following reactions occur:

\[
\begin{align*}
    \text{H}_2\text{O}_2 & \rightarrow 2\text{H}^+ + \text{O}_2 + 2e^- \\
    4\text{H}^+ + \text{O}_2 & \rightarrow 2\text{H}_2\text{O} - 4e^- 
\end{align*}
\]

Any hydrogen peroxide diffusing back into the sample chamber is destroyed by a catalase (EC 1.11.1.6), to prevent interference with the analyses.

**Method.** Plasma and whole blood were obtained from nonhospitalized persons and from hospitalized patients. The blood was drawn into Vacutainer tubes (Becton-Dickinson, Rutherford, N. J. 07070) containing potassium oxalate and sodium fluoride. Blood samples containing no anticoagulant were collected for serum studies. Serum and plasma, promptly separated from the cells, were stored refrigerated until analyses, which were done according to the instrument manufacturers' instructions.

**Reproducibility.** The 2 and 5 g/liter standards provided for the YSI 23A were analyzed alternately on the YSI 23A, 14 times each in the course of one day. Means, standard deviations, and coefficients of variance were, respectively, 2005 ± 12.5 mg/liter, 0.6%, and 4923 ± 71.6 mg/liter, 1.4%. Monitrols, (Dade, Miami, Fla. 33152) with manufacturers' assay values of 880 and 2210 mg/liter were used as quality controls and were analyzed daily for 17 days. The means, SD, and CV were respectively 808 ± 37.8 mg/liter, 4.7% and 2100 ± 57.4 mg/liter, 2.7%. The differences between the manufacturers' values and the measured values may reflect glucose binding to protein (4, 5).

**Analytical recovery.** Glucose (200 to 4000 mg/liter) was added to two urine specimens which contained 120 and 2940 mg of endogenous glucose per liter. Analytical recovery of added glucose in the case of the urine with 120 mg of endogenous glucose ranged from 93.5 to 98.9 (mean, 95.4%); that from the other urine ranged from 96.7 to 99.5% (mean, 98.3%).

**Plasma glucose vs. serum glucose.** Serum and plasma samples from 74 persons were analyzed on the 23A; values ranged from 500 to 2800 mg/liter. The calculated t of 2.042 did not exceed the critical t at the 99% confidence interval, indicating no significant difference between values for serum and plasma glucose.

**Whole-blood glucose vs. serum glucose.** The well-known difference between values to be expected for glucose in whole blood and serum was evident. Whole blood and serum samples from 74 persons showed glucose values ranging from 500 to 2500 mg/liter. By calculation, using least-squares analysis, serum glucose equaled (1.135 × whole blood glucose) + 0.210. The calculated t (15.717) indicated a significant difference (P < 0.01). No correction was made for hematocrit.

**Serum glucose measurements on the YSI 23 A vs. the Beckman Glucose Analyzer.** We analyzed 75 sera by these two methods. The values ranged from 400 to 2400 mg/liter. Calculation of the best-fit line showed the 23A glucose values equaled (1.011 × Beckman glucose) − 0.112. The calculated t (3.177) indicated a significant difference (P < 0.01), although it was not apparent on inspection of the line of best fit. Under certain conditions the t value may be large when both random and constant errors are small (6).

**YSI 23A vs. Du Pont acu.** We analyzed 24 sera by these methods. The values ranged from 640 to 2410 mg/liter. The calculated t value (0.578) indicated no significant difference between the two sets of values.

We observed no significant discrepancies between glucose values obtained with the 23A and those from either the Beckman or the Du Pont systems. We find the 23A glucose analyzer to be reliable in routine laboratory use and have used it for longer than two years. With proper daily maintenance, the membrane lasts for about a week when 40 samples or more are being analyzed per day.

**References**


**Assessment of Thyroxine by Enzyme Immunoassay with the ABA-100 Analyzer**

**To the Editor:** We have evaluated the accuracy and precision of thyroxine assayed by the Enzyme Multiplied Immunoassay Technique (EMIT) with use of the ABA-100 (Abbott Laboratories, Diagnostics Division, South Pasadena, Calif. 91030) and compared results to those by a more widely used radioimmunoassay and to the EMIT AGA Autochemist thyroxine assay.

