ity in other CK immunoassays (12), so we were pleased that we failed to observe any cross-reactivity with the Hybritech assay.

We generated a reference interval for normal patients by assaying sera from 100 randomly selected patients seen in the clinic for reasons other than suspected cardiac pathology. The result, 0–6.0 μg/L, is slightly higher than that quoted by the manufacturer, presumably because of a difference in population sampling. Because the normal reference interval was clearly too restrictive to be used for the diagnosis of AMI, we described a non-AMI reference interval, 0–15.0 μg/L, which was also higher than the one suggested by the manufacturer and reflected the fact that our non-AMI population included several patients with other cardiovascular diseases who had considerable amounts of CK-MB in their sera. Although this clearly results in an area of overlap, we considered that this was more representative of a true in-house patient population and hence made for a more realistic study.

In practical terms, the Hybritech assay is slightly more expensive for batch work than the electrophoretic method. However, unlike electrophoresis, it does not waste reagents when used for urgent ("stat") procedures. The turnaround time is approximately equivalent for the two assays, but the Hybritech assay involves less "hands-on" time, about ½ as much time as electrophoresis. The Hybritech assay is extremely simple to perform and would be ideal for an emergency-room procedure.

In conclusion, both the Hybritech assay and electrophoresis accurately measure CK-MB in serum, show good correlation between their respective results, and yield similar predictive values. Both assays provide useful data for the diagnosis of AMI.

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References

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Reflotron Cholesterol Measurement Evaluated as a Screening Technique

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We evaluated the analytical performance of Boehringer Mannheim Diagnostics’ "Reflotron" analyzer for the measurement of cholesterol. Coefficients of variation (CVs) for whole-blood cholesterol were: within-day 2.0% and 2.2% at 1600 and 2670 mg/L, respectively; between-day 1.8% and 2.4% (n = 9 and 8). Results were similar for serum and heparinized or EDTA-treated single-donor plasma (CV 1.4% to 2.6%). CVs of results for two reconstituted commercial quality-control materials were 3.4% and 4.6%. Heparin and hematocrit were evaluated as interferences, and critical limits for interference were identified for bilirubin, hemoglobin, and triglycerides in blood and plasma or serum. When sample collection and analysis were controlled by trained personnel, results with the Reflotron (y) compared well with those by the Ektachem procedure (x) for both blood and serum samples: r = 0.950, y = 0.944x + 130 mg/L; and r = 0.955, y = 0.93x + 43.5 mg/L, respectively. The same comparability was observed when the analysis was performed by briefly trained high-school students: r = 0.980, y = 0.949x + 23 mg/L. Performance decreased when both collection and analysis were performed by laymen: r = 0.880, y = 0.870x + 186 mg/L.

Additional Keyphrases: sample handling · Ektachem compared · training required for operators · reflectance photometry

The relationship between serum cholesterol and risk of atherosclerosis is central in the National Institutes of Health consensus report (1), and has led to the recommendations from the National Cholesterol Education Program that

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all adults should have their blood cholesterol measured and know their values (2). These reports pose the technical problem of availability of reliable analytical systems for cholesterol testing and the educational problem of "demystifying" laboratory tests for public acceptance. Use of small physician's-office systems offers the possibility of addressing both challenges. Here we evaluated the Reflotron® dry chemistry analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250) for use as a population-screening device for cholesterol. The Reflotron can use, as a 30-µL sample, blood, serum, or plasma obtained by skin puncture (finger stick).

The use of this type of instrument for population screening is acceptable if the results compare well with those obtained with well-recognized laboratory systems; moreover, the level of expertise required for operation does not obviate the accessibility of the instrument to the general public. Our evaluation therefore includes a comparison of results obtained by instrument operators and sample collectors whose training ranged from layman to professional.

