Improved Assessment of Plasma Lipoprotein Patterns: IV. Simple Preparation of a Lyophilized Control Serum Containing Intact Human Plasma Lipoproteins

Heinrich Wieland and Dietrich Seidel

Addition of sucrose (413–825 mmol/L) to human serum allows plasma lipoproteins to be lyophilized without denaturation. A control serum so prepared is especially suited for use in monitoring determinations of apolipoproteins and quantitative lipoprotein electrophoresis. It is clear upon reconstitution; hence, the described procedure may also be useful for preparation of control sera for general clinical chemistry without de-lipoproteinization.

Additional Keyphrases: quality control • lipoprotein electrophoresis

It is now well accepted that, for clinical and epidemiological purposes, the determination of lipids should be extended to the determination of serum lipoproteins and apoproteins. But the precision and accuracy of current methods for assessing plasma lipoproteins or apolipoproteins have been difficult to monitor by use of quality-control material, because plasma lipoproteins cannot be preserved and stored in liquid form for very long. Furthermore, several lipoproteins are insoluble after lyophilization; thus the reconstituted serum is turbid, which interferes with spectrophotometry. Therefore quality-control material is usually de-lipoproteinated and consequently contains only low concentrations of cholesterol and triglycerides. Most lipoproteins are affected by lyophilization and may no longer correspond to native lipoproteins, which makes difficult their assessment by electrophoretic or immunological methods.

Here we describe a simple procedure by which serum lipoproteins are completely protected from denaturation during lyophilization. Control sera prepared according to this procedure are especially suited for quality control of all methods for qualitative and quantitative analysis of plasma lipoproteins and apolipoproteins, and may even serve as reference sera. Furthermore, the serum remains clear upon reconstitution, so this procedure is especially suited also for the production of quality-control material for any kind of clinical-chemical analysis for any compound usually present in human blood.

Materials and Methods

Preparation of the Control Serum

The lipoproteins in any serum or plasma in naturally occurring concentrations are protected from denaturation by adding sucrose before freezing or lyophilizing. The final concentration of sucrose should be between 413 and 825 mmol/L. It can be added in solid form or as a solution. The sucrose-containing serum is shelf-frozen and lyophilized in any kind of test tube, flasks, or vials. It is reconstituted with distilled water. For the present study, many vials containing 0.5-mL aliquots of the same serum were prepared by Immuno GmbH, Vienna, to enable determination of the between-assay precision of methods for quantification of lipoproteins or apoproteins.

Techniques

Lipoprotein electrophoresis: β-, pre-β-, and α-lipoprotein cholesterol was determined with the Lipidophor test kit (Immuno Diagnostica GmbH, Heidelberg, F.R.G.) according to Wieland and Seidel (4). Cholesterol was determined enzymically by the “CHOD-PAP” method (Boehringer, Mannheim, F.R.G.). Triglycerides were determined enzymically by the “peridochrom” method (Boehringer).

HDL cholesterol: HDL cholesterol was determined, after precipitation of low- and very-low-density lipoproteins (VLDL) with phosphotungstic acid and MgCl2 (5), with a commercially available test kit (HDL-Cholesterol, Boehringer).

Determination of apolipoproteins: A-I and apo-B were determined by rate nepholometry (6, 7) in a Beckman Immunochemistry system. Apo-B was additionally determined by radial immunodiffusion, with commercially available test kits (NOR-Partigen; Behringwerke, Marburg/Lahn, F.R.G.).

Ultracentrifugation: Ultracentrifugation was performed on 3-mL aliquots of serum at solution densities of 1.006 and 1.063 kg/L in a Beckman L 275 ultracentrifuge (10 °C, 40 000 rpm, 36 h), in a 50 TI rotor. The sera were dialyzed against sodium chloride solutions of the appropriate densities.

Determination of sample turbidity: Sample turbidity was quantified by light scattering in a Beckman Immunochemistry System after addition of 20 μL of sample to 1 mL of a 9 g/L NaCl solution.

Determination of enzymes and substrates: Na+, K+, Ca2+, glucose, creatinine, uric acid, urea nitrogen, the aminotransferases, glutamytransferase, alkaline phosphatase, and bilirubin were determined by continuous flow (SMAC; Technicon Instruments, Tarrytown, NY 10591). Lactate dehydrogenase, serum amylase, and creatine kinase were determined with reagents supplied by Merck GmbH, Darmstadt, F.R.G.

