Testing the Accuracy of Total Cholesterol Assays in an External Quality-Control Program. Effect of Adding Sucrose to Lyophilized Control Sera Compared with Use of Fresh or Frozen Sera

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We studied the suitability of various types of human serum preparations to test the accuracy of total cholesterol measurements in the External Quality Assessment scheme in The Netherlands, in which ~180 laboratories participate. Checked against the certified Abell/Kendall Reference Method, large reagent-dependent negative biases were observed with lyophilized serum that was insufficiently cryoprotected. The biases for the reagents of Du Pont, Roche, and Beckman averaged −16.7%, −9.2%, and −7.6% respectively; the least bias, −0.4%, was obtained with reagent from Boehringer Mannheim. The beneficial effect of cryoprotection with sucrose was demonstrated by the decrease in interreagent variation from 5.4% to 1.9%, the latter value being comparable with the values for fresh and once-frozen pooled serum (1.3% and 1.7%, respectively). We conclude that the detrimental effect of lyophilization on serum matrix can be minimized by suitable cryoprotection with 200 g/L sucrose.

Indexing Terms: sample handling/cryopreservation/external quality assessment

With the advent of national cholesterol consensus agreements, as now have been reached in many countries, it is no longer acceptable for individual laboratories to use their own lipid reference intervals with inherent, local method-induced biases. Instead, diagnostic strategy requires the application of fixed risk-evaluation cut points. Thus, laboratories must be able to deliver cholesterol results that are standardized to a central accuracy base.

To comply with the US guidelines, issued by the National Cholesterol Education Program, laboratories must have a method bias <3% from the Abell–Kendall (A/K) Reference Method and a between-run method precision better than 3% (1).6 Evaluating inaccuracy in the individual laboratory frequently involves External Quality Assessment (EQA). The existence of method-dependent differences in accuracy of various cholesterol assays has been amply demonstrated (2–6), and part of this variance has been attributed to the lack of commutability of the control sera used (6–11).

With few exceptions, most EQA schemes rely on lyophilized survey material for logistical reasons. However, to obtain unbiased observations concerning the performance of the analyses of the participating laboratories, one must use control material that is in all aspects resembles the characteristics of fresh, unpreserved human serum. Research towards this lack of commutability indicates that one potential source of error may be the use of animal sera; another may be the preparation procedure used for long-term storage. Both can result in lipoproteins that subsequently are suboptimally processed by reagents designed for analysis of fresh human sera. This is especially true after inadequate cryopreservation, which can denature apoprotein B-containing lipoproteins.

Recently (12–15), sucrose was successfully used in the cryoprotection of lipoprotein(a), a lipoprotein that is even more rapidly denatured than the other apoprotein B-containing lipoproteins in serum. These results prompted us to evaluate the use of sucrose as a cryoprotectant in sera used in our EQA surveys. We decided therefore to study the effects of sucrose addition to lyophilized serum preparations with respect to the interlaboratory accuracy—comparing not only the lyophilized preparation with and without added sucrose but also untreated serum, and once-frozen serum preparations.

Materials and Methods
Preparation of Pooled Human Serum

Patients' serum samples that were stored at 4°C for ≤3 days, residuals of the clinical laboratory, were pooled in 200-mL portions. This was done according to the hospital policy of generally asking patients for consent to so proceed. Each pool consisted of material originating from at least 150 different patients. The resulting nominal cholesterol concentration was very constant between pools, being 5.3–5.6 mmol/L. Sera with apparent turbidity, excessive bilirubin, or hemolysis were excluded from pooling. After pooling, the sera were centrifuged in 50-mL volumes at 3000g for 30 min, after which the chylomicrons at the meniscus were removed by manual aspiration. Before distribu-
tion, the subpools were verified to be free of HIV and hepatitis A, B, and C antigens.

Sample preparation and subsequent lyophilization were based on procedures described by Wieland and Seidel (16) and Terlingen et al. (17). In brief, 200-mL portions were combined and processed in four different ways: (a) 5-mL aliquots (n = number of surveys = 1) were dispensed into brown neutral-glass vials and mailed by overnight post (ambient temperature at night, <15°C) to be analyzed within 24 h; (b) 5-mL aliquots (n = 4) were dispensed into the glass vials, frozen at −25°C, and mailed by overnight post in solid CO₂, to be kept frozen until analysis; (c) 5-mL aliquots (n = 27) were dispensed into the glass vials, frozen at −25°C, and lyophilized. All aliquots were dispensed with an imprecision of <0.2% as determined by weighing at random 1 of each 50 vials before and after filling. The aliquots dispensed from the sucrose-containing pools were 7 mL to correct for volume changes. We fine-tuned the dispensing by calculating a factor based on the measured sodium concentration. None of the samples was supplemented with external cholesterol.

