Lipids of Commercial Serum Products Offered as Controls or "Standards" for Cholesterol and Triglyceride Determinations

R. F. Witter, M. Kuchmak, J. H. Williams, Virginia S. Whitner, and Carole L. Winn

The cholesterol and triglyceride content of commercial control products (usually sera), purchased from six manufacturers, were measured. In addition, total fatty acid and qualitative neutral lipid composition of the total lipids were measured by gas-liquid and thin-layer chromatography, respectively. Cholesterol values we found by the Abell-Kendall method differed from the values given for six of the 12 assayed products by 6 to 37 mg/100 ml; triglyceride values, determined by a modified Carlson procedure, differed from the means given for two of the four assayed products by 14 to 17 mg/100 ml. Neutral lipids present in total lipids in 13 of 15 lyophilized samples, and fatty acid composition of the latter in 12, closely resembled those of freshly drawn human serum, indicating that the lipids had not deteriorated grossly. The lipids of one lyophilized product from a bovine serum source included an appreciable amount of linolenic acid, which is not found in human serum; a second contained an unknown lipid-solvent material. Some decomposition of the lipids was noted in a filtered liquid sample. These results strongly support the use of commercial lyophilized preparations as control sera, but not as secondary serum standards.

Additional Keyphrases Abell-Kendall method, cholesterol • Carlson method, triglycerides • control sera, deterioration • gas–liquid chromatography • thin-layer chromatography • fatty acid composition of sera • linolenate in bovine serum

Manufacturers offer a number of products as serum controls for quality-control programs or as serum "standards" for the clinical laboratory, and these preparations are used in many clinical laboratories. They include several commercial control products for the determination of serum total cholesterol and a few for the determination of serum triglycerides.

There is some question as to the usefulness of such preparations. First, how accurate are the cholesterol or triglyceride values assigned to assayed preparations by the manufacturer? Use of products with inaccurate assay values would lead to increased variability in results obtained within and among laboratories. Second, does the composition of such preparations resemble that of fresh human serum? This requirement seems a reasonable one, if a product is to be used to measure variability in analysis of constituents of human serum; it is also of particular importance in the determination of cholesterol or triglyceride, since various analytical procedures for these differ in specificity and in the method or the extent of separation of the given lipid from the complex mixture of serum lipids before the color reaction is applied. Therefore it would be desirable to know how closely the composition of the total lipids in such products resembles that of human serum. Furthermore, it would be useful to ascertain whether the lipids had deteriorated during processing of the commercial sera. Previous studies have shown that simple procedures such as storage of serum samples at refrigerator temperature result in the formation of free fatty acids, mono- and

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diglycerides, and the hydrolysis of triglycerides and various classes of phospholipids. At the same time, peroxidation of the fatty acids combined in the total lipids takes place (1), a process which should result in a decreased proportion of unsaturated acids.

Therefore, in the present study the various classes of neutral lipids present in the total serum lipids and the fatty acid composition of the latter were determined for a number of commercial preparations. The results were compared with those for a pool of fresh human serum. In addition, those products offered as cholesterol and (or) triglyceride controls or "standards" were analyzed in this laboratory for these constituents. The accuracy of the manufacturers' assay value was assessed by comparing the labeled values with those we found.

As far as we are aware, this is the first time the composition of the lipids and the accuracy of the stated triglyceride levels of commercial control serum have been studied. On the other hand, Logan and Allen (2) reported the accuracy of stated cholesterol concentrations for a group of assayed commercial serum preparations.

### Materials and Methods

#### Sources and Storage of Commercial Control Samples

The commercial control sera were purchased from laboratory supply houses or the manufacturers through the usual channels. The vials were refrigerated at 5°C. Lyophilized products were reconstituted with distilled water according to label directions. The products studied and their

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**Table 1. Analyses, in mg/100 ml, of Commercial Control Samples Offered for Determination of Cholesterol**

