A Comparison of Results for Cholesterol in Human Serum Obtained by the Reference Method and by the Definitive Method of the National Reference System for Cholesterol

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Here we compare the Reference Method (I) and the Definitive Method (II) for measurement of cholesterol in serum. For pure cholesterol solutions, values by I agree with values by II, but for fresh, frozen, or lyophilized sera, values by I average 1.6% higher than values by II. We conclude that an undetected interference is associated with I. However, the observed difference does not diminish the usefulness of I as an accuracy base for cholesterol measurements, because it is clinically insignificant at present.

A hierarchy of methods for measuring cholesterol has been developed (I): definitive, reference, and routine methods, in descending order of accuracy and complexity.

A Definitive Method is defined by the National Committee on Clinical Laboratory Standards as an analytical method that has been subjected to in-depth investigation and evaluation for sources of inaccuracy, including nonspecificity (I); the final imprecision and bias are of a magnitude compatible with the method's stated purpose. The mean value determined by the Definitive Method for an analyte is taken as the "true" value. However, the Definitive Method is usually so complicated, technically demanding, and expensive that it cannot be used routinely in the clinical laboratory. Even the materials certified for determining a particular analyte by the Definitive Method normally are too expensive for use as routine calibrators in the clinical laboratory.

A Reference Method is defined as a method whose accuracy and precision are sufficient, as demonstrated by direct comparison with a Definitive Method, and whose low incidence of susceptibility to known interferences is so thoroughly documented that the stated end purposes of the Reference Method may be achieved (2). The Reference Method is technically less demanding than the Definitive Method but generally still too demanding for routine clinical use.

For cholesterol, the currently accepted Definitive Method is an isotope dilution–mass spectrometric method developed at the National Institute of Standards and Technology (NIST) (3), and the Reference Method is the Abell–Levy–Brodie–Kendall (ALBK) assay (4), as modified by the Centers for Disease Control (CDC) (5, 6). For clinical laboratory measurements of serum cholesterol to be accurate, routine methods must be traceable to the Reference Method, which must in turn be traceable to the Definitive Method. This National Reference System for cholesterol, consisting of the Reference Method and the Definitive Method plus a certified Reference Material for which the value has been assigned by use of the Definitive Method, has been accepted by the National Committee for Clinical Laboratory Standards (7).

In 1977, as part of the American Association for Clinical Chemistry Cholesterol Reference Method Study Group project to develop a cholesterol reference method, the original Definitive Method proposed by NIST was used to evaluate two candidate Reference Methods developed by CDC: an enzymatic method (8) and a chemical colorimetric method (the ALBK method (4)). Both candidate Reference Methods gave results slightly lower than the Definitive Method. The enzymatic method could not be brought into agreement with the Definitive Method, so it was abandoned. When the extraction time used in the original ALBK method was increased, the ALBK method could be brought into agreement with the Definitive Method, and so it was chosen as the Reference Method. Results by the modified ALBK method and the Definitive Method agreed when five frozen serum pools were measured (see Table 1 below) later that year.

In 1986, the Definitive Method was modified (9) in part by replacing packed columns in the gas chromatograph with capillary columns. During 1986–1988, the Reference Method and the modified Definitive Method were used to measure cholesterol in cholesterol standard solutions and in human serum pools prepared in various ways, which included samples from three of those frozen serum pools already measured. For the cholesterol standards, results from the Reference Method agreed with those from the Definitive Method, but were about 1.6% higher than the Definitive Method results for all human serum pools. Here we report the results of this comparison study and discuss possible causes for this difference.

Materials and Methods

Materials. Samples of Standard Reference Material (SRM) 909, a lyophilized human serum, and SRM 911a and 911b, cholesterol [cholest-5-en-3-ol(3β)] with a certified purity of 99.8% ± 0.1%, were obtained from the NIST Office of Standard Reference Materials. Samples of lyophilized serum pools used in the Comprehensive Chemistry Surveys of 1985 and 1987 were obtained from the College of American Pathologists, Skokie, IL. Samples of serum pools that were prepared and frozen in 1977 were obtained from CDC. Cholesterol used by CDC in calibration of the Reference Method was SRM 911a, SRM 911b, or manufacturers' preparations as described later. Ethanol was USP grade, 200 proof, and other reagents were as specified in the references for the methods.