For lyophilization we used a Virtis Consol 12 (Gardiner, NY). The samples were placed into the freeze-dryer with a shelf temperature of −20°C. During the next 50 h, the temperature was increased to −14°C and the pressure was reduced to 1.3 Pa. The temperature was increased to 0°C during 40 h, then to 20°C during 7 h, and held there for 10 h. The samples were capped while still under reduced pressure. The moisture content was checked by a Karl Fisher procedure with Metrohm analytical equipment (Metrohm, Herisau, Switzerland); it was always <0.5%.

Commercially Available Sera

To evaluate the suitability (or lack thereof) of commercially available control sera to serve as control for accuracy, we selected 10 different serum preparations for forwarding to the participants in 2 to 13 surveys (see Table 1). All were delivered in the knowledge that they would be used in the Netherlands EQA Scheme (18).

Characterization of Serum Preparations

Lipoprotein integrity was studied by means of density-gradient ultracentrifugation; the serum sample was stained with Coomassie Brilliant Blue before ultracentrifugation, which resulted in blue-colored lipoprotein bands within the density gradient after ultracentrifugation (19). We also characterized serum preparations by lipoprotein electrophoresis with the Paragon system (Beckman Instruments Diagnostics, Brea, CA); staining was performed with Sudan Black. Turbidity of the reconstituted lyophilized serum preparations was measured by absorbance at 620 nm. All serum specimens under study were also analyzed with the Centers for Disease Control and Prevention (Atlanta, GA) standardized A/K Reference Method (20) in the Lipid Reference Laboratory of one of the authors (G.J.M.B.).

Interlaboratory Comparison Surveys

The data presented for the prepared sera stem from the observations of one survey in which untreated serum was analyzed, four surveys in which once-frozen serum was used, and 23 and 27 surveys with lyophilized serum respectively with or without sucrose at 200 g/L. For logistical reasons, the study of the four most important serum preparations (untreated liquid, frozen liquid, lyophilized without, or lyophilized with sucrose) could not be performed with one combined source pool in the same survey. We therefore considered the following two combinations of serum preparations from the same source pool in the same survey: (a) comparing untreated serum with the lyophilized serum without sucrose, evaluated once; or (b) comparing the frozen serum with both lyophilized serum preparations, i.e., without and with sucrose.

The standard procedure allowed for each participant to specify the analytical method used in terms of type of instrumentation and the source of reagents. We analyzed the returned results with respect to the source of the reagents, finding this to be more decisive than differentiation according to the type of instrument because some of the "open analyzer" systems allow users to choose various specific reagents. The users of Boehringer Mannheim (Mannheim, Germany) reagents represented the largest reagent group (80 laboratories, 43%). The next largest group consisted of Kodak Ektachem (Eastman Kodak, Rochester, NY) users (26 laboratories, 14%), followed by Beckman with 15 laboratories (8%), Bayer Diagnostics (Leverkusen, Germany) with 14 laboratories (7%), E. Merck Diagnostics (Darmstadt, Germany) with 9 laboratories (5%), Hoffmann-La Roche (Basel, Switzerland) with 8 laboratories (4%), Du Pont (Wilmington, DE) with 6 laboratories (3%), and Baxter (Düdingen, Switzerland) with 6 laboratories (3%).

The data for commercial control sera reflect serum preparations from Randox Labs, Crumlin, UK (13 surveys with Randox™, lot nos. SE1099, SN 1098, 067SE, 038SN, 002UE, 002UN, 032SN, 078SE, UE1559, SL1097, 064SN); Nycomed A/S, Oslo, Norway (6 surveys with Autonorm™, lot nos. 4003, 11014, 11015, 212043); Bio-Rad Labs., Anaheim, CA (7 surveys with Lyphochek™ bovine, lot nos. 37102, 40301, 40302; 6 surveys with Lyphochek™ human, lot nos. 44101, 44102, 48802, 54801, 54802, 60802; 5 surveys with liquid Liquecheck™, lot nos. 74031, 74032, 59401, 59402); Baxter Healthcare, Dade Division, Miami, FL (2 surveys with Moni-Trol™, lot nos. 615002, 616002); Human GmbH, Taunusstein, Germany (4 surveys with Serodos™, lot nos. 6863, 6865, 6790); Boehringer Mannheim (4 surveys with Precinorm™/Precipath™, lot nos. 187470, 229415); Merck (5 surveys with Quali­trol™, lot nos. 326, 390, 3410, 10937, 11307); and Laboratoires BioMérieux, Marcy l'Etoile, France (4
surveys with Lyotrol™, lot nos. 634361A, 634371A, 644730A, 735530A).

