Performance Characteristics of a Blood Cholesterol Measuring Instrument Used in Screening Programs

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Synopsis

Four experiments were conducted to assess the precision and accuracy of the Boehringer Mannheim Diagnostics Reflotron ®, an instrument that is being adopted by many public health groups to conduct blood cholesterol screening programs. Our study is one of the first to evaluate and document the instrument's performance characteristics. Successive generations of reagent tabs supplied by the manufacturer were tested against a reference laboratory method standardized by the Centers for Disease Control. Three of the experiments also compared two or more Reflotrons to assess intermachine reliability.

The Reflotron analyzer provided precise blood cholesterol measurements both in repeated tests and among instruments. The accuracy of the method varied across reagent lots, with an average negative bias of 21.05 milligrams per deciliter (mg per dl), decreasing steadily to a negative bias of 4.07 mg per dl. Reliability between and within analyzers was high. The data provide support for the use of the instrument in blood cholesterol screening efforts, yet signal the need for attention to quality control procedures, both by the manufacturer and operators, to ensure the validity and accuracy of the results.

THE National Cholesterol Education Program (NCEP) (*1*), the recommendations of the NCEP Adult Treatment Panel (*2*), and the Consensus Conference report "Lowering Blood Cholesterol to Prevent Heart Disease" (*3*) have increased general awareness of high blood-cholesterol levels as a public health problem.

Recent surveys have found that more than 50 percent of American adults have blood cholesterol levels higher than that identified as healthy by the Consensus Conference panel (*4*), and fewer than one-third of Americans report ever having had their blood cholesterol level measured (*5*). Better understanding of the magnitude of the problem has prompted public and private groups nationwide to offer blood cholesterol screenings (*6–8*).

Screening of blood cholesterol among the general population has been criticized as premature, overly expensive, and likely to overwhelm physicians, who will have to treat increasing numbers of patients with high blood cholesterol levels (*9*). However, recent technological advances in rapid, dry chemistry clinical analyzers offer low unit cost, ease of operation, and portability. The often overwhelmingly positive response of the public to the cholesterol mass screenings which have been performed has served to convince many public health professionals that "the time is now" for elevated blood cholesterol identification and management. Parallel developments in physician education efforts have prepared physicians to emphasize blood cholesterol management as part of their practices (*1*).

The present and future efficacy of blood cholesterol screenings rests on the accuracy and reliability of the instruments used to perform the analysis. The performance characteristics of the Kodak Ek-

Table 1. Summary of experimental designs in assessment of Boehringer Mannheim Diagnostics Reflotron®

Experiment number	Number of participants	Number of Reflotrons	Reagent lot	Number of Reflotron measurements	Test objective
1	[1] 134	5	302	2	Reliability, accuracy
2	[1] 153	5	305	2	Reliability, accuracy
3	[1] 169	2	405	1	Reliability, accuracy
4	[2] 30	1	408	1	Accuracy

[1] Study participants, 18–65 years old, were randomly selected from the Community Risk Factor Survey.

[2] Nineteen participants, 18–65 years old, were recruited from weight loss groups and 11 from community cholesterol screenings.

tachem DT60 ® have been described (*10*). In this report the performance of the Boehringer Mannheim Diagnostics Reflotron ® (*A*) is assessed, with respect to both accuracy and precision of four successive reagent lots.

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Methods

For the experiments, the external standard for blood cholesterol measurements was the Lipid Metabolism Laboratory at Miriam Hospital, Providence, RI. The laboratory uses an enzymatic method for the quantification of cholesterol with a Guilford 400 autoanalyzer, as described (*10*). The laboratory participates in three programs for lipid measurement standardization, which are sponsored by the Centers for Disease Control (CDC), the College of American Pathologists, and the National Heart, Lung, and Blood Institute's Committee for the Coordination of Community Demonstration Studies.

The Reflotron is an electrochemical reflectance photometer that measures chromatic changes in blood which are produced by various chemical constituents. Reflectance is measured with a reference beam and an Ulbricht sphere located within the instrument. Two detectors in the optical module compare the emitted light from three diodes with the reflected light from the reagent tab. The amount of reflected light is inversely proportional to the cholesterol concentration in the sample, and a signal processor transmits the associated voltage to the digital display.

Disposable reagent tabs (*B*) contain a separation pad, a reagent pad, and a magnetic code. Each reagent tab is approximately 10 by 0.6 millimeters. The separation pad separates plasma from whole blood by adhesion of erythrocytes and other particles on the surface of a glass fiber paper. The plasma flows to the reagent pad, where cholesterol esterase, cholesterol oxidase, and an indicator are contained for cholesterol measurement. The dye concentration that results from the catalytic and oxidization processes is determined by reflectance photometry.

The magnetic code, located under each reagent tab, is preprogrammed by the manufacturer to allow for test selection, duration of the preincubation and reaction phases, wave length, specifications for calculation of the result from the reflectance measurement, and factors for Standard International and Concentration Units (*11*). In screening kits recently released by the manufacturer, this information is contained on one program tab, rather than on each reagent tab.