“EMIT ABA-100 thyroxine reagent kits,” obtained from Syva Corp., 3181 Porter Drive, Palo Alto, Calif. 94304, included the following reagents. Reagent A: antibody to thyroxine, NAD*, and 0.5 mol/liter glycine buffer, pH 5.0. Reagent B: thyroxine coupled to pig heart mitochondrial malate dehydrogenase (EC 1.1.1.37) in glycerol/phosphate buffer (0.4 mol/liter, pH 8.1), 4/6 by volume. Thyroxine Reagent A diluent: 1.0 mol/liter glycine buffer, pH 5.0. Thyroxine buffer: 291 mmol/liter glycine buffer, pH 7.5, containing L-malic acid, 196 mmol/liter. Serum-Pretreatment Reagent: 0.5 mol/liter NaOH with “Li-Plex.” The reagent kit also included a set of six lyophilized serum-based standard calibrators in the range 20–200 μg/liter. All reagents in the kit were used according to the manufacturer’s directions.

<table>
<thead>
<tr>
<th>Control</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator</td>
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<tr>
<td>Mode</td>
<td>RATE</td>
</tr>
<tr>
<td>Reaction dir.</td>
<td>UP</td>
</tr>
<tr>
<td>Analysis time</td>
<td>5 min</td>
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<tr>
<td>Revolutions</td>
<td>4</td>
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<tr>
<td>Filter</td>
<td>340/380</td>
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<tr>
<td>Syringe plate</td>
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<tr>
<td>Decimal</td>
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</tr>
<tr>
<td>Zero</td>
<td>0.000</td>
</tr>
<tr>
<td>Calibration factor</td>
<td>3070/F.F</td>
</tr>
<tr>
<td>Aug. dispenser</td>
<td>50 μl</td>
</tr>
</tbody>
</table>

Thyroxine buffer was placed in the primary syringe plate reservoir and working Reagent A (Reagent A reconstituted with diluent according to reagent instructions) was placed in the reservoir of the auxiliary dispenser. Twenty microliters of calibrators, controls, or sera were placed, in duplicate, into the cells of an ABA-100 multi-cuvet in positions 2 to 00, with an air-piston pipet. With a repeating dispensing syringe, 20 μl of serum-pre-treatment reagent was subsequently added to each sample cell. The cuvet was covered, its contents mixed by gently agitation, and it was allowed to stand at room temperature for 30 min. Fifty microliters of Reagent B was placed in the sample cups on the ABA-100 carousel in positions 2 to 00. After incubation, the cuvets containing

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the pretreated samples were placed in the ABA-100 incubator and the instrument cycle begun.

The ABA-100 dispensed 25 μl of Reagent B, 250 μl of thyroxine buffer, and 50 μl of working Reagent A into each sample well, an initial absorbance was read 5 and 10 min after reagent addition, and the 5-min change in absorbance was measured. For the standard curve, absorbance change for each calibrator was plotted vs. concentration on the EMI graph paper supplied with the reagents. Unknown concentrations were determined by reading from the standard curve.

Thyroxine values by EMI were determined (at Metpath Labs., Hackensack, N.J. 07606) with the AGA Autochemist (LKB, Bromma, Sweden) by the method of Galen and Foreman (1).

Thyroxine was also radioimmunassayed with reagent kits obtained from Corning Medical, Medfield, Mass. 02052. In this assay system a solid-phase separation technique is used, thyroxine antibody being covalently attached to glass beads and packaged in premeasured assay tubes.

Patients' sera were assayed in duplicate by both EMI technique with the ABA-100 and by radioimmunoassay. The period between analyses was less than 24 h, during which the samples were stored at 4 °C. Linear regression analysis (EMIT-ABA method on the y axis) of the paired data from 198 sera gave a correlation coefficient of 0.96, a slope of 0.96, and a y-intercept of −0.25. The normal ranges for these two methods are similar (45–120 μg/liter for EMI and 45–152 μg/liter for the radioimmunoassay). 96% of our comparative values were diagnostically consistent with these normal ranges. Fifty patients' sera were also assayed by the EMI technique with the ABA-100 and with the AGA Autochemist. Linear regression analysis of these paired data (EMIT-ABA method on the y axis) gave a correlation coefficient of 0.95, a slope of 0.70 and a y-intercept of 1.98.

