Continuous-Flow Enzymatic Determination of Total Serum Cholesterol and Method Standardization with CDC-Calibrated Pooled Sera

M. A. MacAulay, C. L. Jacklyn, J. M. Mathers, and V. A. Storm

We compared Boehringer Mannheim's enzymatic kit for the continuous-flow (AutoAnalyzer II) determination of serum cholesterol with Technicon's N-24a extraction method. Results for patients' samples analyzed by the enzymatic method were higher than those by the comparison method. To evaluate accuracy in the cholesterol determinations, we enrolled the enzymatic method into the Center for Disease Control's (CDC) Lipid Standardization program. We calibrated the method by use of a pooled sera for which cholesterol content was assigned by CDC after analysis by their reference Abell-Kendall procedure. We discuss the difficulties with available calibration material and limitations in the application of some commercial control materials to the enzymatic cholesterol method. The continuous-flow variables, Michaelis-Menten constants, percent ester-hydrolase activity, and other factors affecting the performance of the enzyme-linked cholesterol method are evaluated. We believe pooled sera with an assigned value for cholesterol content is the best calibrator material.

Additional Keyphrases: extraction procedure compared - enzymatic methods - quality control - calibration materials - steroids - analytical error

When we decided to replace our AutoAnalyzer I (AAI) cholesterol system with an enzymatic procedure, the choice of an automated method was limited to one manufacturer, Boehringer Mannheim Co., Canada Ltd. In their method, cholesterol esterase (EC 3.1.1.13, sterol-ester acylhydrolyase) catalyzes the hydrolysis of cholesterol esters to free cholesterol. Cholesterol is then oxidized by use of cholesterol oxidase (EC 1.1.3.6, cholesterol: oxidoreductase) to 4-cholesten-3-one and hydrogen peroxide. The latter is oxidatively coupled to 4-amino-phenazone in the presence of phenol to yield a quinonemine dye (1), the color intensity of which is measured at 505 nm.

Reports in the literature (2, 3) suggested that the enzymatic cholesterol methods are more accurate and precise, and faster, than the traditional cholesterol procedures (4). Increased sample rates imply decreased sample interaction (5), and we believed that the enzyme method would lend itself well to the technique of curve regeneration (6-9). This would, as additional benefits, further increase the sampling rate, decrease the frequency with which samples had to be repeated, and decrease the expense of the enzymatic method by decreasing reagent consumption.

The initial analytical-cartridge design used for enzymatic cholesterol measurements was as outlined by BMC.2 Later, with the advent of Technicon's hydraulic design, we adopted their manifold system. Results with this Technicon hydraulic-BMC enzymatic reagent system were compared with those by the Technicon N-24a (AAI) extraction method we were using. We spent considerable time and effort in selecting an adequate calibration standard for the enzymatic method. This was (9) and apparently still is (10) the major pitfall of enzymatic cholesterol methods.

Here, we report our comparison data. We also discuss shortcomings of the enzymatic method and steps we took to improve its accuracy and precision.

Method and Materials

Equipment

For the initial comparison, we used Technicon's AAI extraction method (N-24a). The enzymatic method was run on a standard AAI system. The analytical cartridge, constructed in this laboratory, made use of the hydraulic design outlined for Technicon's method SE4-0040PC6. We kept all hydraulic connections as short as possible, to obtain good wash characteristics (5). Polyethylene tubing (i.d., 0.034 inches) was used for the sample line; 1.40 mL/min was pumped through the flow-back tube. Sample rate was 60 samples per hour; sample-to-wash ratio was 4/1.

Calibration Material

To calibrate the extraction method, we obtained cholesterol standards (in isopropanol) from BDH Chemicals, Toronto, Ontario M8Z 1K5. For initial calibration of the enzymatic method, we tested three calibration materials: NBS cholesterol material (SRM no. 911a); Technicon's SMA reference T material (No. T03-0912-60), and pooled sera prepared in this laboratory. From NBS material we prepared a 2.000 g/L cholesterol standard for single-point calibration, as follows.