Reference Method. The Reference Method for cholesterol is the ALBK method (4), as modified by the CDC (5, 6). In this method, 0.5 mL of serum is saponified with 5 mL of 0.36 mol/L ethanol KOH for 60 min at 50°C and is extracted with 10 mL of hexane for 15 min. An aliquot of the extract is evaporated, and the residue is reacted for
exactly 30 min with 3.2 mL of Liebermann–Burchard reagent to develop the color, which is measured at 620 nm.

The Reference Method has been revalidated several times as quality of equipment and reagents changed. Organized revalidation experiments were performed during 1975–1978 and again during 1980–1982. Only during the validation studies of 1975–1978 was the Reference Method validated against the Definitive Method. Any change in equipment, reagents, or calibration quality control materials was evaluated with overlapping experiments.

The transferability of the ALBK method to other laboratories by written instructions was tested (6). The ALBK method permits laboratories to attain a CV of <1.5% for one laboratory, and of <3% among laboratories (6). For the optimized ALBK method as performed at CDC the total CV (within- and among-day precision) is <1% (10).

**Definitive Method.** Isotope dilution–mass spectrometry is the preferred technique for Definitive Methods, because the results do not depend on sample recovery and can be tested for bias. Isotope dilution–mass spectrometry is based on adding a known amount of a labeled version of the analyte to the sample as an internal standard, equilibrating the labeled analyte with the endogenous analyte, processing the sample, and then (for cholesterol) measuring the ratio of unlabeled to labeled analyte by gas chromatography–mass spectrometry (GCMS). Full details of the tests of the Definitive Method for bias have been published elsewhere (3).

In 1986 the original Definitive Method was modified to incorporate the improved technology that has become available during the last decade. Packed columns in the GC were replaced by capillary columns, cholesterol-\footnote{\textsuperscript{13}C_5} was replaced by \textsuperscript{13}C\textsubscript{5} cholesterol, and certain instrumental improvements were made to the mass spectrometer (9, 11). Sample-preparation procedures have not been altered.

**Results and Discussion**

**Results from NIST and CDC.** When the results of the candidate Reference Method and the proposed Definitive Method were compared in 1977, the variables in the Reference Method, particularly the vigorous, as opposed to mild, shaking for 15 min, had been chosen so that the Reference Method values would be in agreement with the Definitive Method values. The agreement between the two methods was considered excellent (Table 1).

For about 10 years there were no direct comparisons of the Reference and Definitive Methods. A difference between Definitive and Reference Method results was first evident with the 1985 College of American Pathologists Survey samples, which were prepared in 1985 and analyzed at NIST in 1986 by the original Definitive Method and the Reference Method. The results are shown in Table 2. The Reference Method values for these samples exceeded the Definitive Method values by about 1.5%.

In 1986 NIST modified the original Definitive Method as noted above, and demonstrated that the original and modified Definitive Methods were equivalent when compared on the same pools within about a year (Table 3). Three of the original pools used for the comparison of the Reference Method and Definitive Method were remeasured by the modified Definitive Method. Comparing these values with the original method values obtained 10 years ago showed consistent differences. One might consider that the modification in the Definitive Method caused the difference, but this explanation is not correct, as shown by the values in Table 3, which are the same for pools that have been measured by both methods within a year. Or perhaps the cholesterol in the 10-year-old pools was degrading. Such degradation has been shown to occur in one well-characterized lyophilized pool, NIST SRM 909, at the rate of about 0.1% per year. Similar degradation of frozen pools could account for the difference observed.

Table 4 shows the CDC values in 1977 and 1987 for the same pools shown in Table 1. The value for cholesterol as measured by the Reference Method in these pools in 1987 averaged 1.2% higher than in 1977. Also given are the long-term (nine-year) means for these pools. The Reference Method values do not appear to vary over time, but a small drift might be obscured by the imprecision.

Table 5 gives results from the comparison of the Reference Method and the modified Definitive Method. Samples measured include lyophilized and frozen samples that were prepared recently and about 10 years ago, as well as fresh serum. The Reference Method values are again about 1.6% higher than the Definitive Method values.

**Results from other laboratories.** Other laboratories have used mass-spectrometric methods on pools also measured by the Definitive Method. The Karolinska Institut study involved five pools of frozen sera, with results that differed from the NIST value by -1.1%, 0.2%, 0.0%, 1.3%, and 0.7% (12). Each of the other studies involved only one or two vials of SRM 909. Differences from the NIST value were 0.2% and 0.4% (13), -0.1% (14), and 0.8% (15). The results are therefore not considered to be conclusive confirmations.

