Cholesterol—a Model System to Relate Medical Needs with Analytical Performance

Donald A. Wiebe¹ and James O. Westgard

The evolution of cholesterol testing provides an example of a systematic approach that developed to relate the medical use of a laboratory test with the analytical performance requirements for that test. Laboratories today have the capability to perform cholesterol testing with the accuracy and precision necessary to meet medical needs. This statement can be made because (a) a standard diagnostic process has been established by the National Cholesterol Education Program; (b) an accuracy base is provided through a reference method that is readily available to manufacturers and laboratories; (c) the precision of analytical systems has been improved by manufacturers; (d) operating specifications for all such systems can be established, with statistical quality-control rules to ensure adequate within-run method performance; and (e) analytical performance is monitored by proficiency testing by using national quality requirements defined by CLIA '88 for acceptability. This cholesterol model provides a logical and scientific approach that should be applicable with other analytes to assure that the analytical performance of the laboratory test satisfies medical needs.

Indexing Terms: quality control · standardization · proficiency testing

Clinical laboratories have been routinely measuring cholesterol in patients' serum specimens for 30–40 years or longer. On the basis solely on years of experience, laboratories should be capable of performing cholesterol analysis with a high degree of reliability. The key issue, however, is whether the precision and accuracy achieved by laboratories are meeting medical needs of physicians.

Historically, cholesterol has often received significant attention from the scientific and medical communities. Nobel prizes have been awarded to scientists since 1901 and already 13 of these prestigious honors have gone to researchers whose work specialized on cholesterol. Certainly, the link between cardiovascular disease and cholesterol propelled this ebullience. The National Cholesterol Education Program (NCEP) (1), initiated by the National Institutes of Health (NIH) in 1987, further perpetuates this preoccupation with cholesterol.²

NCEP has three separate missions. First, NCEP proposed to increase awareness in the medical community and the public concerning the relationship between cholesterol and cardiovascular disease. The media blitz has been intense. Many responded overwhelmingly to the phrase “Know Your Cholesterol,” and today everyone is an “expert” on the subject. Second, physicians were asked to be more aggressive in treating patients with hypercholesterolemia. NCEP met this goal with two key initiatives: (a) establishing 5.20 mmol/L (200 mg/dL) as an acceptable cholesterol concentration and 6.24 mmol/L (240 mg/dL) as the critical cholesterol concentration that is considered above normal and requires further medical follow-up, and (b) developing a set of guidelines for physicians to use in treating hypercholesterolemia (2). The 5.20 and 6.24 mmol/L concentrations define the decision interval for medical intervention of the test. The third and final mission of NCEP focused on clinical laboratories, with emphasis on improving accuracy and precision of the cholesterol measurement process (3).

NCEP established the Laboratory Standardization Panel (LSP), composed of leaders throughout the laboratory community, to ensure that cholesterol methods met accuracy and precision criteria. In an unprecedented and innovative move, the LSP set performance standards for bias (accuracy) and reproducibility (precision) that all laboratories were expected to meet or exceed (3). Appropriately, initial laboratory performance standards were set at a realistic level of a ±5% allowable bias and a CV of ±5%; a 5-year period was allowed for laboratories to achieve the final objective of ±3% for both bias and CV. The laboratory community—composed of laboratory, industry, government, and other workers—united to work toward the final goal. At the same time, numerous articles appeared in newspapers and magazines to highlight problems associated with cholesterol measurement inaccuracies, especially between laboratories. The public outcry was heard and probably had a significant role in legislative initiatives passed by Congress in the Clinical Laboratory Improvement Amendments (CLIA) in 1988 (4).

Over the past several years laboratories have been criticized for their inability to perform accurate and precise cholesterol analyses. However, laboratories today are capable of performing cholesterol analyses with the accuracy and precision to meet medical needs. A review of the past 20 years of cholesterol initiatives provides some insight about how a system evolved to achieve medical needs. Evolution of the total cholesterol system has been an iterative process that provides a model that should be used for other critical analytes (Figure 1). In addition, we discuss the within-individual biological variability of cholesterol, to better understand and define the actual medical need. Quality-planning
models are presented to generate process specifications by which laboratories can assess their cholesterol methods to ensure achievement of analytical requirements within the medical need.