Calculations and Statistical Tests
For each survey, the reagent-group-specific mean values were calculated after elimination of statistical outliers. Identification of outliers was carried out at the 1% uncertainty level as described earlier (18). These reagent-specific mean values were thereafter compared with the known A/K Reference Method value to establish the percentage bias with the A/K value. To test whether these percentage biases obtained for the various serum preparations (frozen, or lyophilized without or with 200 g/L sucrose) differed from one another, we used the nonparametric two-tailed Wilcoxon test. For each survey, we also calculated the variation in the reagent-specific mean values and expressed this as the interreagent CV. This is a very sensitive measure for representing the magnitude of a possible matrix-induced intermethod variation. Where appropriate, these CV values were also averaged over the number of surveys.

Results
Serum Characterization
Agarose gel electrophoresis examples of three typical commercial “animal” control sera and a typical commercial “human” control serum are shown in Fig. 1. Also shown are the lipoprotein patterns of both kinds of prepared lyophilized sera: one without and the other with the added 200 g/L sucrose as cryoprotectant. For comparison the pattern for three once-frozen human serum pools are also shown.

For the animal sera the most stain-positive substance is encountered in the α region, with a smear of indiscriminate staining in the β/Pre-β region seen frequently. All lipoprotein bands of the commercial sample of human origin are diffuse, indicating some loss of structure. The sera we prepared, both without and with sucrose, exhibited well-defined α- and β-lipoprotein bands, but the pre-β band is missing somewhat, presumably because of a degree of denaturation during the lyophilization process, even in the presence of sucrose.

The same series of control sera were also analyzed by density-gradient ultracentrifugation (Fig. 2). The animal serum is strongly icteric, resulting in a greenish appearance upon ultracentrifugation (yellow bilirubin mixed with Coomassie Brilliant Blue stain). Animal lipoproteins can be frequently recognized from their hyper-α-lipoproteinemia. In these sera we also frequently detected denatured low-density lipoprotein (LDL), which could be recognized by flakes floating in the LDL density range. The commercially available serum of human origin typically showed a diffuse high-density lipoprotein (HDL) region, in which HDL₂ and HDL₃ could be barely distinguished. The very-low-density lipoprotein (VLDL) fraction also usually appeared to be denatured, with flaky material present in the fraction <1.006 kg/L. The experimental sera we developed displayed sharp and well-separated HDL₂ and HDL₃ bands, independent of the use of sucrose. However, these materials also contained flaky VLDL.

Reconstituted control sera cryoprotected with sucrose at 0, 70, 140, and 200 g/L showed a gradual decrease in turbidity at 620 nm with absorbance values of 1.5, 0.8, 0.4, and 0.3, respectively.

Interlaboratory Comparison Surveys
Averaged over the number of surveys, the percentage bias from the certified A/K Reference Method values found for the various reagent group mean values are shown in Table 1. For comparison, the results for single surveys with material derived from the same pool are also included. The results of these singular surveys did not differ significantly (P = 0.01) from the aggregated data from all surveys for the materials in question. Because the aggregated data consist of results obtained over 2 years, we also looked for possible shifts or trends that might confound conclusions to be drawn from the total data. We specifically looked for any drift or trend in the two reagents giving the most different effects, i.e., the Du Pont and the Boehringer Mannheim results for the in-house-prepared lyophilized sera without sucrose. The percentage difference from the A/K values found in four surveys for the late 1992, mid-1993, and late 1993 surveys was respectively -16.9% (SD 3.2%), -17.7% (SD 1.8%), and -17.9% (SD 2.7%) for the Du Pont reagent, and -0.2% (SD 2.2%), -1.4% (SD 0.9%), and -0.8% (SD 0.6%) for the Boehringer Mannheim reagent. No significant drift or trend was detected.