Materials and Methods

Instrument systems. The Reflotron reagent system and instrument have been previously described (3). In brief, a 30-µL sample aliquot is metered onto an application pad on a system strip. The top layer of the glass-fiber pad retains erythrocytes when whole blood is used, allowing the plasma to diffuse to an adjacent reaction area. The reagent system is impregnated onto a membranous layer on the strip. Insertion of the strip into the Reflotron instrument forces contact of the reagent and sample layers, thus initiating the reaction. The reagent system is as follows:

\[
\text{Cholesterol esters} + H_2O \xrightarrow{\text{cholesterol oxidase}} \text{cholesterol} + RCOOH
\]

\[
\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-ene-3-one} + H_2O_2
\]

\[
H_2O_2 + 3,3',5,5'-\text{tetramethylbenzidine} \xrightarrow{\text{oxidization}} \text{oxidized benzidine} + H_2O \quad \text{(blue)}
\]

The indicator reaction is monitored by reflectance detection at 542 nm.

We compared results with those obtained with an Ektachem 100 Analyzer (Eastman Kodak Co., Rochester, NY 14650) as the reference instrument. In the Ektachem instrument a similar reagent system and reflectance detection are used. Calibration and programming for both instruments are coded by the manufacturer and machine-readable. The calibration of the Ektachem system is traceable to reference methodology, and patients' results have been shown to agree well with those by the Abell-Kendall method (4).

Reagents. Unconjugated bilirubin was obtained from Sigma Chemical Co., St. Louis, MO 63178. Lipozyn II (20% solution) was from Abbott Laboratories, North Chicago, IL 60064. Plasma samples assayed by enzymatic methods of the Northwest Lipid Research Center were from Washington Research Foundation, Seattle, WA 98105. Other such reference material was from the Dutch National Institute for Public Health and Hygiene, Bithoven, The Netherlands.

Samples. The study had two field-trial sections as well as a laboratory-based verification component. For one off-site trial, two blood samples from healthy high-school students were obtained from the antecubital vein, the venipuncture being done by experienced phlebotomists. An unadulterated specimen was collected for serum; the other, EDTA-anticoagulated blood was used without separation. The latter was analyzed on site within 10 min of collection by high-school students operating Reflotron instruments who had been given 15 min of instruction on operation of the instrument. The serum sample was separated from the clot within 3 h of collection, then sent to the laboratory for analysis with the comparison instrument.

The second field study was conducted as follows: (a) Five assembly-line workers volunteered to operate Reflotron instruments; they were instructed as the high-school students had been. (b) Three employees from a YMCA were given brief (1 h) training in obtaining skin-puncture blood specimens. (c) The two groups worked together to screen a factory population by skin puncture sampling. (d) Follow-up venipuncture blood specimens from those subjects whose samples contained increased concentrations of cholesterol (2) were obtained by skilled phlebotomists. (e) Serum assays of the latter samples were then performed in the laboratory.

Patients' samples used in the laboratory section of the study were excess material from a mixed inpatient-outpatient population for whom both lipid and hematological studies were requested. Again, serum and EDTA-anticoagulated blood were used.

For precision and interference studies, blood, serum, and heparin- or EDTA-treated plasma were obtained from two volunteers, one with normal and the other with above-normal cholesterol concentrations. Bilirubin solutions, prepared according to Doumas et al. (5), and a commercial lipid concentrate (Lipozyn II) were used to adulterate plasma and whole blood for interference studies. Similarly, hemoglobin solutions obtained by three freeze-thaw cycles of washed erythrocytes were added to plasma or blood samples. The effect of hematocrit was investigated by removing or adding plasma to blood samples.

Results and Discussion

Precision. The samples used were analyzed as suggested by NCCCLS (6). We used commercially available lyophilized material, serum from volunteers, EDTA-anticoagulated plasma, and EDTA-anticoagulated blood (which could be analyzed in the brief period before clotting occurred). Results for all materials are summarized in Table 1.

Correlation studies. In the laboratory phase of the study, Reflotron-determined values for EDTA-treated blood (y) for samples from 204 hospital patients and the corresponding serum cholesterol values as measured with the Ektachem

| Table 1. Precision of Reflotron Cholesterol Results for Different Specimen Types |
|---------------------------------|-----------------|-----------------|
|                                 | Within-day      | Day-to-day      |
|                                 | Mean, mg/L, CV, % | Mean, mg/L, CV, % |
| Precinorm                       | 1870 ± 3.1      | 1890 ± 3.4      |
| Precipath                       | 2490 ± 4.0      | 2400 ± 4.0      |
| Human serum                     | 1700 ± 1.4      | 1690 ± 1.8      |
| Human plasma (heparinized)      | 1680 ± 3.4      | 1740 ± 2.3      |
| Human plasma (EDTA)             | 1700 ± 3.0      | 1750 ± 2.6      |
| Human blood 1                   | 1670 ± 2.0      | 1800 ± 1.8      |
| Human blood 2                   | 2320 ± 2.2      | 2240 ± 2.4      |