Evolution of Cholesterol Measurements

Here, we review briefly the significant activities that influenced the evolution of cholesterol procedures used throughout clinical laboratories today. In 1952 a "simplified" method for cholesterol analysis was reported (5), and today the Abell–Kendall method is the basis of the Reference Method for cholesterol. By current laboratory standards, the Abell–Kendall procedure is far from a "simplified" approach, yet this method plays a prominent role in the cholesterol standardization effort. The 20-year timeline in Figure 2 represents a list of events and accomplishments that have influenced the current status of cholesterol measurement. Two activities are highlighted for their major impact on laboratory cholesterol testing. First, the 20-year transition period is noted during which clinical laboratories switched from traditional cholesterol assays to the enzyme reagent systems that are the standard methods today. Traditional cholesterol assays required caustic reagents (sulfuric acid, acetic acid, and acetic anhydride) and possibly extraction of the lipid material from the serum.

Fig. 2. The cholesterol timeline highlights various factors and key elements of the evolution for cholesterol standardization. Two important occurrences are shaded and discussed in the text: (1) transition period (1970–1980) during which laboratories changed from traditional to enzyme-based cholesterol methods, and (2) the Lipid Research Clinics program.

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matrix, whereas enzyme-based systems are more laboratory-friendly. The second noteworthy activity was the Lipid Research Clinics (LRC) study and its lasting contribution to lipid measurement in the laboratory. The most publicized impact from the LRC program was the “2 for 1” clinical finding of the intervention trial (6). “Two for one” refers to a 2% lowering of an individual's risk for coronary heart disease (CHD) for each 1% decrease in his or her serum cholesterol. Overlooked are contributions from the LRC laboratories themselves. Even though LRC laboratories are no longer funded by NIH, they continue to play a significant role within the laboratory community. Some LRC laboratories are now active participants as Network Laboratories. In addition, the goal for all laboratories to achieve comparable cholesterol measurements is identical to the primary objective of LRC laboratories. “All laboratories” may refer to the 15,000–20,000 large hospital laboratories or possibly even 250,000 laboratories if clinics and doctors' offices are included. In either case, the task is formidable. Therefore, much has been and may be learned from the experiences of LRC laboratories to meet today's needs.

Transition to Enzyme Assays

Laboratories select analytical methods on the basis of a variety of criteria. Fraser, earlier in this Forum, grouped method-selection criteria into two categories: practicability and reliability. Reliability criteria include accuracy and precision characteristics of the new method. Practicability issues focus more on the conveniences of using the new approach—for example, ease of automation, sample processing or handling, cost effectiveness, and other issues that may have little impact on the actual analytical performance of the method. Rather than reliability, the transition from traditional cholesterol assays to enzymic reagent systems was motivated more by the practicability aspects of the newer methods.

During the early 1970s all clinical laboratories relied on cholesterol assays that required extremely caustic agents. Mixtures of sulfuric acid, acetic anhydride, and acetic acid created a distinctive odor, which permeated laboratories in which cholesterol measurements were in progress. Enzyme systems obviated these caustic and disagreeable reagents while allowing laboratories to eliminate tedious extraction procedures and to automate the cholesterol measurement process; together, these factors offered significant cost reduction. These advantages of the enzyme systems clearly outweighed those of the more traditional methods. Therefore, during the late 1970s and early '80s, laboratories quickly opted for enzyme cholesterol assays over traditional methods.

Unfortunately, the rapid acceptance of enzyme-based assays was not without drawbacks. Accuracy was the loser in the transition to the new enzyme-based cholesterol measurements. Accuracy of traditional cholesterol assays was based on primary standards containing a weighed quantity of pure crystalline cholesterol dissolved in a specified volume of suitable organic solvent. Enzyme activities differ markedly toward cholesterol and cholesterol esters when these are dissolved in organic solvents (as opposed to serum-based media). Thus, use of primary standards to transfer accuracy from Reference Methods to routine laboratory methods is infeasible for enzyme systems. Secondary standards—pooled human serum with a cholesterol value assigned by a Reference Method, such as the Abell–Kendall procedure—are required to transfer accuracy with these new enzymic procedures. Matrix effects of secondary calibrators proved an unexpected complication of accuracy evaluation among enzyme-based analytical systems. Controversy continues among laboratory scientists about whether the matrix effect is an instrument problem, a deficiency of the reference material, or both. By general consensus, lipid experts agree that enzymic cholesterol assays must be judged on their performance with actual patients' specimens compared with reference-method values, thus avoiding matrix problems often associated with commercially processed material (7). The matrix issue with reference materials prevented the transfer of accuracy between laboratories through the use of these materials, and an alternative approach was sought.