Results

Figure 1 demonstrates that lyophilization in the presence of sucrose (619 mmol/L) leads to complete conservation of the electrophoretic properties of serum lipoproteins, whereas lyophilization without sucrose seems predominantly to affect pre-β-lipoproteins. Lower concentrations of sucrose than 400 mmol/L are not sufficient, while concentrations exceeding 900 mmol/L are not suitable for clinical chemical purposes because of the high viscosity of the sample. Figure 2 demonstrates the loss of the pre-β-lipoprotein band and the concomitant broadening of the β-band, and the appearance of material

1 Nonstandard abbreviations: apo, apoprotein; VLDL, HDL, very-low-and high-density lipoprotein(s); and Lp lipoprotein.
remaining at the origin of the lipoprotein electrophoresis, all
induced by lyophilization. The densitogram of a sample ly-
ophili
dized in the presence of sucrose is identical to that of the
responding serum. The ultracentrifugally isolated VLDL-
fraction of lyophilized sample shows, on lipoprotein
 electrophoresis, two fractions that correspond to the changes
demonstrated in Figure 1, i.e., nonmigrating material rem-
aining at the origin and a lipoprotein with β-mobility. Ly-
ophilization in the presence of sucrose (619 mmol/L) does not
change the densitometric pattern.

Table 1. Influence of Lyophilization on the Pattern
Obtained on Quantitative Lipoprotein
Electrophoresis of Serum before and after Lyophilization, with and without Sucrose *

<table>
<thead>
<tr>
<th></th>
<th>Fresh serum</th>
<th>Lyophilized + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lipoproteins</td>
<td>45.9 ± 8.7</td>
<td>55.6 ± 11</td>
</tr>
<tr>
<td>Pre-β-lipoproteins</td>
<td>10.7 ± 4.8</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>α-Lipoproteins</td>
<td>43.3 ± 12</td>
<td>43 ± 11</td>
</tr>
</tbody>
</table>

* Determinations are expressed as percentage of the total integral. The effect
seems to be confined to β- and pre-β-lipoproteins; the α-lipoproteins remain
unchanged. Lyophilization leads to marked decreases of the pre-β-lipoproteins
and a concomitant increase of the percentage of the total integral corresponding
to β-lipoproteins. This effect is abolished by the presence of sucrose during
lyophilization, n = 25 different serum samples.

Table 1 shows the quantitative aspect of the changes in-
duced by lyophilization, determined densitometrically.
Although there is no change in the percentage of the total integ-
ral corresponding to the α-lipoproteins, there is a striking
decrease in the percentage of pre-β-lipoproteins and an in-
crease in the percentage of β-lipoproteins.

Addition of 150 mg of sucrose to 1 mL of serum results in a
10% dilution of the sample—i.e., the concentrations of all
components are decreased to 90%—and an increase in density
to d = 1.072 kg/L.

Table 2 demonstrates the influence of lyophilization on
some lipid and apoprotein variables, including the turbidity of
the sample. The changes presented in Table 2 are almost
obviated by the addition of sucrose before lyophilization. The
values for the samples containing sucrose are corrected for the
above-mentioned dilution. As shown in Table 2, lyophilization
affects more the enzymic determination of cholesterol than
of triglycerides. Also, the values for apolipoproteins A-I and
B are decreased after lyophilization. This decrease is almost
completely prevented by adding sucrose before lyophilization.
The turbidity of the sample increases by threefold after ly-
ophilization without sucrose, but by only 25% with sucrose
present. Thus, control material prepared in the presence of
sucrose is relatively clear upon reconstitution. The presence
of this non-reducing carbohydrate does not influence the re-
results of common clinical chemical determinations. The in-
crease in density of course influences ultracentrifugation at
sample density. If the sample is dialyzed against solutions of
appropriate densities or is diluted, the ultracentrifugal prop-
erties of the lipoproteins of samples lyophilized in the presence
of sucrose also appear to be unchanged. The increased density
caused by the added sucrose also leads to incomplete sedi-
mementation of precipitated lipoproteins in triglyceride-rich
samples during the determination of HDL-cholesterol. If the
precipitate sediments, however, very reproducible HDL-
Table 3. Within- and Between-Assay Precision of Some Lipid, Lipoprotein, and Apoprotein Determinations in Control Serum Containing Sucrose (619 mmol/L)*