The most obvious finding was the large dispersion of the differences of the reagent-specific mean values from the A/K Reference Method values for the commercial control sera. Results for some of them (Lyphochek

7 When the control sera with denatured material were analyzed by conventional sequential ultracentrifugation, a procedure in which no contact with the strongly ionic salts occurs, the d <1.006 kg/L material still contained white flakes.

Fig. 1. Agarose gel electrophoresis of control sera of different origins with α-, β, and pre-β-lipoproteins at the migration distances 1, 2, and 3, respectively.

(a) Experimental serum containing 200 g/L sucrose; (b) human serum from commercial source (BioMérieux Lyotrol 735530A); (c) experimental serum without sucrose; (d, e, f) once-frozen human serum pools; (g, h, i) animal sera from commercial source (Technicon Testpoint IV 09316, Bio-Rad Lyphochek 40302, and Randox 002UE, respectively). Except for d, e, and f, all sera were lyophilized specimens after reconstitution.
human, Precinorm/Precipath, and Lyotrol) resembled the findings for the prepared sera without sucrose; i.e., the reagent-specific group mean values were always lower than the A/K Reference Method values. This large dispersion for the reagent group mean differences is consequently reflected in the relatively high values for the mean interreagent CVs, which were ~3–4%.

The findings for the prepared sera are shown in Fig. 3. Results for the fresh serum, averaged over all reagent groups, showed a positive mean difference of +2.4% from the A/K reference value. Averaged over the four surveys involving once-frozen serum, the results for once-frozen serum also showed a positive mean difference from the A/K values: +2.7% (SD 0.7%). For the lyophilized specimens, the bias between the sucrose-containing sera in relation to the A/K values resembled those for the fresh serum and the frozen sera; i.e., the mean difference was +3.8% (SD 1.1%). In contrast, the sera lyophilized without sucrose gave a mean difference of -5.8% (SD 1.6)—the overall negative bias being highly influenced by the large negative differences found for the reagent groups of Du Pont (-16.7%), Roche (-9.2%), and Beckman (-7.6%).

The reagent-specific mean relative biases from the A/K values for fresh serum, frozen sera, and lyophilized sera with sucrose belonged to one homogeneous statistical population; the results for the lyophilized sera without sucrose belonged to another. The comparability among the first three sample types is also demonstrable by the similarity of their interreagent CVs—1.3%, 1.7%, and 1.9%, respectively, for fresh serum, frozen sera, and lyophilized sera with sucrose; the interreagent CV for lyophilized sera without sucrose, however, was 5.4%.

The data for the sera we prepared can be used to differentiate between pure method-related effects, which arise from the differences between the prepared samples and fresh serum, and effects that are induced by freezing and (or) lyophilization. The effect of lyophilization, differentiated for the presence or absence of sucrose, can be interpreted as the combined effect of...

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**Table 1. Reagent-related mean relative bias from A/K values and mean interreagent CV for various serum preparations.**

<table>
<thead>
<tr>
<th>Type of serum</th>
<th>Matrix</th>
<th>No. of surveys</th>
<th>Mean (SD) % bias with reagent</th>
<th>Interreagent CV, % (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baxter</td>
<td>Beckman</td>
<td>Boehr. M.</td>
<td>Du Pont</td>
<td>Kodak</td>
</tr>
<tr>
<td>Autonorm</td>
<td>Animal</td>
<td>6</td>
<td>2.2 (4.2)</td>
<td>1.2 (0.7)</td>
</tr>
<tr>
<td>Random</td>
<td>Bovine</td>
<td>13</td>
<td>-2.4 (2.9)</td>
<td>-1.5 (1.4)</td>
</tr>
<tr>
<td>Lipochoke</td>
<td>Animal</td>
<td>7</td>
<td>-3.6 (3.2)</td>
<td>-5.5 (6.1)</td>
</tr>
<tr>
<td>Monotrol</td>
<td>Animal</td>
<td>2</td>
<td>1.1 (0.1)</td>
<td>-1.7 (1.0)</td>
</tr>
<tr>
<td>Lipochoke</td>
<td>Human</td>
<td>6</td>
<td>-6.8 (3.7)</td>
<td>-6.4 (4.7)</td>
</tr>
<tr>
<td>Liquitrol</td>
<td>Human</td>
<td>6</td>
<td>-2.2 (2.9)</td>
<td>-1.0 (4.4)</td>
</tr>
<tr>
<td>Saezds</td>
<td>Human</td>
<td>4</td>
<td>-0.4 (1.2)</td>
<td>1.7 (3.3)</td>
</tr>
<tr>
<td>Precip./Precin.</td>
<td>Human</td>
<td>4</td>
<td>-7.2 (2.4)</td>
<td>-7.0 (3.4)</td>
</tr>
<tr>
<td>Qualitrol</td>
<td>Human</td>
<td>5</td>
<td>0.8 (4.0)</td>
<td>1.4 (2.7)</td>
</tr>
<tr>
<td>Lyotrol</td>
<td>Human</td>
<td>4</td>
<td>0.7 (1.4)</td>
<td>-5.8 (3.0)</td>
</tr>
</tbody>
</table>