As noted, the critical analytic component of the instrument is the reagent tab. The tabs are produced in large quantities that are lot-coded and calibrated by the manufacturer. The lots, usually released several times a year, have been subject to changes in calibration methodology in order to standardize them to CDC-based methods for lipid measurement. In practice, this has meant that since the first lot was introduced in January 1986, subsequent lots have been calibrated slightly differently.

For each of the experiments reported, study participants were recruited from ongoing risk factor surveys, weight loss groups, or cholesterol screening programs. Participants were men and women, generally between the ages of 18 and 65 years. Blood was collected from participants, either in their homes or at a central screening site, by trained phlebotomists. Venous samples were drawn into heparinized vacutainers and refrigerated for no more than 1 day before analysis with the Reflotron, or 7 days before laboratory enzyme assay.

Table 2. Summary of analyses of results of experiments

Experiment	Residual DF	Comparison of laboratory assay against Reflotron			Comparison among Reflotrons		
		DF	F-ratio	P	DF	F-ratio	P
1	1469	1	1,387.60	<0.001	4	12.82	<0.001
2	1678	1	1,165.85	<0.001	4	17.19	<0.001
3	336	1	177.35	<0.001	1	2.63	>0.10 (NS)
4	29	1	8.09	<0.01	NA		

NOTE: DF is degrees of freedom. NS is not significant. NA is not applicable.

Table 3. Mean cholesterol values (mg per dl) obtained in comparisons of Reflotrons and laboratory

Experiment	Reagent lot	Reflotron					Laboratory	Standard error of difference [1]
		A	B	C	D	E		
1	302	205.2	204.5	200.8	201.7	203.5	224.2	0.73
2	305	206.4	207.2	203.5	208.4	207.1	223.2	0.63
3	405	...	...	...	205.7	204.4	214.3	0.81
4	408	...	...	...	...	224.1	228.2	1.43

[1] This value is estimated as residual mean square times 2 divided by the number of observations per mean.

Table 4. Mean total cholesterol values (mg per dl) obtained in comparisons of five Reflotrons, within replicates, in two experiments

Experiment and replicate	Observations per mean	Reflotron				
		A	B	C	D	E
1: 1	134	205.6	205.2	200.4	201.9	203.4
1: 2	134	204.8	203.7	201.1	201.6	203.7
Difference (1 – 2)		0.8	1.5	−0.7	0.3	−0.3
2: 1	154	206.7	207.1	203.7	208.2	207.1
2: 2	154	206.0	207.3	203.2	208.6	207.0
Difference (1 – 2)		0.7	−0.2	0.5	−0.4	0.1

Before transfer to the laboratory, sufficient samples were taken from each storage container by means of lithium-heparinized capillary collection tubes (*C*) for analysis by each Reflotron being tested.

The experiments were randomized block designs designating study participants as a random block effect, and method of analysis (either by Reflotron or enzyme assay) as a random treatment effect. Reliability was measured in these experiments as the variability among Reflotrons, compared with the residual variability of the experiment. Accuracy was estimated as the average difference among Reflotrons and laboratory assay for a single lot of reagent tabs. Here the CDC-standardized laboratory value was considered the standard against which the accuracy of the reagent lot was measured.

The four experiments were conducted in sequence over the period of 1 year. Experiments 1 and 2 investigated the reliability of 5 Reflotrons, and estimated the accuracy of reagent lots 302 and 305, respectively. Given that these experiments established analyzer reliability, experiments 3 and 4 were designed primarily to estimate the accuracy of lots 405 and 408. The designs and aims of the experiments are summarized in table 1.

The statistical methodology consisted of the appropriate analysis of variance for each experiment, that is, randomized block design, with replication and random effects. Experiments 1 and 2 also included an orthogonal partition of the treatment

sum of squares into two types, Reflotron versus laboratory assay, and among Reflotrons (by subtraction). The two components were tested separately for significance (using the F-ratio statistic), representing tests of accuracy and reliability, respectively (*12,13*).

Results

Analyses of variance were completed for all four experiments. The mean square among participants was highly significant in all cases, as expected, with F-ratios between 79.52 and 389.03. This large variance component reflects the diversity of participants recruited for the experiments.

Table 2 shows the variance analysis of treatments (Reflotron or laboratory assay) partitioned into the two contrasts described previously. The first contrast (laboratory assay versus average Reflotron result) is highly significant in all cases, indicating differences between the two methods of measuring total cholesterol. The F-ratios partly are particularly large because of the number of degrees of freedom available for estimating the residual mean square.

The contrast of results among instruments in the first three experiments is significantly different from zero only for the first two, which included the same five Reflotrons. However, even these F-ratios are relatively small, and statistical significance is a function of residual degrees of freedom.

