COMMONWEALTH OF AUSTRALIA
Patents Act 1952-1969

CONVENTION APPLICATION FOR A PATENT

BEHRINGWERKE AKTIENGESellschaft,
of D-3550 Marburg 1, Federal Republic of Germany

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

PROCESS FOR REDUCING TURBIDITY IN CONTROL SERA

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered

P33 29 952.8

for a patent or similar protection made in Federal Republic of Germany on 19th August 1983

My address for service is Messr. Edwd. Waters & Sons, Patent Attorneys,

Our

50 Queen Street, Melbourne, Victoria, Australia.

DATED this 16th day of August 1984.

BEHRINGWERKE AKTIENGESellschaft

Lodged at EUI-Office
7 Aug 1984
Melbourne

James Murray
DECLARATION IN SUPPORT OF A CONVENTION APPLICATION
UNDER PART XVI. FOR A PATENT.

In support of the Convention application made under Part XVI. of the Patents Act 1952 by BEHRINGWERKE AKTIENGESELLSCHAFT of Marburg/Lahn, Federal Republic of Germany for a patent for an invention entitled:
"PROCESS FOR REDUCING TURBIDITY IN CONTROL SEAS"

Dr. Philipp Stein    Höhenweg 28, 3550 Marburg

Dr. Siegfried Kamphans, Am Gutshof 4, 3550 Marburg

We do solemnly and sincerely declare as follows:
1. We are authorized by BEHRINGWERKE AKTIENGESELLSCHAFT the applicant for the patent to make this declaration on its behalf.
2. The basic application as defined by Section 141 of the Act was made in München in the Federal Republic of Germany under No. P 33 29 952.8 on the 19th day of August 1983 by BEHRINGWERKE AKTIENGESELLSCHAFT

3. a) Dagmar Steuer, 22a Geschwister-Scholl-Straße, D-3550 Marburg 1
   b) Rudolf Schmidtberger, 1 Salgrund, D-3550 Marburg 1
   a) and b) Federal Republic of Germany

are the actual inventor(s) of the invention and the facts upon which BEHRINGWERKE AKTIENGESELLSCHAFT is entitled to make the application are as follows:

The said BEHRINGWERKE AKTIENGESELLSCHAFT is the assignee of the said Dagmar Steuer and Rudolf Schmidtberger

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

DECLARED at Marburg/Lahn, Federal Republic of Germany this 11th day of July 1984

To the Commissioner of Patents

BEHRINGWERKE AKTIENGESELLSCHAFT

(ppa.Stein)  (ppa.Kamphans)
Prokurist      Prokurist
In order to ensure the storage stability of unstable components, such as, for example, enzymes or lipoproteins, control sera can be lyophilized and stored at a low temperature. Undesirable side effects of lyophilization are turbidity phenomena, which occur after reconstitution of the control sera by a change in the solution properties, especially of the lipoproteins. These turbidity phenomena frequently interfere in spectrophotometric methods, so that a sample blank value is additionally required.

Claim:

1. A process for reducing turbidity in a dried and reconstituted control serum, which comprises adding proline and, if appropriate, Na deoxycholate to the control serum before drying.
S. A control serum in dry form, which contains proline and, if appropriate, Na deoxycholate.
COMMONWEALTH OF AUSTRALIA
PATENTS ACT 1952-69

COMPLETE SPECIFICATION
(ORIGINAL)

Application Number:
Lodged:

Completed Specification Lodged:
Accepted:
Published:

Priority:

Deleted Art:

Name of Applicant:
BEHRINGWERKE AKTIENGESELLSCHAFT

Address of Applicant:
D-3550 Marburg 1, Federal Republic of Germany

Actual Inventor:
DAGMAR STEUER and RUDOLF SCHMIDTBERGER

Address for Service:
EDW. WATERS & SONS,
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

PROCESS FOR REDUCING TURBIDITY IN CONTROL SERA

The following statement is a full description of this invention, including the best method of performing it known to: US
The invention relates to a process for reducing turbidity in a dried and redissolved control serum and to a dried control serum with reduced turbidity after re-solution. The process is also suitable for control sera for lipid determinations, especially those with an increased lipid content.

Control sera are to be understood as sera of human or animal origin which have an optionally modified, but serum-like composition, contain serum constituents in a known concentration and are suitable for the control of determination methods for these serum constituents.

Processes for the preparation of such control sera, including the adjustment of individual constituents to desired concentrations, are known.

In order to ensure the storage stability of unstable components, such as, for example, enzymes or lipoproteins, control sera can be lyophilized and stored at a low temperature. Undesirable side effects of lyophilization are turbidity phenomena, which occur after reconstitution of the control sera by a change in the solution properties, especially of the lipoproteins. These turbidity phenomena frequently interfere in spectrophotometric methods, so that a sample blank value is additionally required. Turbidity presents particular problems in measurements in the region of 340 nm, in which those enzyme activity determinations based on NADH/NAD measurement are chiefly carried out. The extinction of NADH, which is already high per se, is increased further by the turbidity, so that it fre-
quently has to be measured in a range in which precise measurements are not possible. The results become more inaccurate and greatly depend on the quality of the photometer.

Processes are already known for avoiding turbidity phenomena. Shock freezing (German Offenlegungsschrift 2,243,014) requires a great deal of technological effort.