**Notes:**
- *Not further characterized.
- *L* yophilized.
- *L* yophilized and cryoprotected with 200 g/L sucrose.
- **Prepared** from the same respective pool and combined into one survey.

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are likewise obtained by subtracting the values for the frozen specimens from the corresponding the values for the fresh specimen from the mean values obtained for the frozen samples. "Lyoph. Effect 0% sucrose" and "Lyoph. Effect 20% sucrose" values with 200 g/L sucrose (20% sucrose) on the different reagent-specific relative biases with the A/K Reference Method values.

**Method effect**

**Fig. 4. Differentiation of the effects of differences in methods, frozen vs fresh, frozen vs lyophilized without sucrose, and frozen vs lyophilized with 200 g/L sucrose (20% sucrose) on the different reagent-specific relative biases with the A/K Reference Method values.**

Method effect bars represent the percentages difference for the native fresh serum (same as in Fig. 3). Values for the freezing effect are obtained by subtracting the values for the fresh specimen from the mean values obtained for the frozen samples. "Lyoph. Effect 0% sucrose" and "Lyoph. Effect 20% sucrose" values are likewise obtained by subtracting the values for the frozen specimens from the corresponding 0% and 20% sucrose values (Table 1 and Fig. 3).

**Discussion**

To assess the quality of the determination of total serum cholesterol, one must consider two factors: intralaboratory imprecision, which has to be low, and concentrations with most reagents, the reagents of Du Pont, Beckman, and Roche being affected most. The data for lyophilization with sucrose show that the presence of sucrose results in only minor deviations (<3%) for all reagents studied.

Frozen, freeze-drying, and reconstitution processes. Fig. 4 illustrates the effect of these processes on the assayed results. The inherent method-related differences with the A/K value are shown as "Method effect." The freezing effect, obtained by subtracting the values for "fresh" from those for frozen sera (Table 1), shows that the reagents of Bayer (+3.7%), Kodak (+3.7%), and Du Pont (-3.6%) are most affected by the freeze-thaw cycle. Lyophilization without sucrose (data after subtraction from the "Frozen" data in Table 1) results in greatly underestimated cholesterol concentrations.
accuracy. The US guidelines recommend a maximum intralaboratory CV of 3%—a value we believe can be reached by the majority of laboratories, as judged from our own experience (21) and the literature (22). As for accuracy, reliable performance evaluation of both participants and methods, as classically carried out by EQA surveys, is frequently hampered by the fact that the control sera used in such evaluations are not comparable with the fresh human serum matrix and thereby induce serious method/reagent/analyzer-dependent differences that obscure assessment of the real laboratory performance with patients' specimens. This drawback is especially true in analyses for total serum cholesterol. We therefore have studied some of the above-mentioned variables in a special survey with unadulterated human serum.

In our approach, control sera were evaluated by using variations in the species from which the serum originated, either commercially available or prepared by us and either cryoprotected with 200 g/L sucrose or not. By density-gradient ultracentrifugation, agarose gel electrophoresis, and turbidity measurements, we could show that the physical aspects of the various control sera were sometimes strikingly different from those obtained for the fresh controls: Lipoprotein bands of pooled serum lyophiliized in the absence of sucrose were less sharp and contained more denatured lipoproteins than when lyophiliized in the presence of sucrose. For the commercially available sera, the method used for cryoprotection is unknown, but some sera clearly contained large flakes, indicating LDL denaturation.