Quantitatively transfer 200 mg of dried NBS (desiccated at 0 °C) cholesterol into a 100-mL volumetric flask. Rinse the weighing vessel with 2-3 mL of chloroform and allow the washings to run into the volumetric flask. Swirl the contents of the flask under a stream of hot water (60-70 °C) until all the cholesterol is dissolved. Add 20-30 mL of surfactant (Pegosperse) and swirl the flask for 5-10 min. Add 20 mL of sodium taurocholate (250 g/L) and swirl to mix. Dilute to volume with saline (9 g/L), swirling the flask during its addition. Mix well and store in a glass container at 4 °C.

The computer-assisted system was calibrated with pooled sera that had been assayed for cholesterol by the Center for

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1 Nonstandard abbreviations used: AAI, AutoAnalyzer I; AAI, AutoAnalyzer II; BMC, Boehringer Mannheim Corp., Canada Ltd.; NBS, National Bureau of Standards, Washington, DC 20254; CDC, Center for Disease Control, Atlanta, GA 30333; CAP, College of American Pathologists, Traverse City, MI 49684; and SRM, Standard Reference Material.


3 The saturated cholesterol esters (stearate and palmitate) were difficult to prepare in high concentrations. "Pegosperse," the trade name for a Technicon serum-clearing agent (Product T01-0879), apparently can maintain cholesterol and esters in an aqueous micellar dispersion. It is a solid at 25 °C, but dilute aqueous solutions can be maintained at −4 °C.
Disease Control, Atlanta, GA 30333. This material was prepared in bulk, apportioned into test tubes, and stored at -20 °C. On analysis days, the calibration pool was thawed at room temperature for 30 min, thoroughly mixed, and transferred to the sample plate to serve as a calibrator.

Procedure

Figure 1 shows the flow diagram for the enzymatic cholesterol method. During the initial comparison phase, we determined serum cholesterol in quality-control material and in patients' samples over three different periods, using different calibration materials for standardization. The calibration set-point concentration for the NBS material was 2.000 g/L. The value for the pooled sera was determined by multiple measurements with the AAI extraction method. We also compared the three calibration materials with regard to linearity of the method, analytical recovery, percent sample interaction, percent ester hydrolysis, effect of high concentrations of bilirubin, and effect of BDH serum separator filter tubes on the enzymatic method.

Because inadequacies of the available commercial material for both calibration and control of the enzymatic system became apparent, we asked CDC for advice on the standardization of the enzymatic procedure and were accepted into their Lipid Standardization Program. In the short term, this satisfactorily solved our immediate difficulties with the method; in the long term, this surveillance of our system would ensure that standards for precision and accuracy would be maintained. CDC's program consisted of three phases: during Phase I our results, with use of the secondary standard (pooled sera), were aligned with those obtained by CDC's reference (Abell-Kendall) method. In Phase II we analyzed unknown samples to establish short-term accuracy and precision. In Phase III, we analyzed 120 unknown samples at five concentrations during 15 weeks, to obtain insight into long-term precision and accuracy; the concentrations reported for these blind samples had to be within CDC's specifications for precise and accurate measurements. Using the computer-assisted enzymatic cholesterol method, we successfully completed the standardization program in about 14 months.

Kinetic Measurements

In determining the Michaelis–Menten constant for the cholesterol oxidase and cholesterol esterase reactions, we obtained from Boehringer Mannheim Corp. cholesterol oxidase prepared from Nocardia erythropoda and cholesterol esterase prepared "from microorganisms".4 We also determined the $K_m$ value by using cholesterol oxidase from myco-

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4 Boehringer Mannheim does not reveal the source of their cholesterol esterase.
Table 1. Results for Some Quality-Control Materials as Analyzed by Technicon's N-24a Extraction Method and the BMC Enzymatic Method Standardized with Three Different Calibration Materials