The results indicate that almost all of the variability between measurement methods is in the first contrast (laboratory verses Reflotron), indicating a lack of accuracy of the Reflotron (particularly for the earlier reagent lots). Reliability among Reflotrons appears to be relatively good in comparison. The findings are demonstrated more clearly in table 3, which presents mean cholesterol levels for each measurement method.

The differences between the laboratory value and the average Reflotron value are 21.05, 16.73, 9.30, and 4.07 milligrams per deciliter (mg per dl) for experiments 1 through 4, respectively. Given that the experiments were performed sequentially on new reagent lots, the improvements in reagent standardization are well documented. The difference of 4.07 mg per dl in experiment 4 is of the same order as differences among Reflotrons.

The range of means among Reflotrons is 4.4 and 4.9 mg per dl for the five instruments in experiments 1 and 2, respectively. Comparing analyzers D and E only, for the first three experiments, the corresponding differences are 1.8, 1.3, and 1.3 mg per dl. The values are reasonably consistent across experiments, providing evidence of matching reliability over time. Rank order in means is maintained for three of the five analyzers (B, C, and E) across experiments 1 and 2.

Table 5. Estimated accuracy and precision of four reagent lots

Reagent lot	Estimated mean cholesterol	Estimated standard deviation	Estimated relative standard deviation [1]	Estimated analyzer bias [2]
304	203.14	8.44	4.15	21.05
305	206.50	7.85	3.80	16.73
405	205.02	7.41	3.61	9.30
408	224.10	5.54	NA	4.07

[1] Column 2 divided by column 1 (coefficient of variation percentage).
[2] Estimated bias of the mean Reflotron assay in column 1, through subtraction from the laboratory mean value.
NOTE: NA is not applicable; one instrument was tested.

An additional demonstration of Reflotron reliability is given in table 4, which compares total cholesterol means from each instrument, for each replication, in the first two experiments (table 1). The differences among replicates for each analyzer appear small and essentially random. The differences are considerably smaller than differences among analyzers.

Discussion

Summary data from these studies, shown in table 5, provide strong evidence of the precision of the Reflotron in terms of both highly reproducible results across repeated measurements, and among analyzers.

The results document the increasing accuracy of reagent lot generations. While lots 302 and 305 each showed significant negative biases of 21.05 mg per dl and 16.73 mg per dl, respectively, lot 405 had a bias of 9.30 mg per dl, which was further reduced in lot 408 to 4.07 mg per dl. All lots had coefficients of variation (CVs) well within the ±5 percent range recommended by the recent report of the NCEP Lipid Standardization Panel (LSP) (*14*). The negative bias of lot 408 is within the ±3 percent range recommended by the LSP as a national goal to be achieved for cholesterol-method biases by 1990.

In the larger context of present-day laboratory standardization, data from the College of American Pathologists (CAP), Quality Assurance Service, show that in 1985, intra-laboratory CV precision was about 3.5 percent across about 2,000 participating laboratories. A second CAP program, the

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Chemistry Proficiency Testing Survey (CPTS), provided about 5,000 laboratories with blood pools to assess cholesterol measurement accuracy. In 1985, CDC reference materials had a true value of 262.2 mg per dl. Various laboratory methods used in the CPTS produced mean values ranging from 243.4 mg per dl (−15.6 percent CV) to 281.2 mg per dl (+13.7 percent CV) (*14*).

The data on lot 408 warrant the conclusion that the Reflotron is an accurate and precise method for blood cholesterol screening. However, these features may be compromised unless adequate attention is given to other aspects of the cholesterol measurement process, such as

- A commitment to quality performance by the manufacturer
- Regularly scheduled maintenance and quality control procedures by the user
- The use of competent, well-trained staff in collecting the capillary blood samples, and operating the Reflotron
- Established methods for identifying and correcting problems by both the manufacturer and user. This issue is especially important as new lot generations are introduced to the market.
- Use of CDC-traceable reference materials for performance monitoring (*14*).

If blood cholesterol screenings are to be successful and made widely available to the public, the accuracy and precision of the analyzer must be assured. Studies of recent lot generations of reagent tabs for the Reflotron suggest that standardized characteristics have been achieved for the instrument itself.

However, the performance characteristics hinge on the production of well-standardized reagent tabs. Health professionals currently using, or contemplating using, this or any other analyzer must be informed users. Manufacturers should be required to provide objective data documenting the accuracy and precision of the instruments against well-standardized laboratory methods before deploying the instruments in screening sites or clinical offices. Investigators who use data obtained from the Reflotron need to provide the lot number of the reagent tabs, so that misleading and possibly conflicting conclusions will not be drawn from their work.

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Equipment

A. Boehringer Mannheim Diagnostics Reflotron ®, Boehringer Mannheim Diagnostics, 9115 Hague Rd., Indianapolis, IN 46250.
B. Boehringer Mannheim Diagnostics Reflotron Cholesterol reagent tabs.
C. Boehringer Mannheim Diagnostics Reflotron capillary collection tubes.