The analytical methods used, density-gradient ultracentrifugation and agarose electrophoresis, lack discriminatory power to identify slight differences in denaturation. However, by comparing the relative performance of the various control sera, preparations could be identified for which the interreagent variation was comparable with that for fresh serum. In this way, we could show that, for short-term storage, once-frozen serum, sucrose-preserved serum, and some commercially available sera were more reliable than others. By appropriate rearrangement of the data we showed that some reagents or reagent/analyzer combinations were influenced by even the slightest denaturation of lipoproteins. For simple freezing and thawing, for instance, the reagents from Baxter, Du Pont, and Kodak were influenced by $>3\%$, albeit not always in the same direction.

The detrimental effect of lyophilization on the accuracy of the determination of total cholesterol has also been studied by the groups of Ross et al. (6), Kroll et al. (8, 9), and Naito et al. (10), whose studies revealed comparable reagent-related differences. Whereas some reagents, including those from Boehringer Mannheim, appear to have no or modest matrix-induced bias, others show considerable matrix error with lyophilized control sera. The reported errors (6, 8, 10), to a large extent, agree with our findings. The Du Pont/Dimension method group showed the largest matrix-induced bias $[-11.6\% (6), -14.3\% (8), -8.9\% (10), and -16.3\% (this publication; corrected data of Fig. 4)]$, followed by Beckman/Synchron $[-3.6\% (6), -5.5\% (10), and -11.1\% (this publication)]$. The negative matrix bias we saw for Roche reagents, $-11.4\%$, can be compared with the finding of Ross et al. (6) of $-4.4\%$.

Crucial in these observations is the conclusion that lyophilization as such induces matrix biases having different consequences for the various (enzymatic) reagents used in the total cholesterol assay. Demacker et al. (2), for example, already indicated that the activity and specificity of the cholesterol esterase used can be critical. Moreover, the cholesteryl esters in the lipoprotein core can be hydrolyzed only after disruption of the lipoproteins by a suitable detergent (23). The effect of the detergent may be sufficient when pancreatic cholesterol esterase is used but insufficient for complete hydrolysis by the bacterial esterase. Comparable information on the importance of the kind of cholesterol esterase is given in the studies of Noel et al. (24) and Tel and Berends (25).

The lyophilization of serum pools may affect some or all classes of lipoproteins. In the process of lyophilization, the freezing step is the most crucial one. As ice crystals are formed, the remaining interstitial fluid becomes overconcentrated; thus, the freezing cycle means a dehydrating process with its accompanying denaturing effect. After reconstitution, therefore, the lyophiliized pools are more turbid than untreated or frozen serum. The change in turbidity thus reflects the change in the size and shape of the lipoproteins. This denaturation gives rise to a decreased solubility, thereby hampering the availability of the lipoproteins for the enzymatic esterase and oxidase processes. Several studies with commercially available sera have shown that these kinds of phenomena play an important role. Kroll and Chesler (9) reported prolonged enzymatic reaction times before lyophilized specimens reach absorbance equilibrium and noted differing susceptibilities for the different enzymatic formulations used in the Du Pont acc and Technicon RA-1000 systems to have suboptimal reaction rates. They also commented on the optimal surfactant concentration in tests with lyophilized control material (26). The formulation of the Boehringer Mannheim reagent kit contains additional lipolytic enzymes, which may help in sufficiently disrupting also the denatured lipoproteins, thereby rendering this kit one of the most so-called matrix-robust methods. Finally, our present findings support earlier reports of Wieland and Seidel (16), Sgoutas and Tuten (12, 13), and Borque et al. (14) on the beneficial effect of sucrose as cryoprotectant during the freeze-drying process.

In conclusion, we have demonstrated the significant improvement of the interreagent variation in the determination of total cholesterol and the overall much closer resemblance of sucrose-protected lyophilized control serum to fresh or once-frozen serum, apparently, therefore, ameliorating the detrimental lyophilization effects. Accordingly, lyophilized control sera
with added sucrose may better serve the purpose of judging analytical accuracy in most routine cholesterol assay techniques than do unprotected control sera. In spite of having these tools for carrying out better intercomparability cholesterol studies, we still urge manufacturers to continue their search for developing and producing stabilized control material that will best mimic the characteristics of fresh human serum for use as calibrators. Only the fairly large-scale availability of such serum products can satisfy the need for cholesterol assays to be traceable to the necessary accuracy level as provided by official and recognized reference laboratories.

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