<table>
<thead>
<tr>
<th></th>
<th>Given value</th>
<th>Extraction method</th>
<th>Enzymatic method</th>
<th>Pooled sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>QAS II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} ), g/L</td>
<td>1.340</td>
<td>1.339</td>
<td>1.495</td>
<td>1.349</td>
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<tr>
<td>SD, mg/L</td>
<td></td>
<td>136.1</td>
<td>71.4</td>
<td>64.6</td>
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<tr>
<td>CV, %</td>
<td></td>
<td>10.16</td>
<td>4.77</td>
<td>4.79</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>39</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>Pooled sera</td>
<td></td>
<td>1.950</td>
<td>1.936</td>
<td>2.115</td>
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<td>SD, mg/L</td>
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<td>87.5</td>
<td>97.4</td>
<td>107.0</td>
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<tr>
<td>CV, %</td>
<td></td>
<td>4.51</td>
<td>4.61</td>
<td>5.06</td>
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<tr>
<td>n</td>
<td></td>
<td>62</td>
<td>33</td>
<td>50</td>
</tr>
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<td>Validate EL</td>
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<td>3.560</td>
<td>3.586</td>
<td>3.182</td>
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<td>SD, mg/L</td>
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<td>174.7</td>
<td>144.6</td>
<td>172.9</td>
</tr>
<tr>
<td>CV, %</td>
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<td>4.87</td>
<td>4.54</td>
<td>5.09</td>
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<tr>
<td>n</td>
<td></td>
<td>33</td>
<td>14</td>
<td>30</td>
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<tr>
<td>Precilip</td>
<td></td>
<td>1.380</td>
<td>1.476</td>
<td>1.433</td>
</tr>
<tr>
<td>SD, mg/L</td>
<td></td>
<td>56.6</td>
<td>176.4</td>
<td>70.0</td>
</tr>
<tr>
<td>CV, %</td>
<td></td>
<td>3.83</td>
<td>12.3</td>
<td>4.68</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>26</td>
<td>12</td>
<td>34</td>
</tr>
</tbody>
</table>

* Extraction method with multi-point cholesterol in isopropanol standards (0–4.000 g/L).

b Enzymatic method used single-point calibration. Set-point concentrations were: NBS SRM 911a, 2.000 g/L; SMA Reference T, 1.440 g/L; and pooled sera 1.916 g/L.

coil in their manifold design (incubation interval, 5.3 min). For these reasons, we adopted Technicon's manifold design for enzymatic cholesterol determination.

Table 1 shows the results obtained by analyzing quality-control materials by the AAI extraction method and by the AAII system with BMC enzymatic reagents for serum cholesterol determinations. Results are given for cholesterol in control samples measured with each of the three calibration materials. The enzymatic method gave improved precision over that obtained by our automated extraction method. Accuracy in measurement, as judged by the closeness to the extraction-determined value, was acceptable for the low-level control. The mean for the intermediate concentration (1.986 g/L) was higher than that obtained by the extraction method (1.936 g/L). In contrast, the mean result for the enzymatic method at the high concentration (3.260 g/L) was lower than that obtained by the extraction method (3.586 g/L). Table 1 shows that the accuracy of the extraction method was satisfactory; we found good agreement between the extraction-determined values and the manufacturer's assigned value for each material tested.

Patient-sample comparisons (Table 2, Figure 3) showed that our enzymatic method gave higher results for cholesterol than those obtained for the AAI extraction method, even when the enzymatic calibration material had its mean (1.916 g/L) value assigned by our extraction method. When we calibrated the enzymatic method with SMA reference T material (Figure 3B), we noted increased dispersion of data points in the intermediate- and high-concentration regions. We believe this

Fig. 3. Comparison of serum cholesterol data for reference material and patients' sera analyzed by Technicon's N-24a extraction method (AAI) and Boehringer-Mannheim's enzymatic cholesterol method (AAII)

Enzymatic method calibration material: A, NBS (911a) material (2.000 g/L); B, Technicon SMA reference material (1.440 g/L); C, pooled sera (1.916 g/L).
reflected the low concentration (1.440 g/L) of cholesterol in this material and concluded it was unacceptable for calibration purposes.