<table>
<thead>
<tr>
<th>No.</th>
<th>Company</th>
<th>Product designation</th>
<th>Mean cholesterol concn.</th>
<th>SD</th>
<th>Standard error of obsd. mean</th>
<th>Mfrs' stated acceptable limits</th>
<th>95% confidence limits of obsd. mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dade</td>
<td>Moni-trol II</td>
<td>138</td>
<td>3.6</td>
<td>0.8</td>
<td>128–148</td>
<td>136–140</td>
</tr>
<tr>
<td>2</td>
<td>Dade</td>
<td>Choles-trol</td>
<td>207</td>
<td>6.0</td>
<td>1.8</td>
<td>None</td>
<td>203–211</td>
</tr>
<tr>
<td>3</td>
<td>Hyland</td>
<td>Elevated Cholesterol Control Serum</td>
<td>406</td>
<td>10.1</td>
<td>2.7</td>
<td>390–430</td>
<td>400–412</td>
</tr>
<tr>
<td>4</td>
<td>Hyland</td>
<td>Special Clinical Chemistry Control Serum</td>
<td>162</td>
<td>4.5</td>
<td>1.3</td>
<td>170–190</td>
<td>159–165</td>
</tr>
<tr>
<td>5</td>
<td>Roselawn</td>
<td>Roselawn Serum</td>
<td>195</td>
<td>5.3</td>
<td>1.5</td>
<td>None</td>
<td>192–198</td>
</tr>
<tr>
<td>6</td>
<td>General Diagnostic</td>
<td>Versatol-A Alternate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>93</td>
<td>3.7</td>
<td>1.4</td>
<td>None</td>
<td>89–97</td>
</tr>
<tr>
<td>7</td>
<td>Dade</td>
<td>Moni-trol I</td>
<td>187</td>
<td>4.2</td>
<td>1.2</td>
<td>179–199</td>
<td>184–190</td>
</tr>
<tr>
<td>8</td>
<td>Dade</td>
<td>Choles-trol &quot;D&quot;</td>
<td>345</td>
<td>10.4</td>
<td>3.0</td>
<td>None</td>
<td>338–352</td>
</tr>
<tr>
<td>9</td>
<td>Hyland</td>
<td>Normal Clinical Chemistry Control Serum</td>
<td>176</td>
<td>3.3</td>
<td>0.9</td>
<td>195–215</td>
<td>174–178</td>
</tr>
<tr>
<td>10</td>
<td>General Diagnostic</td>
<td>Serachol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>370</td>
<td>6.4</td>
<td>2.5</td>
<td>None</td>
<td>364–376</td>
</tr>
<tr>
<td>11</td>
<td>General Diagnostic</td>
<td>Versatol-A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>94</td>
<td>3.0</td>
<td>1.2</td>
<td>None</td>
<td>91–97</td>
</tr>
<tr>
<td>12</td>
<td>Reese Foundation</td>
<td>Serum Standard and Control</td>
<td>200</td>
<td>5.0</td>
<td>1.3</td>
<td>None</td>
<td>197–203</td>
</tr>
<tr>
<td>13</td>
<td>Dade</td>
<td>Moni-trol I · X</td>
<td>185</td>
<td>5.3</td>
<td>1.5</td>
<td>...</td>
<td>182–188</td>
</tr>
<tr>
<td>14</td>
<td>Dade</td>
<td>Moni-trol II · X</td>
<td>154</td>
<td>4.0</td>
<td>1.1</td>
<td>...</td>
<td>151–157</td>
</tr>
<tr>
<td>15</td>
<td>Hyland</td>
<td>Abnormal Clinical Chemistry Control Serum</td>
<td>151</td>
<td>5.4</td>
<td>2.0</td>
<td>...</td>
<td>146–156</td>
</tr>
<tr>
<td>16</td>
<td>General Diagnostic</td>
<td>Versatol</td>
<td>116</td>
<td>4.3</td>
<td>1.2</td>
<td>...</td>
<td>113–119</td>
</tr>
<tr>
<td>17</td>
<td>Chek Lab</td>
<td>Sterile Human Serum</td>
<td>183</td>
<td>3.5</td>
<td>1.0</td>
<td>...</td>
<td>181–185</td>
</tr>
</tbody>
</table>

<sup>a</sup> When stated values based on more than one method were tested by a manufacturer, only the value based on the Abell–Kendall (1) method is given.