Adequate availability of the Reference Method provides an alternative approach to establishing an accuracy-based system that is dependent on results of patient-specimen comparison. Fortunately, the LRC program developed several specialized lipid laboratories that have become active participants in the National Reference System for Cholesterol (NRSC) and the Cholesterol Reference Method Laboratory Network (8).

Impact of the Lipid Research Clinics Program

The LRC program (a 12-year, NIH-funded project) included prevalence and intervention trials to study hypercholesterolemia in men. Information from the prevalence study is often cited as the reference range for lipids and lipoproteins within a population of healthy individuals. As mentioned above, the most far-reaching contribution of the LRC program was the “2 for 1” finding of the intervention trial. This LRC finding that related lower serum cholesterol values and lower CHD risk is the basis on which NCEP was established. However, often overlooked is the contribution of the LRC program to laboratory medicine and laboratories specializing in lipid and lipoprotein analyses.

Eleven laboratories, strategically located throughout the US and Canada, focused on the task of generating comparable lipid data (Table 1). The expectation was that if LRC participants were to have their samples assayed for serum cholesterol at any one of the other LRC laboratories, the results would be similar. Obviously, standardization of cholesterol measurements was a key component of the LRC program (9). The Centers for Disease Control (CDC) served as the standardization operations center, providing primary standards, secondary calibrators, and serum-based control materials (knowns and unknowns) to each LRC laboratory. The modified Abell–Kendall cholesterol assay became the cholesterol Reference Method against which all LRC
Method

The influence of CDC on the LRC program cannot be overemphasized. The laboratory success of the study can be traced to the insistence from CDC that NIH not allow the study to begin before all these laboratories were standardized. That laboratory needs should sway or dictate the start of a study of this magnitude is a rare occurrence.

Although LRC laboratories are no longer active as such, they continue to have a strong influence on several current activities in lipid and lipoprotein programs. LRC laboratories form the backbone of the NRSC Network Laboratories (Table 1). Obviously, CDC continues in a significant role regarding standardization efforts for lipid measurements. Standardization programs are structured for the Network Laboratories by using approaches similar to those that were successful in the LRC program. In addition, the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards) developed the Definitive Method for cholesterol (10) and, together with CDC, embodies the central component of the national cholesterol standardization effort.

Network Laboratories service both laboratories and manufacturers (similar to the way that CDC served LRC laboratories) by providing the basis through which all cholesterol methods can be documented to provide accurate measurements. Standardizing 16,000–20,000 individual laboratories could be considered an impossible task, at least by traditional approaches. Therefore, the objective of Network Laboratories is to standardize cholesterol methods through manufacturers of instruments, reagents, and control materials, or through calibrators commercially available to individual laboratories.

Analytical Performance Capabilities for Cholesterol

Accuracy and precision are the two key analytical performance criteria by which judgments are made with respect to acceptability of the method. Accurate cholesterol procedures are the byproduct of collaborative efforts from several groups. First, instrument and reagent manufacturers have actively documented the accuracy capabilities of their systems through the NRSC Network Laboratories. Next, laboratories have the option of documenting the accuracy of their cholesterol method through manufacturers' efforts or by certifying their system directly with a Network Laboratory. Documentation of precision characteristics of cholesterol procedures is the responsibility of each laboratory. Quality control materials must be used on a daily basis to assure that cholesterol assays are precise. College of American Pathologists precision data and actual cholesterol performance figures from the University of Wisconsin Hospital and Clinics (UWHC) document current status for cholesterol reproducibility (Table 2).