We also used analytical recovery experiments to judge the accuracy of our enzymatic cholesterol method. Using an albumin-based lyophilized control (3.420 mg/L) and patients’ sera, we measured 43 analytical recoveries during a month. The mean recovery was 103%, with a range of values from 92–112.5%. For 15 recovery experiments performed by the extraction cholesterol method the mean was 97.2%, the range 93–101%.

During the developmental stage, we noted that certain commercial controls were not suitable for use in making judgments regarding the accuracy of the enzymatic cholesterol method. The enzymatically determined mean of 1.514 g/L (SD, 0.661; n, 14) for Technicon’s SMA reference serum (T01-8050) was 20–30% lower than the mean of 2.165 g/L (SD, 0.961; n, 16) determined by the extraction method. General Diagnostics’ serum cholesterol calibration material, “Serachol,” which contains cholesterol (4.610 g/L) primarily in the form of the acetic ester (81.8%), gave extremely low results when estimated by the enzymatic method. We obtained means of 2.530 g/L (SD, 0.215; n, 15) by the enzymatic method and 4.500 g/L (SD, 0.260; n, 14) by the extraction method for this material. Cholesterol esterase attacks the acetate ester extremely slowly (10), resulting in a significant underestimate of total cholesterol. We also noted that high concentrations of organic solvents (isopropanol, ethanol), used in calibration materials to solubilize cholesterol, changed the flow-characteristics (streaming) of the hydraulic components of the automated system and invariably led to atypical peak tracings.

Abnormally high bilirubin concentrations reportedly (10, 12) have various effects on cholesterol as determined by enzymatic methods. Figure 4 shows the effect of bilirubin on cholesterol as determined by our enzymatic method. We added aliquots of Dade Bilirubin Standard (B5132) to a sample of a clear, non-icteric serum to obtain a curve for bilirubin-in-cholesterol, covering the concentration range 0.093–1.860 g of cholesterol and 0–41.7 mg of bilirubin per liter. Concurrently, we analyzed a blank curve (sample + water). The presence of 10 mg of bilirubin increased the observed cholesterol concentration by 35 mg/L.

The linearity of our enzymatic cholesterol method was established by analyzing a series of aqueous-based standards (in Pegosperse) and by analyzing abnormal patients’ samples covering the concentration range 0.50 to 4.000 g/L. Absorbance and concentration were linearly related to at least 4.000 g of cholesterol per liter.

We studied the effect of sample hemolysis on cholesterol results by analyzing 28 patients’ samples in duplicate. To promote hemolysis we added two drops of water to one of the duplicates; as control, we added two drops of physiological NaCl solution (0.15 mol/L) to the other. After serum separation, the pairs were analyzed concurrently. The mean cholesterol concentration for the hemolyzed samples was 2.274 g/L; the mean for the samples treated with NaCl solution was 2.218 g/L. A paired Student’s t-test (t = −4.113) showed that this difference was statistically significant. Witte et al. (2), using Abbott enzymatic reagents and the Abbott Bichromatic Analyzer (ABA-100), and Pesce et al. (12), using BMC reagents and the Centrifichem centrifugal analyzer, reported no hemoglobin interference when erythrocyte lysate preparations were added to pooled sera. In contrast, our data suggest that sample hemolysis increases apparent serum cholesterol concentration.