<table>
<thead>
<tr>
<th></th>
<th>Control serum</th>
<th>Fresh serum, within assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Between assay</td>
<td>Within assay</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.1</td>
<td>1.59</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>3.6</td>
<td>2.37</td>
</tr>
<tr>
<td>A-I</td>
<td>2.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Apo-B</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>β-Lp.-Chol.</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Pre-β-Lp.-Chol.</td>
<td>8.4</td>
<td>7.06</td>
</tr>
<tr>
<td>α-Lp.-Chol.</td>
<td>5.43</td>
<td>5.2</td>
</tr>
<tr>
<td>β-Lp./α-Lp.</td>
<td>5.2</td>
<td>3.97</td>
</tr>
<tr>
<td>HDL-Chol.</td>
<td>4.3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

* The within-assay precision (CV, %) as determined from 25 determinations made in parallel. Between-assay precision was calculated from determinations done on 60 consecutive working days (not weekends). Lp, lipoprotein.

cholesterol values can be obtained (Table 3). Lyophilization without sucrose strongly affects the results of HDL-cholesterol determination: values average 120 mg/dL lower.

Table 3 also shows the between- and within-assay precision obtained with the new control serum for some lipid, lipoprotein, and apoprotein measurements. The within-assay precision of the lyophilized control material is very similar to that obtained with fresh serum.

The electrophoretic and immunological properties of the lyophilized plasma lipoproteins remain unchanged, even after storage at 37 °C for a year.

Discussion

The mechanisms by which sucrose protects lipoproteins from the denaturation induced by lyophilization is not clear. It probably is incorporated into the hydration shell of the molecule and replaces water during the freeze-drying process. On reconstitution, it may readily accept water, with the consequent restoration of the shell. Lipoproteins so protected behave similar to, if not identical with, native plasma lipoproteins in all the kinds of separation procedures and analytical steps that we examined. In our opinion, the methods most influenced by the physical-chemical state of the analyte under investigation are lipoprotein electrophoresis and rate-nephelometric determination of apolipoprotein B. With both of these methods, results with the lyophilized control serum cannot be distinguished from those for fresh whole serum. An additional advantage of lyophilization with sucrose is the complete absence of any insoluble material after reconstitution, which is usually complete after 5 to 10 min. In contrast to most other control sera, all lipids occurring naturally in human serum can still be determined and the supersorbed material can be used as reference serum. This is important progress, especially in view of the fatty acid moiety of triacylglycerols. With the new control serum, the accuracy and precision of both the separation and the determination of normal and also abnormal (lipoprotein-X, β-VLDL) lipoproteins can be monitored. Electrophoretic lipoprotein patterns can be conserved, which should make phenotyping of hyperlipoproteinemias more comparable among different laboratories. Inter-laboratory comparability of HDL-cholesterol determination, either by ultracentrifugation or by precipitation techniques, should also improve.

The new technique also makes it possible to prepare a stable reference material for the determination of all the apolipoproteins present in human serum. So far, only a fairly well-defined group of normolipemic subjects could be used for the comparison of different methods. This fact and a variety of standardization procedures may have led to the wide range of normal values reported for apolipoproteins B (810–1290 mg/dL), A-I (1000–1430 mg/dL) and A-I (10, 11). If all groups determining apolipoproteins would agree to use such a material as a secondary standard, the accuracy and precision of apoprotein determinations probably would improve and a normal concentration range for apolipoproteins could be established. This would make determinations of apoproteins much more meaningful for clinicians and is a prerequisite for the incorporation of apolipoprotein determinations in epidemiological studies. Moreover, with a common reference material, individual properties of antisera produced by different laboratories may be detected and characterized.

We hope that this new way of preserving lipoproteins will lead to a stringent system of quality control for plasma lipoprotein analyses, benefitting clinicians, epidemiologists, and, not least, patients at risk for coronary heart disease.

References