* n = 9; ** n = 8; all others, n = 20.
system (x) compared well: \( r = 0.950, y = 0.944x + 130 \text{ mg/L} \) (\( S_{xy} = 143.0 \text{ mg/L} \); the range observed was 1120 to 3370 mg/L. When serum samples were analyzed with both instruments, the results were similar: \( r = 0.955, y = 0.930x + 43.5 \text{ mg/L} \) (\( S_{xy} = 119 \text{ mg/L}, n = 125 \)).

Results of the first field-trial phase of the study, in which high-school students operated the instrument(s), were also favorable. Results for Reflotron-analyzed venous EDTA-treated blood (y) compared well with Ektachem-analyzed venous serum (x): \( r = 0.980, y = 0.943x + 23 \text{ mg/L} \) (\( S_{xy} = 83 \text{ mg/L} \); range = 950 to 3000 mg/L; \( n = 132 \)). The second field study put both collection and analysis in the hands of laymen, with the following results: \( r = 0.880, y = 0.870x + 186 \text{ mg/L} \) (\( S_{xy} = 132 \text{ mg/L} \); \( n = 108 \)). The last set of results demonstrate a positive correlation, but are not similar to laboratory-derived values. The consistent underestimation is not explicable by the difference of skin-puncture sampling compared with values for serum (7). It is probably a result of improper collection technique, i.e., excessive squeezing of the puncture site, causing dilution of the specimen by tissue fluids.

**Linearity and analytical-recovery studies.** Linearity was assessed by mixing fractions of sera with low and elevated cholesterol concentrations and assaying. Results appear consistent from 1000 to 3250 mg/L. Reference material that had been assayed by enzymatic methods (Northwest Lipid Research Center Pools 1 through 9) were also analyzed. Omitting aberrant results traceable to chylomicronemia, it again appears consistent to 3250 mg/L (Figure 1).

Analytical recovery for assayed material from the Dutch National Institute (cat. no. 8067), added in five intervals to a patient’s serum sample in the range 1910 to 2580 mg/L, ranged from 106% to 95%. We also used an assayed commercial material traceable to the Abell–Kendall methodology (Omega, Cooper Biomedical Diagnostic Division, Freehold, NJ 07728). A pool of material was prepared at 2.5 times the normal concentration for use as an adulterant. This pool was used to reconstitute the lyophilized form in regular increments, e.g., Vial 1 was reconstituted with 4.0 mL of diluent + 1.0 mL concentrate; Vial 2, 3.0 mL/2.0 mL, etc. In the range 2240 to 3300 mg/L, recovery ranged from 102% to 105%. Results for the several externally assessed assay materials showed, for the Reflotron, a linear relation to concentration in the range 1000 to 3350 mg of cholesterol per liter, and recovery of added cholesterol to be between 95% and 106% for that range.

**Interference study.** Hematocrit (by packed cell volume), varied from 15% to 59% by manipulation of plasma volume from a single donor, had no discernible effect on blood cholesterol measured at a concentration of 1700 mg/L. At a hematocrit greater than 60%, a negative deviation was observed.

Heparin in a concentration up to 108 USP units/mL, fivefold the normal concentration used for anticoagulation, was without effect.

Added hemoglobin and bilirubin caused a decrease in apparent cholesterol concentration in the analysis of both serum and whole blood. Negative interference was proportional to bilirubin concentration above a threshold of 30 mg/L; at 200 mg/L a decrease in apparent cholesterol of 33% was observed for both blood and serum (Figure 2). We presume the interference occurs at the indicator reaction. Bilirubin interferes with the peroxidase-coupled indicator reaction (8), whereas hemoglobin itself can function as a peroxidase.

Hemoglobin from hemolysis caused by improper skin puncture would be the most common source of interference. If whole blood were used for analysis, even a trained laboratory specialist could not detect hemolysis at the levels that affected results. It is possible, however, to detect gross hemolysis by looking at the used reagent strip.