<sup>b</sup> Observed minus stated mean.

<sup>c</sup> Analysis of variance technique was used (12).

<sup>d</sup> Bilirubin present.

All products were lyophilized preparations of human serum except Products 2, 8, and 17, which were a solution of cholesterol in an alcohol, a lyophilized bovine serum fraction, and filtered sterile human serum, respectively.
corresponding identifying numbers are listed in Table 1.

Cholesterol and Triglyceride Methods

Total cholesterol was determined by the method of Abell et al. (3). Triglyceride was assayed by Carlson’s procedure (4). Standards at four concentrations and a control sample from a frozen pool prepared in this laboratory were run in duplicate with each run for these two classes of lipids. The standard for the determination of triglyceride was a mixture (molecular weight, 859) of trioilein and tripalmitin 2:1 by weight.

Duplicates were taken from six vials of each of the 17 preparations for the determination of serum cholesterol. These analyses were randomized in eight separate runs so that no more than one analysis in duplicate was performed in the same run on any product. A similar scheme was used for the four preparations with stated triglyceride values.

Gas–Liquid Chromatography

Serum lipids were extracted from the reconstituted or liquid sample by the method of Folch et al. (5), in which chloroform and methanol containing 5 mg of 2,6-di-tert-butyl-p-cresol per 100 ml is used to prevent oxidation of the lipids (6). The lipids were saponified with methanolic potassium hydroxide. The fatty acids were liberated with hydrochloric acid, extracted with petroleum ether, and esterified on a basic ion-exchange resin (sodium form, IRA-400, Amberlite) with anhydrous methanolic-hydrochloric acid. This procedure was adapted from that of Hornstein et al. (7, 8).

Gas–liquid chromatography was done on a Model 400 chromatograph (F & M Scientific Div., Hewlett-Packard Co., Avondale, Pa. 19311) equipped with a hydrogen-flame ionization detector and disc-chart integrator. The chromatographic column was packed with Chromosorb W impregnated with 17% ethylene glycol adipate. The identities of the fatty acids were established by directly comparing the retention time of the methyl esters in the lipid extract with those of reference compounds obtained from Applied Science Laboratories (State College, Pa. 16801). The data were calculated as weight percent, which is proportional to the peak area when a flame-ionization detector is used (9).

Two vials of each commercial control sera and fresh pooled human sera were used in these experiments. A single extract was prepared from each vial, and the fatty acids were analyzed in duplicate.

Thin-Layer Chromatography

The lipid extracts were chromatographed on thin-layer plates prepared according to Stahl (10). We coated the plates with silica-gel G (Brinkmann Instruments, Westbury, N.Y. 11590) in layers 250-μ thick. The equivalent of 50 μl of serum was spotted for chromatography in hexane-diethyl ether–acetic acid, 80:20:1.5 by volume, for separation of the neutral lipids. After development, the spots were made visible by spraying the plate with sulfuric acid–dichromate and heating to 180°C for 30 min (11).

Results

Cholesterol Concentrations of Commercial Control Sera

Cholesterol concentrations were determined on 17 control sera offered by six manufacturers (Table 1).

Five products were unassayed by their manufacturers. The mean cholesterol levels found in this laboratory for these five control sera (hereafter designated “observed mean”) ranged from 116–185 mg/100 ml with four of the five products in the range of 151–185 mg/100 ml. The manufacturers’ stated mean values for the 12

<table>
<thead>
<tr>
<th>No.</th>
<th>Company</th>
<th>Product designation</th>
<th>Mean triglyceride level</th>
<th>SE, obsd. mean, mg/100 ml</th>
<th>95% confidence limits of obsd. mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dade</td>
<td>Moni-trol II</td>
<td>55</td>
<td>3.4</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>Hyland</td>
<td>Special Clinical Chemistry Control Serum</td>
<td>63</td>
<td>3.1</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>Dade</td>
<td>Moni-trol I</td>
<td>107</td>
<td>4.3</td>
<td>1.2</td>
</tr>
<tr>
<td>12</td>
<td>Reese</td>
<td>Serum Standard and Control</td>
<td>111</td>
<td>3.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* All products were prepared from lyophilized human serum. The triglyceride levels used to determine the observed mean were calculated assuming a molecular weight of 859 for the serum triglyceride. No acceptable limits stated by the manufacturer.