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**Table 1. 1977–1978 Comparison of Reference Method and Original Definitive Method**

<table>
<thead>
<tr>
<th>CDC frozen pool no.</th>
<th>Original Definitive Method</th>
<th>Reference Method</th>
<th>Percent difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>777</td>
<td>1326</td>
<td>1322</td>
<td>-0.30</td>
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<tr>
<td>877</td>
<td>1825</td>
<td>1838</td>
<td>+0.71</td>
</tr>
<tr>
<td>977</td>
<td>2378</td>
<td>2371</td>
<td>-0.29</td>
</tr>
<tr>
<td>1077</td>
<td>2883</td>
<td>2868</td>
<td>-0.52</td>
</tr>
<tr>
<td>1177</td>
<td>3398</td>
<td>3383</td>
<td>-0.44</td>
</tr>
<tr>
<td>Average</td>
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<td></td>
<td>-0.17</td>
</tr>
</tbody>
</table>

* Calculated as (Reference Method value – Definitive Method value)/100/Definitive Method value.

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**Table 2. 1986 Comparison of Reference Method and Original Definitive Method**

<table>
<thead>
<tr>
<th>Cholesterol concn, mg/L</th>
<th>1985 CAP lyophilized pools</th>
<th>Original Definitive Method</th>
<th>Reference Method</th>
<th>Percent difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4</td>
<td>2581</td>
<td>2624</td>
<td>+1.7</td>
</tr>
<tr>
<td></td>
<td>C5</td>
<td>3062</td>
<td>3114</td>
<td>+1.7</td>
</tr>
<tr>
<td></td>
<td>C10</td>
<td>3472</td>
<td>3526</td>
<td>+1.6</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>2985</td>
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<tr>
<td></td>
<td>C17</td>
<td>2585</td>
<td>2615</td>
<td>+1.2</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td>+1.5</td>
</tr>
</tbody>
</table>

* Calculated as (Reference Method value – Definitive Method value)/100/Definitive Method value.
of the Definitive Method, but they do demonstrate that the difference of both the original and modified Definitive Methods with other mass-spectrometric methods is smaller than the difference between the Definitive and Reference Methods.

Possible Sources of Error in the Methods

In this section the possible sources of error in both Reference and Definitive Methods are analyzed to try to account for the inter-method difference.

Preparation of standard solutions. In the Definitive Method, weighing errors in standards preparation are routinely tested for by independently preparing multiple sets of standards and intercomparing them, according to the published protocol (9). The bias among standard sets in the values for cholesterol as measured with the modified Definitive Method has typically been <0.08%.

In the Reference Method, a primary 10 g/L standard stock solution of cholesterol is prepared in ethanol. From this stock solution, working standard solutions at six different cholesterol concentrations are prepared. These working standards are immediately aliquoted into five sets of standards, each set of which has each of the six cholesterol concentrations. These five sets are stored in a desiccator jar containing ethanol, which is placed in a refrigerator, and have been found to be stable for at least six months. The working standards are analyzed randomly in each run, and the results are used to prepare a standard curve for the run by the method of least squares. Each standard curve is checked for linearity, proportionality, and drift. Acceptable deviations of each standard point on the curve may be up to 2% at 1 g/L and 0.75% at 4 g/L. In addition, the correlation coefficient (r) for the daily standard curve must be ≥0.9995.
for the curve to be accepted. Results for standards with the Reference Method observed for 80 runs done in 1984 showed overall coefficient of variations for each concentration of working standard ranging from 0.76% for 1 g/L to 0.24% for 4 g/L.

We tested whether the Reference Method and the Definitive Method would agree for measurement of solutions of pure cholesterol. A chemist at NIST and one at CDC each made up three solutions of cholesterol in ethanol, which were placed in ampules and exchanged between NIST and CDC. Another chemist at NIST and another at CDC analyzed all six solutions—at NIST by the Definitive Method, at CDC by the Reference Method. The person analyzing the samples did not know the gravimetric values for any of the solutions. The results (Table 6) demonstrate that the bias observed for cholesterol in the serum matrix is not seen for pure cholesterol in ethanolic solutions.