Accuracy

Clinical laboratories generally use a single manufacturer's instrument, reagents, and calibrator to analyze

<table>
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<tr>
<th>Table 1. Location of LRC and Network Laboratories</th>
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<tr>
<td><strong>LRC</strong></td>
</tr>
<tr>
<td>Baylor College of Medicine, Houston, TX</td>
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<tr>
<td>University of Minnesota, Minneapolis, MN</td>
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<tr>
<td>University of Washington, Seattle, WA</td>
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<tr>
<td>Washington University of Medicine, St. Louis, MO</td>
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<td>Johns Hopkins University, Baltimore, MD</td>
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<td>University of Toronto, Toronto, Canada</td>
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<td>Stanford University, Stanford, CA</td>
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<td>University of Iowa, Iowa City, IA</td>
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<tr>
<td>University of Cincinnati, Cincinnati, OH</td>
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<tr>
<td>Oklahoma Medical Research Foundation, Oklahoma City, OK</td>
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<tr>
<td>George Washington University, Washington, DC</td>
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<td>VA Hospital, Rotterdam, The Netherlands</td>
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<th>Table 2. Analytical Precision at the University of Wisconsin Hospital and Clinics</th>
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<td><strong>Control material</strong></td>
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* Mean expressed as mmol/L (mg/dL).
patients' specimens for cholesterol. Use of a homogeneous (single manufacturer) cholesterol system permits the laboratory (user) to accept the manufacturer's documentation of accuracy. The caveat is that the laboratory must operate the system in accordance with the manufacturer's recommendations. However, several laboratories rely on heterogeneous cholesterol systems, in which the instrument, reagents, and calibrators are obtained through a variety of manufacturers. Under these circumstances, the individual laboratory must assume the responsibility for documenting the accuracy of its system. Figure 3 illustrates the process by which individual laboratories can interact with the NRSC program to document the accuracy of their cholesterol method. All laboratories should determine whether their cholesterol methods are homogeneous or heterogeneous, to determine where responsibility lies for the accuracy of their method.

UWHC has three analytical systems to perform cholesterol measurements: the Hitachi 747 distributed by Boehringer Mannheim Corp. (Indianapolis, IN), Ektachem 700 manufactured by Eastman Kodak Co. (Rochester, NY), and Cobas Fara from Roche Diagnostic Systems (Montclair, NJ). Each cholesterol system is meant to provide a different service for the hospital. The Hitachi 747 system provides cholesterol as part of a routine chemistry test panel (16 different analytes). The Cobas Fara performs batch analyses of all routine requests for specialized lipid profiles and individual or packaged cholesterol tests. Finally, the Ektachem 700 is utilized for fast turnaround requirements with specialized outpatient clinics that treat hyperlipidemic patients.

A key characteristic of the three cholesterol systems at UWHC is that two are homogeneous (Hitachi 747 and Ektachem 700) and the other heterogeneous (Cobas Fara). As illustrated in Figure 4, accuracy of the first two systems can be "traced" (i.e., assessed and documented through the manufacturer. However, Boehringer Mannheim enzyme reagents are used with the Cobas Fara and calibrators are prepared in-house, so documentation of accuracy becomes the responsibility of UWHC. UWHC performed a method-comparison study with a Network Laboratory and certified the accuracy of the cholesterol method.

Even with a homogeneous cholesterol assay, laboratories should contact a Network Laboratory to perform a methods-comparison study. Information gained from such an effort can be used in conjunction with manufacturer's data to further document actual performance characteristics in their own laboratory.

Laboratories can gain useful information from proficiency-testing data with respect to the accuracy of their cholesterol method. However, matrix issues limit the unrestricted reliability of this approach and caution must be applied to judge accuracy from proficiency-testing data. To assess accuracy, the future may see improved processing and manufacturing techniques.

Fig. 3. Options by which clinical laboratories can make use of the services from the Network Laboratories to document the accuracy of their cholesterol procedure.

Fig. 4. How instrument manufacturers can interact with the Network Laboratories to document the accuracy of their system for customers.
that provide laboratories with stable and matrix-free human-based reference materials for cholesterol. In the meantime, manufacturers and the Network Laboratories remain key elements to ensure that accurate cholesterol methods are an achievable and realistic goal today.