### Table 3. Accuracy and Precision for BMC Enzymatic Cholesterol Method for Serum Samples Supplied by CDC

<table>
<thead>
<tr>
<th>Program Phase I</th>
<th>mg/L</th>
<th>n</th>
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<tbody>
<tr>
<td>1250 ± 70</td>
<td>1240</td>
<td>≤70.0</td>
</tr>
<tr>
<td>2690 ± 130</td>
<td>2680</td>
<td>≤80.0</td>
</tr>
<tr>
<td>3210 ± 160</td>
<td>3280</td>
<td>≤90.0</td>
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</table>

<table>
<thead>
<tr>
<th>Program Phase II</th>
<th>mg/L</th>
<th>n</th>
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<tbody>
<tr>
<td>1620 ± 80</td>
<td>1620</td>
<td>≤70.0</td>
</tr>
<tr>
<td>2440 ± 120</td>
<td>2360</td>
<td>≤80.0</td>
</tr>
<tr>
<td>3460 ± 170</td>
<td>3370</td>
<td>≤90.0</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Program Phase III</th>
<th>mg/L</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>1270 ± 70</td>
<td>1290</td>
<td>≤70.0</td>
</tr>
<tr>
<td>1620 ± 80</td>
<td>1690</td>
<td>≤70.0</td>
</tr>
<tr>
<td>2020 ± 100</td>
<td>2080</td>
<td>≤80.0</td>
</tr>
<tr>
<td>2440 ± 120</td>
<td>2410</td>
<td>≤80.0</td>
</tr>
<tr>
<td>3460 ± 170</td>
<td>3440</td>
<td>≤90.0</td>
</tr>
</tbody>
</table>

* Calibration material was pooled sera (1860 mg/L), value assigned by CDC’s (Abell–Kendall) method. Sampling rate 72 h, sample-to-wash ratio was 4/1.
CDC Standardization

Being unable to obtain the reported relationship (2) between results by our enzymatic and extraction methods, we sought the advice of CDC regarding standardization of the enzymatic cholesterol method. CDC assigned a value (1.860 g/L) to our pooled-sera calibrator and we were accepted into their cooperative cholesterol and triglyceride Standardization Program. Table 3 shows our results for the initial three phases of their program. Our method precision for the Phase I intermediate-concentration (2.690 g/L) and high-concentration (3.210 g/L) cholesterol samples were both outside of CDC’s acceptable limits, but the accuracy was acceptable at the three concentrations tested. Results for Phase II showed acceptable precision for the low and intermediate concentrations; precision for the high concentration (3.460 g/L) remained outside of CDC’s acceptable range. We analyzed 120 samples covering five different concentrations during over a 15-week period in Phase III of the program. Results for both Phases II and III were blind to participants. Our accuracy for each of the five concentrations was within CDC limits. However, the precision for the 2.440 g/L concentration was outside of CDC’s acceptable range—though this was a result of our measuring system or due to inherent difficulties with the sample is not known (our precision for triglycerides at this concentration was also outside the acceptable limits). Our laboratory completed the cholesterol standardization program in 14 months and we were certified by CDC as meeting their criteria for cholesterol standardization.

The major corrective action required to maintain adequate precision with BMC’s cholesterol reagents was to control standard drift. This problem was evident in our initial experimental work and the cause remains unknown. To cope with the situation, we programmed our computer with a drift-correction routine. Whether or not this technique has actually improved our system, we cannot yet say.

Continuous-Flow Variables

We determined the magnitude of sample-to-sample interaction (5) for the enzymatic cholesterol method by running interaction test patterns during 10 days. We estimated the mean percent carryover at a cholesterol concentration of 1.400 g/L to be +0.2%. Our estimates of continuous-flow variables, the lag (a) and exponential (b) phases, are shown in Table 4. Using the mean estimate of the exponential variable (3.32 s) we calculated (5) the maximum sampling rate (<1.0% carryover) to be 93 samples/h. Contrary to our experience (8) with other systems, the lag phase (12.04 s) was larger than the exponential variable. This implies that hydraulic components other than the sample line and flow-cell contribute significantly to the total sample interaction (5) in the system.