Sample-preparation errors. In the Definitive Method, weighing errors in the preparation of labeled cholesterol solutions for addition to samples are routinely tested for by preparing multiple independent sets of samples, and after measuring all samples, determining whether a given set of samples is unacceptably biased. This event has not occurred in the cholesterol measurements. For example, the modified Definitive Method was used for the 1987 measurements of three sets of SRM 909 samples and results between sets agreed to within 0.2%, which limits the possible errors in weighing to <0.2%. In the Reference Method no special sample preparation is required, except for reconstitution of lyophilized samples. Analysis of the three lyophilized College of American Pathologists reference materials in 1988 showed an average CV of 0.76%. Because this CV is similar to that obtained with frozen samples, error due to reconstitution is likely to be small. Therefore, sample preparation is not likely to be the cause of the 1.6% difference.

Hydrolysis. If the cholesterol ester hydrolysis were incomplete, the Definitive Method value would be low. The tests used during the development of the Definitive Method to show complete ester hydrolysis can detect 0.1% unhydrolyzed material; actual test results indicated no observable unhydrolyzed ester (3). Hydrolysis conditions on serum for the Reference Method were chosen to give at least 97.7% hydrolysis of cholesterol esters. If hydrolysis were incomplete, the Reference Method results would still be unaffected (16). Therefore, incomplete hydrolysis is not likely to be the cause of the difference.

Analytical recovery. Low recovery after the labeled cholesterol has been equilibrated with the serum sample would not affect the results of the Definitive Method, because it is the ratio of unlabeled to labeled analyte that is measured. (It has been demonstrated that the two are in equilibration in the samples.) Recovery of cholesterol from serum by hexane extraction is about 99% (3).

In contrast, recovery of cholesterol must be considered throughout the Reference Method. All primary standard solutions are carried through all steps of the Reference Method, and the average recovery was 100.0%. The average recovery of added cholesterol to serum was 100.5%. Recovery problems are unlikely to be the cause of the difference.

Isotope effects. Isotope effects could lead to bias and imprecision in the Definitive Method. An isotope effect during extraction of the cholesterol from the hydrolysis mixture is not expected, and because 99% of the cholesterol is recovered (3), the possible error from an isotope effect is negligible. An isotope effect in the derivatization reaction or on the GC column, as is observed (3), will not affect the results since both standards and samples will be affected equally. Therefore, an isotope effect is not likely to be a source of error for the Definitive Method. Such an effect is not relevant to the Reference Method.

Interferences. GCMS was the technique chosen for the Definitive Method because of the excellent separation of the analyte from interferences and the highly specific detection. Nevertheless, interferences are tested for in every group of samples. After all samples in all sets are measured with use of a principal ion, a representative subset of samples is measured by using other ions, with other GC columns, and or with another mode of ionization. For an interference to be undetected, it would have to have the same retention time as the cholesterol derivative on each column, have to have ions at all the masses that are being used for measurement, and have to have the same abundance ratios under all conditions examined—a highly unlikely series of circumstances.

In the Reference Method, potential known interferences were examined during the method-validation studies. Interference studies with 15 potentially interfering sterols involved adding the given sterol, at 200 mg/L, to aliquots of a 2000 mg/L primary standard solution. Results deviated from the expected value of 2000 mg/L by +13% when 7-dehydrocholesterol was added, by +16% for lathosterol, by +16% for ergosterol, and by +13% for epicholesterol (5a-ol); 5β-pregn-3β,20β-diol, lanosterol, 4,6-cholestan-3-one, 5α-cholestan-3β-ol, 5β-cholestan-3β-ol, 4-cholesten-3-one, 5-cholesten-3β,25-diol, sitosterol, 5-cholesten-3-one, 5β-cholesten-3-one, and cholesterol-5α,6α-epoxide all deviated by <6% from the expected 2000 mg/L value. None of these 15 sterols has been reported to be present in

