49 and 50 in contact with protein solution such as anti-serum containing solution, due to electrolytic phenomenon, so that some error is made for the measurement of the membrane potential, with poor reproducibility and tedious cleaning of the electrodes 49 and 50.

FIG. 5 shows a schematic cross-sectional view of a syphilis diagnosis apparatus used for the third syphilis diagnosis method similar to FIG. 4.

Referring to FIG. 5, smaller cells 64 and 65 filled with the electrolyte are disposed in a larger cell 61 filled with the solution to be tested. An immobilized antigen membrane 62 and an antigen-free membrane 63 are stretched at the bottoms of the smaller cells 64 and 65, respectively. The
lower surface of immobilized antigen membrane 62 and the antigen-free membrane 63 are in contact with the solution 71 to be tested and contained in the larger cell 61. Electrodes 66 and 67, for example, made of calomel or Ag-AgCl, are disposed in the cells 64 and 65 respectively. The electrodes 66 and 67 are connected to input terminals of an amplifier 68, and an output terminal of the amplifier 68 is connected to a detector 69 for measuring the membrane potential of the immobilized antigen membrane.

The smaller cells 64 and 65 may be separated from each other, or they may be integrally with each other at their one side wall. The immobilized antigen membrane 62 and the antigen-free membrane 63 are preferably fixed at higher portions of the smaller cells 64 and 65 than the lower ends of the smaller cells 64 and 65. That is more advantageous in the prevention of the break of the membranes 62 and 63.

The smaller cells 64 and 65 need only to be arranged so that the lower surfaces of the immobilized antigen membrane 62 and the antigen-free membrane 63 are in contact with the solution 71 to be tested. For example, a bar 70 may be fitted to the opening of the larger cell 61 to fix the electrodes 66 and 67, and to fix demountably the smaller cells 64 and 65. That is very convenient for the exchange operation of the cells 64 and 65 after used. It is required that the lower ends of the cells 64 and 65 should not be in contact with the bottom of the larger cell 61. However, legs extending to the bottom of the larger cell 61 may be fixed to the lower ends of the smaller cells 64 and 65.

In the syphilis diagnosis, a requisite amount of physiological saline 71 containing the anti-serum is poured into the cell 61, while a requisite amount of physiological
saline 72 is poured into the cells 64 and 65. The antibody reacts with the antigen exposed on the surface of the immobilized antigen membrane 62 in contact with the physiological saline 71 containing the anti-serum. An antigen-antibody complex is formed on the surface of the antigen membrane 62. The antigen membrane 62 becomes asymmetric. The one surface of the antigen membrane 62 in contact with the physiological saline 72 is electrically charged due to the property of the membrane itself, while the other surface of the antigen membrane 62 in contact with the physiological saline 71 containing the anti-serum is electrically charged due to the property of the antigen-antibody complex. There is some difference of the charge density between the upper and lower surfaces. The difference of the charge densities is remarkably exhibited as membrane potential. Accordingly, the antigen-antibody reaction is followed by the measurement of the membrane potential. The membrane potential is led to the amplifier 68 through the electrodes 66 and 67, and amplified thereby. The output of the amplifier 68 is applied to the detector 69. The existence of the syphilis antibody is detected with the output of the detector 69. The membrane potential $\Delta \phi$ is expressed by the following equation:

$$
\Delta \phi = \frac{RT}{F} \ln \frac{Q_2 \left( \frac{Q_1^2 + \Delta C^2}{Q_1 + \left( \frac{Q_1^2 + \Delta C^2}{2} \right)} \right)^{1/2}}
$$

where $Q_1$ represents the charge density of the surface of the antigen membrane in contact with the physiological saline; $Q_2$, the charge density of the surface of the antigen membrane on which the antigen-antibody complex is formed; $C$, the concentration of the electrolyte; $R$, gas constant; $T$, absolute
temperature ($^0\text{K}$); and $F$, Faraday constant.

When the concentration of the electrolyte, for example, physiological saline, contained in the cell 61, is equal to the concentration of the electrolyte contained in the cells 64 and 65, the membrane potential of the antigen membrane is zero. No antigen-antibody reaction occurs on the antigen-free membrane 63.

The reason why the electrodes 66 and 67 are separated from the solution to be tested is that insulating films are otherwise formed on the electrodes 66 and 67 in contact with protein solution such as a solution containing anti-serum, due to electrolytic phenomenon, so that some error is made for the measurement of the membrane potential the reproducibility is poor, and the electrode 66 and 67 need to be cleaned.

FIG. 6 shows the effect of serum dilution on membrane potential at low concentration cardiolipin membrane, and FIG. 7 shows the effect of serum dilution on membrane potential at high concentration cardiolipin membrane.

According to this inversion, as described above, antigen reacting with a substance to be detected is immobilized to a membrane, and the objective substance, namely syphilis antibody, can be selectively and directly detected with the measurement of the membrane potential of the immobilized antigen membrane. In contrast to the conventional diagnosis with naked eye, the final decision is made by an electric signal from the membrane potential. Accordingly, erroneous reading is very small, and there is little difference in diagnosis carried out by different persons. The separation of the antigen-antibody complex is not required. The diagnosis operation is very simple. The syphilis diagnosis is exact and reproducible.
Next, one example will be described.