* Observed minus stated mean.

* Standard deviation represents the variability of a single analysis repeated on different days.

* Analysis of variance technique (12) was used.

* Stated values are averages of several determinations, not the results of numerous replicate analyses as are the stated mean values given for other constituents.

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sayed control sera (hereafter designated “stated mean”) encompassed a wider range of concentration, from 88–410 mg/100 ml. Three stated means were in the range of 88–137 mg/100 ml, six were in the range of 180–214 mg/100 ml, and another three ranged from 356–410 mg/100 ml.

The stated means obtained by the manufacturer and the observed mean cholesterol concentration that we found are compared in Table 1. Disregarding plus or minus signs, the difference between the two sets of means ranged from 0.7 to 14% of the stated mean or from 1–37 mg/100 ml. Four of the stated means differed from the observed means by 1–2 mg/100 ml, two by 3–4 mg/100 ml, four by 6–18 mg/100 ml, and two by 29–37 mg/100 ml. Stated means that were within the 95% confidence limits of their respective observed means were not considered significantly different from the observed means. By this criterion, the stated values for six products differed significantly from their observed means: the differences ranged from 6–37 mg/100 ml.

Triglyceride Concentrations of Commercial Control Sera

The triglyceride levels of the four commercial control sera in which a mean value for this constituent was given are listed in Table 2. The stated mean values ranged from 52–110 mg/100 ml; the observed means differed from the manufacturers’ stated means by 1–17 mg/100 ml or by 1 to 21%. If the criterion given in the section on cholesterol content is applied, stated values of two of the four products differed significantly from their observed means.

Fatty Acid Composition of the Total Lipid of Commercial Control Sera

The fatty acid composition of 15 of the 17 commercial control products is listed in Table 3. For comparison, we give the fatty acid composition for the total lipids of pooled fresh human serum analyzed at the same time as the commercial products.

The fatty acid composition of the total lipids of the lyophilized products made from human serum was similar to that of fresh serum from nonfasting persons (no. 18). Saturated fatty acids constituted about one-third of the acids, with palmitate dominating in this group. About equal amounts of the major unsaturated acids oleate and linoleate were present. Their combined contribution to the total fatty acid present was about 57%. The fatty acid composition of the total lipids in these sera
agrees with that reported in the literature for human serum by other workers (13, 14). Chek Lab's "Sterile Human Serum," no. 17, a filtered pooled human serum, contained less polyunsaturated acids than did the corresponding lyophilized or fresh serum. The concentration of arachidonate was only about half and of linoleate about two-thirds that in lyophilized sera or pooled fresh human serum. Furthermore, the content of both saturated or mono-unsaturated fatty acids was higher in the Chek Lab product (no. 17) than in the other human serum samples.

The fatty acid composition of the total lipids of Dade's "Choles-trol D" (no. 8), a lyophilized preparation from bovine serum, did not resemble that of any of the human sera analyzed. For example, only about half the concentration of palmitate and oleate, about one-fourth as much arachidonate, but twice as much stearate were present as is found in the total lipid of normal human serum. The most striking difference was the appearance of 13.8% linolenate in the bovine lipid as contrasted to none in the human serum lipid. The identity of this acid was confirmed by co-chromatography with reference methyl linolenate.

Thin-Layer Chromatography of Lipid in Commercial Control Sera

The results of thin-layer chromatography of 15 of the commercial products derived from serum are shown in Figure 1. The chromatograms illustrate that the qualitative composition of the neutral lipids of most of the samples resembles that of human serum. Dade's "Choles-trol D" (no. 8) was an exception, since no triglyceride spot was detected. In addition, with General Diagnostics' "Serachol" (no. 10) an additional spot was seen, with an Rf somewhat greater than that of triglycerides. The spot did not appear in any of the other samples, including the fresh serum. During the extraction of this lot of "Serachol" (no. 10), foaming and emulsion formation were noted. Whether the unknown component is an emulsifying agent added to solubilize the lipids is not known. Two faint spots running with the Rf's of mono- and diglyceride also were noted with this sample. The chromatograph of Chek Lab's "Sterile Human Serum" (no. 17) showed that this sample contained more free fatty acids than either the control serum or the other lyophilized samples.