<table>
<thead>
<tr>
<th>Solution made by</th>
<th>Definitive Method</th>
<th>Reference Method</th>
<th>Percent difference*</th>
<th>Prepared by weighing</th>
<th>Percent difference*</th>
<th>Percent difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST-A</td>
<td>945.0</td>
<td>937.0</td>
<td>-0.85</td>
<td>946.8</td>
<td>-0.19</td>
<td>-1.0</td>
</tr>
<tr>
<td>NIST-B</td>
<td>1005.0</td>
<td>998.5</td>
<td>-0.67</td>
<td>1008.4</td>
<td>-0.34</td>
<td>-0.99</td>
</tr>
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<td>NIST-C</td>
<td>1026.6</td>
<td>1026.0</td>
<td>-0.06</td>
<td>1029.1</td>
<td>-0.24</td>
<td>-0.30</td>
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<td>CDC-1</td>
<td>1897.6</td>
<td>1892.5</td>
<td>-0.27</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>CDC-2</td>
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<td>2419.5</td>
<td>+0.10</td>
<td>n.a.</td>
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<tr>
<td>CDC-3</td>
<td>2776.5</td>
<td>2790.5</td>
<td>+0.50</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

* Calculated as (Reference Method value - Definitive Method value)100/Definitive Method value. · Calculated as (Definitive Method value - weighed-in value)100/weighed-in value. · Calculated as (Reference Method value - weighed-in value)100/weighed-in value. n.a., not available.
normal sera in concentrations that, on the basis of these reported deviations, would cause an obvious alteration in the Reference Method results for cholesterol in sera. In separate interference studies, nine redox-active compounds, such as bilirubin and ascorbic acid, were added in high concentrations to aliquots of an 1800 mg/L solution of pure cholesterol; none produced measurable interferences. However, it is not possible to prepare a true serum blank, because serum cannot be treated to remove cholesterol without affecting the other components in serum. Therefore, substances that are in the serum itself and are unknown to us cannot be tested as interferences in the Reference Method. None of the substances studied as possible interferences in the Reference Method would interfere with the Definitive Method, even at higher concentrations.

At the present time, CDC attributes the gain in absorbance caused by increased shaking time to either increased extraction of cholesterol or extraction of interferants. Such an interferant would have to be commonly present in serum, at a constant percentage of the cholesterol concentration, and be unaffected by the different sample-preparation processes (Table 5). Both methods can be tested for the effect of a specific compound that might interfere, and with neither can we be absolutely sure that there is no interference. However, the Definitive Method is inherently more specific, because it involves a more powerful separation method, GC, and a more specific detector, a mass spectrometer. Thus interference is less likely to occur in the Definitive Method than in the Reference Method. In addition, the Definitive Method includes a scheme of confirmatory measurements as part of the method, which make it likely that even unknown interferences will be detected. This is not the case for the Reference Method. A specific interference has not been identified.

GC column memory. A GC column “memory” effect exists when the result from one injection onto a GC column is influenced by a previous injection. Analysis of the tests for memory effects in both the original and modified Definitive Methods indicate a worst-case single measurement error of <0.06%. This possible error is further reduced by the requirement in the measurement protocol of reversing the order of measurement on the second day. Such an effect is not relevant to the Reference Method. Memory effects are not likely to be the cause of the difference.

Definitive Method modification. Because the original Definitive Method was modified, several samples were measured by both protocols. The results (Table 3) indicate the two methods to be equivalent. The results in Tables 2 and 5 show a consistent bias between the Reference Method and the Definitive Method, whether the original or modified Definitive Method is used. Therefore, the difference between the Reference Method and the Definitive Method results is not caused by the changes made in the original Definitive Method.

The value for cholesterol as measured by the Definitive Method in SRM 909 has been slightly decreasing during eight years at the rate of about 0.1% a year. The probable explanation for this is that the cholesterol in SRM 909 is degrading slightly over the years since its preparation, most likely by oxidation. The products are unknown, but in any case the Definitive Method will not measure these because of the method’s high specificity. The decrease in the value for SRM 909 was seen by both the original and modified methods. Thus the decrease in value of cholesterol in SRM 909 cannot be attributed to method modification.

Purity of standard cholesterol. The purity of the labeled cholesterol (added to both standards and sera) used in the Definitive Method is not significant, because the purity factor cancels out of the equations for calculating cholesterol concentrations in serum. The purity of the unlabeled cholesterol is critical to both the Reference and Definitive Methods. SRM 911a cholesterol, and its successor 911b, both with a certified purity of 95.8% ± 0.1%, have been used in preparation of all standards for the Definitive Method. During the development and original validation of the Reference Method, SRM 911a was used as a primary standard to check the secondary standards from commercial sources (17, 18). These standards were then used in the Reference Method. CDC, starting in 1988, used SRM 911b exclusively for calibration of the Reference Method. The difference between the two methods has been relatively constant in recent years, so the standards used for calibration of the Reference Method are probably not a significant contributor to the difference.