Kinetic Measurements

We prepared an aqueous-based (including surfactant) standard containing cholesterol and cholesterol esters. This standard contained, per liter, 414 mg of cholesterol, 431 mg of cholesteryl oleate, 179 mg of cholesteryl palmitate, and 274 mg of cholesteryl linolate. The total cholesterol content was 1,790 g/L. We used this standard to evaluate the percent hydrolysis and thereby the activity of cholesterol esterase in the

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Table 4. Estimates of Continuous-Flow Variables from Rise Curves of Steady-State Tracings BMC Enzymatic Cholesterol Method with Hydraulics of AAII

<table>
<thead>
<tr>
<th>Trial no.</th>
<th>a</th>
<th>Time, s</th>
<th>b</th>
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<tbody>
<tr>
<td>1</td>
<td>16.8</td>
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<tr>
<td>2</td>
<td>9.4</td>
<td>10.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>7.9</td>
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<td>4</td>
<td>10.6</td>
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<td>5</td>
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<td>8</td>
<td>12.3</td>
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</tr>
<tr>
<td>9</td>
<td>12.6</td>
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</tr>
<tr>
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<tr>
<td>x̄</td>
<td>12.04</td>
<td>8.32</td>
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<tr>
<td>SD</td>
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<td>0.82</td>
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<tr>
<td>CV, %</td>
<td>23.58</td>
<td>9.86</td>
<td></td>
</tr>
</tbody>
</table>

*One variable estimate per day.*

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Fig. 5. Analytical recovery of cholesterol for various concentrations of cholesterol esters analyzed by the automated enzymatic method and read from the CDC standardized pooled-sera calibration curve

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Fig. 4. Effect of bilirubin on serum cholesterol concentrations determined by the AAII system with BMC enzymatic reagents. △, bilirubin present; O, no bilirubin
### Table 5. Michaelis–Menten Constants for Cholesterol and Cholesterol Esters

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme</th>
<th>Source (and supplier)</th>
<th>Michaelis–Menten constant, $\text{mol/L} \times 10^{-5}$</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Cholesterol oxidase</td>
<td><em>N. erythropolis</em> (BMC)</td>
<td>1.1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol oxidase</td>
<td><em>M. smegmatis</em> (Miles)</td>
<td>6.3</td>
</tr>
<tr>
<td>Cholesteryl oleate</td>
<td>Cholesterol esterase</td>
<td>Microorganisms (BMC)*</td>
<td>3.9</td>
</tr>
<tr>
<td>Cholesteryl oleate</td>
<td>Cholesterol esterase</td>
<td>Beef pancreas (Miles)</td>
<td>1.3</td>
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<td>Cholesteryl linoleate</td>
<td>Cholesterol esterase</td>
<td>Microorganisms (BMC)</td>
<td>5.1</td>
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<td>Cholesteryl acetate</td>
<td>Cholesterol esterase</td>
<td>Microorganisms (BMC)</td>
<td>8.2</td>
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</table>

* Substrates prepared in solution containing, per liter, 20 mL of chloroform, 250 mL of Pegosperse, 200 mL of sodium taurocholate solution (100 g/L), and 530 mL of distilled and de-ionized water.

Enzymatic method. We calculated percentage esterase activity according to the equation

$$\% \text{ activity} = \frac{[\text{determined cholesterol (g/L)} - 0.414 \text{ g/L}]}{[\text{given cholesterol (g/L)} - 0.414 \text{ g/L}]} \times 100.\text{.}$$

The mean recovery was 95%. Evidently the major esters of cholesterol in serum are quantitated.