Discussion

The stated cholesterol levels of six of the 12 commercial products and the triglyceride concentrations of two of the four assayed preparations differed significantly from the values found in this laboratory. Logan and Allen (2) also noted such differences in control products offered for cholesterol determinations.

We saw no correlation between concentrations of cholesterol and (or) triglyceride and the size of the differences between their observed and stated mean values. Also, the difference did not correlate with the method used for assay by the manufacturer. Complete references for the methods of analysis were given for only five of the 12 cholesterol products and for none of the triglyceride preparations. Also, triglyceride values were given as "mg percent" rather than as molar units. Hence, some of the differences between stated and observed values could have been caused by differences between the molecular weight of the triglyceride

![Fig. 1. Thin-layer chromatography of neutral lipids](image-url)

Numbers correspond to those in Table 1. The extracts of these samples were spotted in random order; hence, the numbers are not in sequence. Symbols are PL, phospholipids; CH, cholesterol; FFA, free fatty acids; TG, triglycerides; CHE, cholesteryl esters; BHT, 2,6-di-tert-butyl-p-cresol; FS, fresh pooled human serum; ST, reference lipid mixture.
standards employed by the manufacturer and the present investigators. Certainly the molecular weight of the triglyceride standard either should be supplied or the concentration expressed in molar units.

Our results do not support the use of commercial serum "standards" for the determination of cholesterol or triglyceride. Differences in accuracy of stated values among manufacturers alone could lead to marked variation in results. For example, the stated cholesterol values of the lots of Hyland's "Elevated Cholesterol Control Serum" (no. 3), Dade's "Cholesterol D" (no. 8), and General Diagnostics' "Serachol" (no. 10) studied, which are in the range of 350 and 400 mg/100 ml, differ by -4, -37, and +14 mg/100 ml, respectively, from the observed values. Thus if an unknown were analyzed on three successive days with one of each product used as a standard in the order named, the values obtained would be 33 mg/100 ml more the second day and 18 mg/100 ml less the third day than was observed on the first day, just from the change in "standards."

Caraway (15) has suggested that assayed products could be used as a guide in setting up methods if products from two companies were used. Our results would not support this proposal.

Other workers also have concluded that assayed commercial control samples should not be used as standards (16). Radin (17), and more recently, Logan and Allen (2) have discussed the role of primary and secondary serum standards in clinical chemistry and the objections to the use of serum "standards." In some instances, the clinical chemist must use secondary serum standards since the serum constituent to be analyzed cannot be obtained in pure form. However, this is not the case with cholesterol and triglyceride since suitable primary standards are available from commercial sources. In addition, the National Bureau of Standards now issues a primary cholesterol standard.

The neutral lipids present in the total lipids in 13 and the fatty acid composition of the latter in 12 of 15 lyophilized sera closely resembled those of freshly drawn human serum, indicating no gross deterioration of the lipids. Furthermore, no difference was noted in the qualitative composition of the lipids between assayed and unassayed products, although the latter did appear to have a low triglyceride content when examined by thin-layer chromatography. None of the unassayed products were offered as control sera for the triglyceride determination. These results strongly support the use of these lyophilized preparations as quality-control sera for cholesterol determinations.

On the other hand, the composition of the total lipids of three products showed some differences from that of fresh human serum. One lyophilized preparation from a bovine source contained linolenic acid, a second an unknown material that was soluble in lipid solvents. A liquid product, a filtered human serum, had an increased level of free fatty acids and a decreased amount of polyunsaturated acids, indicating some decomposition of the lipids.

These results do not support the use of the three products as quality-control sera, at least as far as lipid composition is concerned. On the other hand, it remains to be proved whether the differences noted in lipid composition would influence results obtained in various methods for cholesterol and (or) triglyceride. It seems reasonable, however, to avoid the use of materials containing unknown additives or grossly decomposed lipids.

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References