
**Measurement of Blood Cholesterol with the Reflotron® Analyzer Evaluated**

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The Boehringer-Mannheim Reflotron®, a rapid, convenient blood chemistry analyzer intended for use in nonlaboratory settings, is widely used for screening to detect increased concentrations of blood cholesterol. Whole blood, usually a capillary-blood sample, plasma, or serum, is placed on a reagent strip that contains cholesterol oxidase, and undergoes enzymatic determination. The strips are read in a reflectance photometer, providing a value within 3 min. We evaluated this machine for precision, accuracy, and linearity in two environments. The first was a laboratory where we compared in duplicate Reflotron results for capillary blood, venous blood, and plasma samples (y) with the plasma results (x) obtained with the modified Abell–Kendall reference method (n = 43, mean = 6.11, SD = 1.74 mmol/L, CV = 1.4%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (and SD) cholesterol, mmol/L</th>
<th>CV, %</th>
<th>Slope</th>
<th>y-intercept, mmol/L</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary</td>
<td>5.58 (1.51)</td>
<td>4.7</td>
<td>0.85</td>
<td>0.37</td>
<td>0.979</td>
</tr>
<tr>
<td>Venous</td>
<td>5.62 (1.39)</td>
<td>3.2</td>
<td>0.77</td>
<td>0.89</td>
<td>0.973</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.63 (1.31)</td>
<td>3.1</td>
<td>0.75</td>
<td>1.09</td>
<td>0.991</td>
</tr>
</tbody>
</table>

We also tested the analyzer in the field, measuring capillary blood from 304 adult participants, in duplicate, with the Reflotron, and compared the results with those for venous plasma samples drawn simultaneously and measured in the reference laboratory (ABA-100, Abbott; n = 403, mean = 5.31, SD = 1.10 mmol/L).

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<th>Slope</th>
<th>y-intercept, mmol/L</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary (first)</td>
<td>5.06 (1.03)</td>
<td>0.87</td>
<td>0.45</td>
<td>0.929</td>
</tr>
<tr>
<td>Capillary (second)</td>
<td>5.10 (1.03)</td>
<td>0.86</td>
<td>0.55</td>
<td>0.916</td>
</tr>
</tbody>
</table>

Although the precision in both the laboratory and field settings was acceptable, there was significant negative bias for the Reflotron. In the field setting, results by the Reflotron were not linearly related to cholesterol concentrations. A second-degree polynomial (y = −57.3 + 1.60x − 0.0017x², where y is the Reflotron and x the reference method) best described the relationship.

Recognizing the inaccuracy, the manufacturer has recalibrated the system. However, use of this analyzer for screening should always include ongoing quality control, with split samples being assayed by a reliable laboratory.

**Automated Ektachem Method for Measuring Sodium in Sweat, Robert V. Coyne and David F. Walton (Dept. of Laboratories, Kaiser-Permanente Med. Center, 2025 Morse Ave., Sacramento, CA 95825)**

Recent improvements in iontophoresis equipment and sweat-collection devices provide the potential for significantly reducing the errors inherent in some steps of the sweat-test procedure used in diagnosing cystic fibrosis (1).

The "Macroduct" system (2), which provides for collection of undiluted sweat, obviates errors caused by sample manipulation and dilution before analysis. Even so, subsequent flame photometry, commonly used to measure the sodium concentration in sweat (3), remains a relatively time-consuming, labor-intensive, and only more-or-less accurate procedure.

We describe here an automated technique for quantifying sodium in Macroduct-collected sweat, with use of ion-selective electrodes for the simultaneous analysis for sodium and potassium (Ektachem 400 Analyzer; Eastman Kodak Co., Rochester, NY 14650).

Our first attempts at using the serum sodium channel were unsuccessful; the instrument did not "recognize" the sweat specimen on the sample slide and indicated "Invalid slide reading" (error code IR). However, when the sweat sample was prepared and presented as if it were urine, the instrument recognized the sample and proceeded with the analysis. Therefore, we used the Kodak urine sodium methodology without modification for the sweat samples (4), diluting 10 µL of sweat specimen to 50 µL with Kodak Ektachem Urine Electrolyte Diluent; printed results were multiplied by the five-fold-dilution factor.

In our initial series of 28 patients, sodium values from the Ektachem 400 ranged from 7 to 97.5 mmol/L; results of concurrent analyses with a "KLiNa" flame photometer (Beckman Instruments, Inc., Brea, CA 92621) ranged from 8 to 94 mmol/L (mean KLiNa flame (x) = 24.946 mmol/L; mean Ektachem 400 (y) = 26.375 mmol/L). The difference in means is statistically significant (paired t-test; P = 0.0025) but clinically insignificant, and is consistent with results obtained for serum samples analyzed by these two methodologies. Linear regression analysis yielded r = 0.994 (P <0.0001), y-intercept = 0.95, and slope = 1.0192. Between-run precision studies (n = 17; x = 15.74) indicated 1 SD = 0.52 mmol/L and CV = 3.3% for the Ektachem 400 procedure; each of the 17 sodium values represents the result from a separate dilution of a pooled normal sweat specimen. Within-run precision (n = 20; x = 28.1) indicated 1 SD = 0.34 mmol/L and CV = 1.2%.