Precision

Most analytical systems available commercially for cholesterol are stable and offer clinical laboratories sensitive and reproducible procedures. The three methods used at UWHC demonstrate precision performance that should be achievable within any laboratory that follows the manufacturer's directions and standard quality-control practices. Table 2 lists the day-to-day precision obtained from five different reference materials during a single month (September 1992) with the three systems. All three cholesterol systems exhibit acceptable reproducibility, with CVs of <1.5% across the concentration range. Evidently, meeting the NCEP goal of precision of <3% CV is readily achievable for all clinical laboratories.

Intralaboratory precision data from the College of American Pathologists Quality Assurance Service demonstrate significant improvement in cholesterol procedures during the past 15 years (11). In 1975 the average intralaboratory CVs estimated at 5.20 and 6.24 mmol/L (200 and 240 mg/dL) were 4.32% and 4.18%, respectively. These CVs improved to 3.81% and 3.94%, respectively, during 1980; by 1990, precision was enhanced further, to 2.56% at both concentrations. The 1975 data were obtained during the transition time, when many clinical laboratories still used traditional methods for cholesterol, and therefore those data represent the poor reproducibility expected for these methods. By 1980, the vast majority of clinical laboratories used enzyme systems for cholesterol analysis, and precision improved. The change observed between 1980 and 1990 for CV probably is due to the efforts of NCEP to improve laboratory performance.

Instrument manufacturers have provided clinical laboratories with automated systems that are capable of achieving extremely precise measurements. These instruments are microprocessor-controlled to the extent that all fluid transfers, both serum specimen and reagents, are reproducible to microliter quantities. The instrument and reagent manufacturers deserve considerable credit for the improved accuracy and precision performance achieved for cholesterol during the past several years.

Quality Requirements for Cholesterol Assay

Clinical laboratories often set specific performance requirements for precision and accuracy to judge acceptability of analytical methods. NCEP and CLIA provided these analytical performance standards for cholesterol. NCEP established interpretation guidelines for cholesterol that define medical requirements, and CLIA '88 established proficiency-testing criteria for analytical performance.

Medical Need for Cholesterol Values

A key issue for every analyte measured by clinical laboratories is what constitutes the medical need against which analytical characteristics of the procedure can be judged. The medical need for a given analyte can be addressed only by the physician who is using the laboratory information. Various approaches have been attempted to determine medical need, and other presentations at this Forum have thoroughly discussed this important issue but with no agreement or consensus as to the approach by which medical need is determined. On the basis of the earlier presentations here, focused on different approaches that may be beneficial to determine medical usefulness, within-individual biological variability for a given analyte has a major impact on medical need. The assumption can be made that an analyte exhibiting considerable variability can also tolerate more deviation in the analytical procedure. Similarly, an analyte that is tightly controlled biologically must have a precise measurement to detect a clinically important change.

Biological (CVB) and analytical (CV_A) components can be combined mathematically to derive the total within-individual variability (CV): CV^2 = CV_B^2 + CV_A^2. CDC has suggested that CV_A should be <5% to ensure reliable CHD risk assessment. CDC coworkers (12) developed the following tabulation (Table 3), based on a typical CV_B of 6.5%, to illustrate the relationship between analytical variability, number of replicate determinations, and number of specimens required to achieve the goal of CV_A <5%. The last column was added to highlight the CV_A obtainable if the "perfect" analytical system (no imprecision or bias) is used. Two messages become obvious from the relationship of CV_A and CV_B to CV_A. First, an analytical assay that exhibits high variability, such as 5%, requires replicate analysis to improve the significance of the measurement. Most important, even with a "perfect" assay, multiple specimens must be collected to account for the CV_B of 6.5% and reduce CV_A to <5%. Therefore, before a physician can confidently assess an individual's "true" cholesterol value, multiple specimens must be collected from that individual and assayed.