In another experiment, we prepared calibration curves (0–4.000 g/L) by use of various cholesterol esters and determined their cholesterol concentrations by the enzymatic method, using CDC standardization pooled sera for method calibration. We used the expression

$$\% \text{ recovery} = \frac{([\text{determined cholesterol (g/L)} \times \text{ester}] - [\text{theoretical cholesterol (g/L) in ester}])}{[\text{theoretical cholesterol (g/L) in ester}]} \times 100.\text{.}$$

to obtain the percentage cholesterol accounted for, for each ester concentration on the respective calibration curve (Figure 5). Under our conditions, the mean analytical recovery for cholesteryl oleate was 99.4%. Cholesteryl acetate gave the poorest recovery; only 16.6% of the ester was measured, a finding that agrees with that of Chu et al. (10), who previously showed that cholesteryl acetate requires longer incubation than do free or other cholesterol esters for complete color development. Figure 5 also shows the recoveries for a calibration curve prepared using NBS SRM No. 911a cholesterol. Results for serum cholesterol from this curve were higher than those obtained when we calibrated with CDC standardized pooled sera.

Table 5 gives our results for determination of the Michaelis–Menten constant for cholesterol oxidase and cholesterol esterase. The $K_M$ determined for the cholesterol oxidase reaction with use of BMC’s cholesterol oxidase (from *Nocardia erythropolis*) was $1.8 \times 10^{-5}$ mol/L. Using BMC’s cholesterol esterase (microbial source) we determined $K_M$ values $3.9 \times 10^{-5}$, $5.1 \times 10^{-5}$, and $8.2 \times 10^{-4}$ mol/L when the substrates were cholesteryl oleate, -linoleate, and -acetate esters, respectively. Of the substrates tested, cholesteryl acetate gave the largest $K_M$ value. The affinity of cholesterol esterase for this substrate is considerably less than its affinities for the oleate and linoleate esters of cholesterol and this supports our findings for the ester response curves illustrated in Figure 5.

### Discussion

Enzymatic cholesterol methods are less tedious than extraction methods. Their inherent simplicity and specificity, the increased analysis rate, and the use of non-corrosive reagents have perhaps been responsible for their popularity.

In view of these attributes, we expected better accuracy and precision as compared with our extraction method. Our accuracy did improve, but we were only able to maintain long-term precision by devoting considerable time and effort to policing the enzymatic method. A careful scrutiny of the measurement conditions revealed no methodological troubles other than intermittent drift in the calibration standard. Long-term analyses of CDC samples suggest that our attempts to correct this problem met with only marginal improvement in reproducibility for high cholesterol concentrations.

We experienced difficulty in assessing the accuracy of the enzymatic method in the early stages of development, largely owing to the lack of adequate calibration materials and the many subtle demands of the method. Our report indicates that most reported difficulties (4, 9) attributed to extraction methods, such as method standardization, bilirubin interference, and variability in reaction rates of cholesterol esters, also apply to enzymatic methods.

Cholesterol oxidase and cholesterol esterase can be denuetred by many of the more commonly used extraction calibration materials. To obviate this, techniques have been developed to allow preparation of essentially aqueous-based cholesterol calibration materials. Micellar dispersions of cholesterol or its esters can be maintained by using wetting agents such as Triton X-100 and Pegosperse. We believe these matrix-type calibration materials have a definite use in the research laboratory, but their composition and behavior are so dissimilar to human serum that we do not believe they should be used in the clinical laboratory.

Having evaluated several different types of calibration materials for standardization of the enzymatic cholesterol method, we recommend pooled sera, for which the cholesterol concentration has been assigned by a reputable reference method. Our use of pooled sera in conjunction with CDC’s Lipid Standardization Program has demonstrated that Boehringer-Mannheim’s enzymatic cholesterol method is capable of yielding acceptable long-term accuracy in measurement.

Reference to the 1977–78 surveys conducted by the College of American Pathologists (CAP) vividly demonstrates the rather wide range of cholesterol estimates obtained by enzymatic methods. Precision estimates are also, on the average, not as good as those obtained by the extraction or direct cholesterol methodologies. There appear to be as many variations of the enzymatic cholesterol method as there are of the extraction or direct cholesterol procedures. Overall, enzymatic cholesterol method performance as indicated by the CAP data suggests that the underlying reason for the popularity of these procedures is the more agreeable procedure rather than their accuracy and precision.

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### References