Conclusions
There is a statistically significant difference of about 1.6% between values measured by the Reference Method and the Definitive Method on fresh, frozen, and lyophilized serum samples. In 1977, on frozen pools of serum, the Reference Method and the Definitive Method agreed. For these 10-year-old pools, the values measured in 1987 by both the Reference Method and the Definitive Method differ from those measured in 1977: the Definitive Method values have decreased, whereas the Reference Method values have increased. The decrease in the Definitive Method values may be due to aging; however, an aging effect is not relevant to the 1.6% difference, because the difference is seen even with fresh sera.

It is probable, as discussed above, that problems with standard or sample preparation, the hydrolysis procedure, recovery of cholesterol, isotope effects, column memory, the Definitive Method modification, or purity of cholesterol standards do not provide an explanation for such a difference between the methods.

For all materials except the 10-year-old CDC frozen pools, the 1.6% difference exists. One explanation for the difference that cannot be ruled out is that of undetected interference in the Reference Method. The Definitive Method has the built-in checks for undetected interferences in the confirmatory measurements, as well as the general specificity of the GCMS. The interferences in the Reference Methods cannot be detected unless the chemical substance is known and can be added in a recovery experiment. NIST and CDC are cooperating in the search for an explanation.

The difference observed between the Reference and Definitive Methods does not diminish the usefulness of this accuracy base for cholesterol measurements. Continued use of the Reference Method in its defined role to provide a check on the routine methods used in the clinical environment is justified for the following reasons. First, the actual size of the difference is small compared with present clinical laboratory CVs, so clinically the difference is not yet important. Second, the relatively constant difference found, regardless of the serum matrix, provides a means of estimating the “true” value for studies using the Reference Method. Third, the Definitive Method is so lengthy and expensive that it is impracticable to use in the manner that the Reference Method is being used.
We are grateful to the College of American Pathologists for their support of Polly Ellerbe. We are indebted to Charlene Griffin, CDC, for maintaining the highest quality in performing the Reference Method for cholesterol. CDC work was supported in part under an inter-agency agreement between CDC and the National Heart, Lung, and Blood Institute, NIH.

References

In this two-step automated assay of the MB isoenzyme of creatine kinase (CK-MM), developed for the Abbott "IMx" immunoassay analyzer, monoclonal anti-CK-MM antibody immobilized onto latex microparticles and polyclonal anti-CK-MM antibody coupled to alkaline phosphatase are used. Within-run CVs ranged from 3.9% to 9.0%, between-run CVs from 0.0% to 5.6%, and the sensitivity was 0.2 μg/L. Twenty-four results can be obtained in about 37 min. Analytical recovery of CK-MM added to human serum or plasma ranged from 89% to 109%. Icteric, lipemic, or hemolyzed samples did not interfere with CK-MM recovery. Cross-reactivity with CK-MM and CK-BB was 0.012% and 0.001%, respectively. The normal reference interval was 0–5 μg/L. IMx CK-MM results correlated well with CK-MM enzyme activity as determined by electrophoresis (n = 203; r = 0.97; slope = 0.90; y-intercept = −4.29) and with commercial immunonasays. We think that this assay will be useful for confirmation of acute myocardial infarction, both in critical-care units and in the clinical laboratory.

Additional Keyphrases: myocardial infarct, immunoassay

Assay of creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MM) for the confirmation of acute myocardial infarction is well accepted (1). CK-MM is routinely quantified by one of four techniques: electrophoresis, enzyme immunoabsorption, column chromatography, or immunonasay. Details and limitations of each of these methods have been discussed (2). Briefly, the disadvantages of current methodologies include one or more of the following: time required to obtain results, precision, accuracy, specificity, and (or) sensitivity. Electrophoresis offers good sensitivity and specificity, and currently it is the reference technique for CK-MM determinations. Although enzyme immunometric assays offer the potential for good sensitivity and specificity, they are not widely used in clinical laboratories, in part because of the poor performance of currently available assays and the multiple manual pipetting steps required. Until recently, no automated immunometric assays for CK-MM have been available. The object of our studies was

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3 Nonstandard abbreviations: CK-BB, CK-MM, isoenzymes of creatine kinase.