We thank Dr. A. Leong, Dept. of Pediatrics, for his clinical assistance.

References
Nephelometric and Immunodiffusion Assays for the Fourth Component of Complement in Acquired C1 Esterase Inhibitor Deficiency, Lynn B. Keil and Vincent A. DeBari (The Renal Lab., St. Joseph's Hosp. and Med. Center, 703 Main St., Paterson, NJ 07503)

Severe deficiency states, resulting in a variety of clinical syndromes, have been described for most of the components of the complement system. In most cases these are hereditary, and the well-known disease entity, hereditary angioedema, results from a virtual absence of C1 esterase inhibitor. Much less common, with fewer than 40 cases having been reported in the literature, is the acquired form of C1 esterase inhibitor deficiency (AC1ID). In this syndrome, the early components of the classical pathway present severely decreased values when measured both by functional assays for bioactivity and by chemical assays for immunoreactivity ("antigen assays") (1). Invariably the value of C3, as well as C5–C9, is normal or near normal by both functional and antigen assays.

In our laboratory, we routinely use end-point radial immunodiffusion (RID) to assay C3 and C4. Our detection limit for C4, assayed with the kit from Kallestad Laboratories (Chaska, MN), is 14 mg/L. Despite this, sera from seven individuals with AC1ID, each having measurable quantities of C4 by rate immunonephelometry (ICS Immunochemy Analyzer; Beckman Instruments, Fullerton, CA) that exceeded the lower limit of detection of our RID assay, failed to produce a precipitin ring. The values for C4 (means of duplicate measurements by nephelometry) in serum from these patients were 381, 84.9, 48.8, 96.6, 34.2, 67.2, and 41.4 mg/L for patients 1–7, respectively.

In Figure 1, the upper panels demonstrate the apparent inability to detect C4 by RID. In the lower panels, the failure of sera from cases 2–7 to precipitate with anti-C4 (Figure 1D) is contrasted with the reactions for a series of standards ranging in C4 concentration from 170 to 6 mg/L (Figure 1C).

Previously, case 1 was presented as a possible example of a patient with "macro C4" (2). We suggested, based on the discrepant immunochemical C4 values and on immunoelectrophoretic observations, that this subject had circulating C4 that was precipitated by antibodies in a homogenous system but that, in its bound form, was too large to migrate through the pores of a 1.5% agarose gel. We strengthened that conclusion by demonstrating that the same nephelometric antiserum that precipitated the C4 in a suspension system did not react with the serum from case 1, either on a double-diffusion plate or on immunoelectrophoresis. The serum from this patient also demonstrated preformed protein complexes that migrated toward the cathode in a 0.6% ("high electroendosmosis") agarose gel, but values for circulating immune complexes were normal by both Raji cell and C1q binding assays.

These experiments, of course, do not allow us to discount the possibility that the C4 detected by nephelometry is artifactual, possibly some interfering substance. We believe, however, that this is unlikely, given our experiments with case 1.

In conclusion, we suggest that in laboratories where nephelometric methods are used for the assay of C4, the simple application of RID may greatly facilitate the diagnostic process for AC1ID when there is other presumptive evidence for this disease. Additionally, a normal C3 value, in the face of a depressed value of C4, should be treated with some degree of suspicion. Although that situation is not uncommon in some disease states, the inability to detect C4 by RID could be a valuable adjunct in the diagnosis of this extremely rare disease.

We thank Dr. H. Shah for referring Case 1 and Dr. B. Zuraw for the sera of cases 2–7. Dr. W. F. Kolb provided invaluable advice on AC1ID, and Dr. I. S. Kampa graciously allowed us the use of his Beckman ICS.

References

Determination of Ephedrine Bioavailability, A. Bailey, B. Lau, I. D. Watson, and M. J. Stewart (Glasgow Royal Infirmary, Glasgow G4 0SF, Scotland, U.K.; 1 on secondment from Clinical Pathology Unit, Princess Margaret Hospital, Hong Kong; 2 author for correspondence)

Ephedrine, a sympathomimetic compound, has hitherto been measured in plasma by complex gas–liquid chromatographic procedures (1). Owing to its low molar absorptivity, it has not hitherto been determined in plasma by liquid chromatography at concentrations that may be found during therapy. We have developed a simple procedure for use in bioavailability studies, involving liquid–solid extraction,