What does CV_B = 6.5% represent in terms of confidence interval? For an individual with a "true" cholesterol of 5.20 mmol/L (200 mg/dL) and a CV_B of 6.5%, and an error-free analytical procedure, the expected range of

<table>
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<tr>
<th>Specimen</th>
<th>CV_A, %</th>
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<tr>
<td>1</td>
<td>8.2, 7.4&lt;sup&gt;b&lt;/sup&gt; 7.2, 6.8 6.8, 6.7 6.6, 6.5 6.5, 6.5</td>
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<td>2</td>
<td>5.8, 5.2 5.1, 4.8 4.8, 4.7 4.7, 4.7 4.6, 4.6</td>
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<td>4.7, 4.3 4.1, 3.9 3.9, 3.8 3.8, 3.8 3.7, 3.7</td>
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<td>4</td>
<td>4.1, 3.7 3.6, 3.4 3.4, 3.3 3.3, 3.3 3.2, 3.2</td>
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<sup>a</sup> "Perfect" analytical system; no analytical variability.
<sup>b</sup> Results shown are CV_A values (CV_A = CV_B^2 + CV_A^2) for duplicate determinations; CV_A = 6.5% for all.
cholesterol values would be 4.52–5.88 mmol/L (174–226 mg/dL) with >95% confidence. This range of cholesterol values seems excessively broad and underscores the need for multiple specimen collections to improve the confidence interval for reliable cholesterol assessment. That the “perfect” analytical system cannot reduce this variable component cannot be overemphasized. Therefore, to adequately assess a patient’s cholesterol status, a minimum of three specimens should be collected 2–3 weeks apart while the patient maintains his or her usual lifestyle, and the specimens should be assayed by the same laboratory.

If physicians are expected to deal with biological variability issues and within-individual changes, laboratories must provide reliable information concerning the imprecision of assays. Thus, an additional recommendation is for laboratories to include the variability component as part of their laboratory report. Therefore, a laboratory that has a cholesterol method with a CV of 3% should report a 5.20 mmol/L value with ±0.31 mmol/L (200 ± 12 mg/dL), and a laboratory with a CV of 1% should report the same 5.20 mmol/L value with ±1.04 mmol/L (200 ± 40 mg/dL). The general use of computer systems throughout clinical laboratories should make straightforward this process of providing specific method-performance information.

Analytical Performance Requirements

The 1980s will always be remembered as a period of increased regulation throughout laboratory medicine. The Health Care Financing Administration is the federal agency responsible for implementing and enforcing CLIA regulations by using the laboratory’s proficiency-testing performance as a key component of their plan (13). Failure to meet CLIA regulatory guidelines places the laboratory at risk for lost revenue, lost service, financial penalties, and possible closure. Thus, laboratories must take a more active role to assure that all analytical procedures meet CLIA performance criteria. Moreover, these CLIA performance criteria become more significant and pertinent than cholesterol recommendations established by NCEP.

Operating Specifications

Quality-planning models can be based on either medical or analytical performance criteria, and the output will provide the necessary reproducibility, bias, and quality-control rules that the procedure must meet to satisfy requirements. Both clinical and analytical models have been presented for cholesterol in which NCEP guidelines are used for the medical-decision interval and the CLIA cholesterol performance standard for the analytical requirement. The quality-planning model permits the laboratory to define operational specifications and to control the process within stated quality requirements. The basic approach for quality planning is presented and thoroughly discussed in articles by Westgard et al. (14, 15).

NCEP guidelines for interpreting a patient’s cholesterol value have set 5.20 mmol/L as the upper limit for “desirable” cholesterol and 6.24 mmol/L as the lower level for “high” cholesterol. Therefore, the critical decision interval is the difference between the desirable and high cholesterol limits, or 1.04 mmol/L (40 mg/dL). Now, the quality-planning model can determine the allowable bias (systematic error) and imprecision (random error) at a critical concentration of cholesterol 5.20 mmol/L at which the analytical procedure must perform to assure that the 1.04 mmol/L limit is not exceeded. A key component of the clinical model is the need to include all possible preanalytical issues that may cause excessive bias or imprecision. Included in preanalytical components is the within-individual biological variability of 6.5%. The following mathematical equation expresses the relationships of the various factors:

$$D_{int} = \text{Bias}_{spec} + \text{Bias}_{mess} + \Delta \text{SE}_{cont} + \text{mess}$$

$$+ z \sqrt{\frac{s_{wub}^2}{n_{test}} + \frac{s_{spec}^2}{n_{test}} + \frac{(\Delta \text{RE}_{cont} + \text{mess})^2}{n_{test}n_{spec}^2}}$$