The use of purified lipid fractions (literature: Clin. Chem. 22, (1976), 456-490 and 1299-1305) causes high raw materials costs, and the addition of detergents may interfere with the particular test. Addition of sugars, sugar-alcohols or amino-sugars (German Patent 2,825,391, Research Disclosure Oct. 1977, No. 16, 229, Clinical Abstracts, volume 87, 1977, No. 196, 807 g and volume 90, 1979, No. 511 11c) causes a high viscosity and interferes with glucose determination methods. The addition of organic substances which are not sugar-like, such as methanol, alanine, triethylene glycol, valine, acetate, lactate or sodium 2-hydroxymethylbutyrate (German Patent 3,107,060) can lead to interference in enzyme reactions in the case of alanine and methylbutyrate. Addition of methanol generally constitutes a health hazard; if sodium acetate is used, the control serum can no longer be used as a universal control serum for electrolyte determinations; addition of ammonium compounds interferes in urea determinations; other substances can cause general test interferences.

The invention was therefore based on the object of preparing a universal control serum, and in particular a lipid control serum, which has a reduced tendency towards
turbidity after reconstitution of a dried form and does not have the disadvantages described for the known processes.

Surprisingly, it has been found that reconstituted control sera of relatively low turbidity can be prepared if proline is added to the control serum before drying.

It has furthermore been found that turbidity as a result of added lipids, in particular triglycerides, is further reduced by combination of the aminoacid mentioned with Na deoxycholate, especially if the control serum has a reduced electrolyte content (is low in electrolyte). Na deoxycholate is particularly suitable for this purpose because it does not itself cause interfering turbidity.

The invention thus relates to a process for reducing turbidity in a dried and reconstituted control serum, which comprises adding proline to the control serum.

A particular embodiment of the invention comprises also adding Na deoxycholate to a control serum with an increased lipid content. This is particularly advantageous if the ionic strength of the control serum is low, that is to say if it has a low electrolyte content.

The effective concentration of the above aminoacid is between 5 and 100 g/liter, the amount required depending on the lipid content of the serum. Sodium deoxycholate is used in a concentration of 0.5-5 g/liter.

"Low in electrolytes" in the context of the present invention means that the concentration of sodium is 40 to 80 mmols/liter and that of chloride ions is 20 to 70 mmols/liter. The process according to the invention is
suitable for the preparation of clinical-chemical control sera, i.e. products which are to be used for the quality control of clinically useful serum parameters, such as the enzyme, substrate, metabolite, hormone or electrolyte content. It is also suitable for the preparation of a particular lipid control or a lipid calibrator. The reduction in the turbidity is measured as follows: the extinction of the dried control serum reconstituted with distilled water and containing one or both of the additives according to the invention was measured with a light path of photometer at 546 nm in a cell with a layer thickness of 1 cm and was compared with the extinction of the same control serum without the addition.

Reconstitution is understood as meaning the solution of a dried material in the amount of solvent which it contained before drying.

The following examples illustrate the invention.

**EXAMPLES**

Pooled human serum from healthy donors or human serum with a reduced NaCl content was used as the serum base of the control serum. Egg yolk extract was added to increase the triglyceride concentration. Cholesterol was added as bovine cholesterol concentrate. Proline and, if appropriate, Na deoxycholate were then added in the concentration shown, and the mixture was then filtered free from germs, filled into bottles and lyophilized. After reconstitution with distilled water, the turbidity was measured.

The following table shows the results of the extinc-
<table>
<thead>
<tr>
<th>Basis material</th>
<th>Cholesterol (mmol/liter)</th>
<th>Triglyceride (mmol/liter)</th>
<th>Na deoxycholate (g/liter)</th>
<th>Preline (g/liter)</th>
<th>Extinction 546 nm</th>
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<td>Normal serum*</td>
<td>3.1</td>
<td>0.91</td>
<td>0</td>
<td>0</td>
<td>0.616</td>
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<td>0</td>
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<td></td>
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<td>1.035</td>
</tr>
<tr>
<td></td>
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<td>2.29*</td>
<td>2</td>
<td>50</td>
<td>0.614</td>
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<tr>
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<td>2.29*</td>
<td>2</td>
<td>10</td>
<td>1.219</td>
</tr>
<tr>
<td></td>
<td>7.76*</td>
<td>2.29*</td>
<td>2</td>
<td>50</td>
<td>0.728</td>
</tr>
<tr>
<td>Serum low in electrolytes</td>
<td>4.58</td>
<td>0.67</td>
<td>0</td>
<td>0</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>8.53*</td>
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<td>0</td>
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<tr>
<td></td>
<td>8.53*</td>
<td>3.49*</td>
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<td>50</td>
<td>0.6-0.8</td>
</tr>
<tr>
<td></td>
<td>11.4*</td>
<td>5.28*</td>
<td>0</td>
<td>0</td>
<td>&gt; 3</td>
</tr>
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<td></td>
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</table>

* The concentrations thus labelled were obtained by addition of cholesterol or triglycerides.
<table>
<thead>
<tr>
<th>Basal material</th>
<th>Cholesterol (mmol/liter)</th>
<th>Triglyceride (mmol/liter)</th>
<th>Na deoxycholate (g/liter)</th>
<th>Proline (g/liter)</th>
<th>Extinction 540</th>
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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for reducing turbidity in a dried and reconstituted control serum, which comprises adding proline and, if appropriate, Na deoxycholate to the control serum before drying.

2. The process as claimed in claim 1, wherein the concentration of sodium in the liquid control serum is 40-80 mmols/liter and that of the chloride ions is 20-70 mmols/liter.

3. The process as claimed in either of claims 1 and 2, wherein the concentration of the proline in the liquid control serum is 5-100 g/liter.

4. The process as claimed in either of claims 2 and 3, wherein the concentration of sodium deoxycholate in the liquid control serum is 0.5-5 g/liter.

5. A control serum in dry form, which contains proline and, if appropriate, Na deoxycholate.

DATED this 16th day of August 1984.

BEHRINGWERKE AKTIENGESELLSCHAFT

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MELBOURNE, VIC. 3000.