Example:

(1) Preparation of the immobilized antigen membrane.

Acetyl cellulose (38% of acetyl value; manufactured by Wako Junyaku Co., Ltd.) and Ogata antigen (manufactured by Sumitomo Chemical Industrial Co., Ltd., for syphilis Wassermann reaction; consisting of 0.01% cardiolipin, 0.04% lecithin, 0.20% cholesterol in ethanol) were used to prepare an immobilized antigen membrane.

One milliliter of the above described Ogata antigen was mixed with 6 milliliters of acetone solution containing 250 milligrams of acetyl cellulose. The mixed solution was cast on a glass plate (18 \times 10 \text{ cm}^2) and dried at room temperature under reduced pressure. After dried, the film was peeled from the glass plate. Thus, an immobilized antigen membrane was obtained.

Acetone solution of acetyl cellulose was used to prepare an acetyl cellulose membrane containing no antigen in the above described manner.

(2) Diagnosis experiment on syphilis serum to be detected.

(a) Physiological saline was used as electrolyte. Positive serology control serum for syphilis diagnosis (manufactured by DADE) diluted to $10^{-3}$, $10^{-2}$ and $10^{-1}$ was used as the solutions to be tested. Membrane potentials were measured for the respective control serum solutions.

The results are shown in FIG. 1.

The positive serology control serum exhibits a weak potential at a dilution of 1/16 by the VDRL method, and at a dilution of 1/32 by the RPR and USR methods.

(b) The comparison of the present method (ICES) and conventional methods (VDRL, TPHA and Ogata Method) is shown in
Table 1. Samples 1 to 11 are patients' serum.

<table>
<thead>
<tr>
<th>Sample</th>
<th>VDRL</th>
<th>TPHA</th>
<th>Ogata Method</th>
<th>ICES (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.01</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>x 2</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>x 2</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>x 4</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>x 4</td>
<td>0.07</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>x 16</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>x 16</td>
<td>0.11</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>x 32</td>
<td>0.15</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>x 32</td>
<td>0.17</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>x 320</td>
<td>0.17</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>x 320</td>
<td>0.14</td>
</tr>
</tbody>
</table>
The claims defining the invention are as follows:--
1. An antigen membrane for syphilis diagnosis comprising cardiolipin immobilized on a polymer matrix.
2. The antigen membrane for syphilis diagnosis of Claim 1, in which said polymer is a cellulose derivative.
3. A method for diagnosing syphilis comprising bringing one surface of the antigen membrane of Claim 1 into contact with a solution to be tested; bringing the other surface of said antigen membrane into contact with a normal serum solution; and measuring electrochemically the membrane potential generated across said antigen membrane.
4. A method of diagnosing syphilis comprising piling the antigen membrane of Claim 1 and an antigen-free membrane to form a piled membrane body; bringing both surfaces of said piled membrane body into contact with a solution to be tested; and measuring electrochemically the membrane potential generated across said piled membrane body.
5. A method for diagnosing syphilis comprising bringing one surface of the antigen membrane of Claim 1 and one surface of an antigen-free membrane into contact with a solution to be tested; bringing the other surface of said antigen membrane and said antigen-free membrane into contact with an electrolyte; and measuring the membrane potential generated across said antigen membrane.
6. The method Claim 5, wherein said electrolyte is physiological saline.
7. An apparatus for use in diagnosing syphilis comprising an electrolytic cell partitioned into two compartments by the antigen membrane of claim 1; electrodes disposed in said compartments; and a detector connected to said electrodes for measuring the membrane potential across said antigen membrane.
8. An apparatus for use in diagnosing syphilis comprising, an electrolytic cell partitioned into two compartments by the piled membrane body of Claim 4; electrodes disposed in said
two compartments; and a detector connected to said electrodes for measuring the membrane potential of said piled membrane body.

9. An apparatus for use in diagnosing syphilis comprising an electro-nic cell partitioned into three compartments by the antigen membrane of Claim 1, and by an antigen-free membrane, electrodes disposed in the two outer said compartments; and a detector connected to said electrodes for measuring the membrane potential of said antigen membrane.

10. The apparatus of Claim 9, wherein the central one of said three compartments is adapted to be filled with the solution to be tested and the outer two compartments are filled with an electrolyte.

11. An apparatus for diagnosing syphilis comprising a first
electrolytic cell; a second pair of electrolytic cells disposed inside said first electrolytic cell; the antigen membrane of Claim 1 being stretched across the bottom of one of said second pair of electrolytic cells, and an antigen-free membrane being stretched across the bottom of the other of said cells; an electrode disposed in each of said second pair of electrolytic cells; and a detector connected to said electrodes for measuring the membrane potential of said antigen membrane.

17. The apparatus of Claim 11 wherein said inner cells contain electrolyte and said first cell is adapted to contain the solution to be tested.

DATED this 29th day of August, 1980

NIPPON CHEMIPHAR CO., LTD.

Patent Attorneys for the Applicant:

F.B. RICE & CO.
FIG. 1

[Graph showing a curve and a horizontal line with logarithmic scales on the x-axis and linear scales on the y-axis.]