(1)

where $D_{int} = \text{decision interval, Bias}_{spec} = \text{sampling bias,}$

$\text{Bias}_{mess} = \text{analytical measurement bias,} \text{s}_{mess} = \text{standard deviation of the analytical measurement,} \Delta \text{SE}_{cont} = \text{change in systematic error detected by a quality-control procedure with selected probability criteria,} z$ is related to the maximum allowable defect rate, $s_{wub} = \text{within-subject biological variation,} s_{spec} = \text{between-specimen variation,} \Delta \text{RE}_{cont} = \text{change in random error to be detected by a selected quality-control procedure with a given probability,} n_{test} = \text{number of tests performed with each specimen,}$

$n_{spec} = \text{number of specimens analyzed,}$ and $n_{samp} = \text{number of replicate measurements.}$

This equation describes the relationship of all these factors as systematic and random components at a critical decision concentration ($x_e$). The analytical model can use the CLIA proficiency-testing requirement that cholesterol results must be within 10% of the target value to set the total allowable error ($T_{ea}$). Given 5.20 mmol/L as the critical decision concentration for cholesterol, $T_{ea} = 0.52 \text{ mmol/L (20 mg/dL).}$ The preanalytical components accounted for in the clinical model are absent in the analytical model, as noted in the following mathematical model:

$$T_{ea} = \text{Bias}_{mess} + \Delta \text{SE}_{cont} + \text{mess} + z \sqrt{\frac{(\Delta \text{RE}_{cont} + \text{mess})^2}{n_{test}n_{spec}^2}}$$

(2)

These mathematical models were applied for both the clinical and analytical goals to detect systematic error (SE) with use of the following conditions: $T_{ea} = 200 \text{ mg/dL,} D_{int} = 400 \text{ mg/dL,} s_{wub} = 6.5\%, \text{Bias}_{spec}$ and $s_{spec} = 0, z = 1.65,$ and $\Delta \text{RE}_{cont} = 1.$

Figures 5 and 6 show the allowable limits for precision and accuracy given common quality-control procedures. NCEP performance standards for cholesterol of 3% and 5% bias and imprecision are noted on both
Comparison of the operating specifications for the clinical requirement to that of the analytical requirement, as illustrated in Figures 5 and 6, demonstrates that CLIA performance criteria are more stringent and demanding than the clinical requirements. For example, a laboratory whose cholesterol procedure has 2% bias and 2% imprecision could use most quality-control rules with \( n = 2 \) and meet the NCEP clinical requirement, but only the multirule quality-control procedure with \( n = 4 \) would ensure that the method would meet the CLIA requirement.

Extending this comparison may answer the question regarding accuracy and precision of cholesterol procedures and whether they meet the medical need. Because operational process specifications required to meet CLIA criteria (analytical) are tighter than those required for the NCEP guidelines (medical need), any laboratory that satisfies the CLIA operating specifications will also achieve the medical need.

Summary

During the past two decades a system has evolved that provides the capability to perform cholesterol measurements with the necessary accuracy and precision. The transition toward enzyme-based cholesterol methods played a significant role, as did the automated analytical instruments developed to deliver precise cholesterol results. The NRSC Network Laboratories provided a much-needed accuracy base that enzyme assays could not derive from primary standards or secondary calibrators. Implementation of new CLIA regulations and establishment of the NCEP precision and accuracy requirements resulted in an unprecedented collaborative effort between manufacturers, laboratory scientists, and professional organizations to ensure that cholesterol performance goals could be met. Finally, NCEP established treatment guidelines for adults with hypercholesterolemia to aid physicians in managing patients. These treatment guidelines provide the basis for defining the medical need for cholesterol testing. All these initiatives form a unique total cholesterol system to manage both analytical and medical requirements for laboratory methods.

Within-individual biological variability has become an important issue for assessing a patient's cholesterol status. A major component of the uncertainty of an individual's cholesterol value over a series of specimens is the biological variability, as opposed to error inherent in the analytical method. Clinicians must be educated to incorporate biological variability into the cholesterol management process. Three or more specimens collected 2–3 weeks apart must be used to assess a patient's cholesterol status properly. Even the "perfect" analytical system will not circumvent this requirement.