Triglycerides in concentrations up to 14 700 mg/L (Liposyn II 20%) had no apparent effect on results measured with the Reflotron. Concentrations greater than this significantly decreased the observed cholesterol results, possibly explained by variation in volume equivalent of the sample applied. The Reflotron analyzer requires 30 \( \mu L \) of blood; therefore, the dilution of blood by chylomicrons could have critically decreased the actual volume of blood applied. Separate tests suggest the critical difference in volume to be \( \sim 5 \mu L \), extrapolating to a critical applied volume of 25 \( \mu L \).

Precision with the Reflotron was acceptable (Table 1). Its accuracy, as assessed by assay of independently assayed materials, was acceptable, i.e., usually within 5% of the target values, in contrast to a previous report (9). (We presume that the manufacturer has responded to the previous report with improvements in the calibration protocol for the instrument.) Results compared exceptionally well with laboratory values for serum, after correction for plasma-serum bias (10). As a whole, the Reflotron provided results
comparable to those obtainable with a laboratory system, regardless of the level of training of the instrument operator. However, the results were acceptable only when a properly collected blood sample was presented for analysis. The risk in the proliferation of this and similar systems is the assumption that sample collection is trivial, but a proper, professionally collected blood sample is essential. Instrumentation cannot compensate for a contaminated specimen.

We conclude that under proper conditions, the Reflotron is suitable for use in cholesterol screening programs. Its ease of operation, comparability with accepted systems, and, most importantly, its speed of analysis of whole-blood samples facilitate population studies. Its use is justified when all analytical aspects, especially sample collection, are controlled.

We thank Boehringer Mannheim Diagnostics, Indianapolis, IN, for the instruments and supplies used in the field studies described.

References

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Stability of Serum Fructosamine during Storage
Pertti Koskinen and Kerttu Irjala

Stability of serum fructosamine during storage was evaluated in serum specimens obtained from 27 diabetic individuals. The samples were divided into six aliquots, which were stored at −20 °C and −70 °C for two, eight, and 16 months. The minor systematic differences between the six treatments contrasted with the considerable variation of individual specimens. The mean percentage changes in the six treatments ranged between −4.6% and 7.5%, whereas the changes in individual specimens ranged from −20% to 26.7%. Several factors evidently contribute to this variation, one being progressive in vitro glycation, especially at −20 °C. Small changes in fructosamine concentrations between consecutively drawn specimens, determined after storage, evidently should be interpreted cautiously. Low temperatures, at least −70 °C, are preferable to minimize pre-analytical variation during storage.

Determination of glycated proteins in serum by their ability to reduce nitroblue tetrazolium in alkaline medium (the "fructosamine" assay) has been described as a means of evaluating glycemic control during the preceding one to three weeks in diabetes mellitus (1–4). Such assay is merit by its reasonable costs and technical simplicity, although its feasibility may be compromised by the problems associated with its standardization, the effects of variation in the reaction matrix, and the lack of specificity of the redox reaction involved (5–6). Furthermore, it is not possible to set the reaction parameters as precisely as required in all automatic analyzers (7). Fructosamine assay might be useful in follow-up studies where simple processing and subsequent analysis of large batches would be desirable. Such an approach assumes adequate stability of fructosamine-containing specimens during storage. The present study was designed to evaluate the effect of storage on results for serum fructosamine.

Materials and Methods

Serum fructosamine concentrations were determined in 27 serum specimens obtained from type I and type II diabetic individuals. Commercially available reagents (Fructosamine Test; Roche, Basle, Switzerland) were used. All serum specimens were then divided into six aliquots, three of which were stored at −20 °C and three at −70 °C. Samples stored at each of the two temperatures were analyzed two, eight, and 16 months later.

Specimens for the determination of blood glucose with the GA-1220 automatic glucose analyzer (DIC, Kagaku, Japan) were drawn at the same time as those for serum fructosamine. Two pools of sera were used for assessing the between-assay variation of the fructosamine method. Repeated measurements of analysis of variance were used for evaluating the effects of temperature and duration of storage. Blood-glucose concentrations were correlated with the changes of serum fructosamine found after storage at the various combinations of temperature and duration of storage.

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