Necessary analytical performance can be derived from quality-planning models that translate stated medical or analytical quality requirements into operational specifications for accuracy, precision, and quality control. Using the quality-planning models for cholesterol shows that CLIA proficiency-testing standards are more demanding than medical criteria derived from the NCEP guidelines. Therefore, laboratories that establish operational specifications based on the CLIA requirements will satisfy the NCEP medical requirements.
Discussion

Basil Doumas: Dr. Wiebe, you mentioned, and I agree with you, that we should provide the physician the analytical error (SD), but I don't think we can do this with every result we report; we will have very cluttered computer printouts. Fifteen years ago, we started using the following approach: In the "Laboratory Guide for Physicians" we include for all quantitative tests the analytical error (SD) and the concentration of the analyte in the control specimen. This reasonable approach is well-accepted by physicians in our hospitals. My question is this: We just heard Dr. Schectman say that for triglycerides he doesn't feel we need much improvement in precision. You are saying we need a lot more than we have. Which is true?

Donald Wiebe: As long as we are using the triglyceride value as part of the Friedewald equation, I think we really do need better precision. Triglycerides are subject to very broad biological variability. Perhaps measuring fasting triglycerides is not really what the laboratory ought to be doing. Maybe the clinician ought to be determining the 24-h average triglyceride for an individual. Also, I don't think we have come close to transferring accuracy for determinations of triglycerides. Reference methods for triglycerides are not in place. To my knowledge, there is no Definitive Method for triglycerides.

Basil Doumas: There can be no Definitive Method because the analyte cannot be defined; it is a mixture of several substances.

Jack Levine: One of the ways that we in the industry have been able to remove bias from the calculation of error is that the lipid Laboratory Network is available to everybody. We, as manufacturers, can use it to get our methods certified. Our customers can also get certified by a similar type of study. This is a big plus for all of us trying to get to a better answer; it removes the major bias components from our calculations. It has really helped us in the industry, and it helps the individual users to make sure they are on track and to check on their suppliers.

Your concerns about biological variation and laboratory variation and explaining this to physicians and the general public will add a new dimension to their understanding of laboratory data. When we tell the public there is a ±30 or 40 mg/dL variation at a cholesterol concentration of 200 mg/dL, they are not going to be able to accept that cognitively. We simply have to say the result is 200 and that’s it. We can’t start telling the public there is this plus or minus level of uncertainty. They can’t deal with it; they can’t be educated to deal with uncertainty.

Donald Wiebe: I agree. If you give an individual a cholesterol result of 199 mg/dL, they will fight you to the death if you tell them their cholesterol is 206 mg/dL.
"No, no, it's 199; I've got the test printout." The public doesn't understand the concept of variability, but I think we must get that information across to our clinicians. 

Wiveka Elion-Gerritzen: If we really want to relay data on analytical variability to the clinician, there is an easy way. In the case of results with decimal points, e.g., for potassium or calcium, we are used to not reporting more decimal points than we can account for. Likewise, we can incorporate the data on analytical variability and biological variability if we want to do so—in results without decimal points. We would report for glucose, for example, 105, 110, 115 mg/dL, thus increasing the smallest difference between two results from ~1% to ~5%. It is interesting to note that, for glucose, the smallest difference between two results in mmol/L—5.4, 5.5—amounts to ~2%. For calcium the smallest difference between two results in mg/dL is ~1%, but in mmol/L is ~0.4%!

John Ross: Neither Dr. Schectman nor Dr. Wiebe has said much about analytical bias. The bias term is very important; for example, if the analytical competence of a result is to be recorded, the bias term as well as the imprecision term should be included. The replication experiments and the effect of replication they discussed do not include the presence or absence of the bias term. Those data are essentially meaningless without that term in the equation.

Donald Wiebe: Your comment is exactly right. We factored bias out; we assumed zero bias.

Bradley Copeland: When you say bias, are you referring to bias with respect to the Definitive Method for cholesterol?

Donald Wiebe: The bias I look at is the difference between the result the national reference laboratories obtain and what our laboratory gets. As we know, there is bias between their laboratories and the Definitive Method—albeit small, it does exist; it is just something we have to deal with. I don't think we will ever control it, but we have to understand that it exists.