Determination of the within-day precision of the EMI thyroxine assay for three sample concentrations gave coefficients of variation of 11.0% (mean, 25.0 ng/ml; range, 32.1–20.0 ng/ml; 3.1% (mean, 125.7 μg/liter); and 3.1% (mean, 125.7 μg/liter). Determination of day-to-day precision by assaying two control sera on each of 20 days gave coefficients of variation of 3.5% (mean 53.4 μg/liter) and 3.3% (mean 103.9 μg/liter). Analytical recoveries of thyroxine added to thyroxine-free sera averaged 95% over the range of 25–160 μg/liter.

To determine the stability of the EMI thyroxine reagent, we compiled the absorbance changes for each calibrator from 26 standard curves over a 20-day period. The variations in the calibrator raw data were determined and coefficients of variation in the range 2.4–2.9% were obtained.

The EMI ABA-100 thyroxine assay yields results that agree well with those obtained by a radioimmunoassay. Agreement was also found with the original application of the EMI thyroxine method on the AGA Autochemist, although there was more bias. Precision of the assay was excellent and the analytical recoveries were satisfactory. The major advantage in using this enzyme immunoassay for thyroxine is the stability of the standard curves within a given lot of reagents. The reagent stability allows longer use of a reagent kit than is possible with radioimmunoassay.

We conclude that the EMI ABA-100 thyroxine assay is a viable alternative to radioimmunoassay.

Reference

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Antisera as a Source of Inaccuracy in Protein Immunoassays

To the Editor:

The Letter from Pratt (1) on the anti-antiserum requirements for accurate assays of peptide hormones prompts me to point out that similar problems arise in the measurement of protein concentrations by automated immunoprecipitin and related techniques. Many human serum proteins, such as α₁-antitrypsin, display genetic variations in structure. It is relatively easy to produce antisera that are specific and do not give unwanted reactions with other proteins. However, for concentration measurements to be accurate, all the genetic variants of the prot-in should react to the same extent with the particular antiserum under the reaction conditions chosen—that is, an equal number of antibody molecules must be bound. It follows that a suitable antiserum must not contain antibodies to antigenic determinants present in some, but not all, of the protein variants.

In a single serum a large number of IgG proteins of widely different structure are present. Again, an antiserum for quantitative applications should contain only antibodies specific for the antigenic determinants present in all human IgG proteins. Grubb (2), using monoclonal IgG proteins, showed that selected antisera gave reasonably accurate immunoassay results for several preparations containing single IgG proteins, as judged by an independent chemical assay, and would therefore be likely to give accurate results for sera containing oligo- and polyclonal IgGs. We have suggested one technique by which such antisera might be prepared (3). However, the cost of producing antisera guaranteed to give accurate results is likely to be so high for some protein antigens as to restrict the use of immunoassays severely.

References

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Source of Error in Determination of Erythrocyte Folate by Competitive Binding Radioassay—Comments

To the Editor:

Netteland and Bakke (1) have identified problems in preparing whole-blood samples for radioassay of erythrocyte folate. In the protocol for our folate radioassay kit, the methodology for release of folate reactive in our competitive protein binding system involves a 21-fold dilution of ethylenediaminetraacetic acid-treated blood in ascorbic acid, 2 g/liter solution, (pH ~4.2) and subsequent incubation of the diluted sample for 90 min at room temperature. According to Netteland and Bakke (1), this kind of procedure yields suboptimal values for erythrocyte folate. However, cells are lysed and oligoglutamates hydrolyzed to monoglutamate under these conditions, and these effects, which were established in our laboratory, are based on the following findings (2):

(a) In binding reactions with milk protein at pH 9.3, pteroylmoglutamate (PteGlu) and 5-CH₃FH₄ are equally reactive (3). However, PteGlu3 and PteGlu7 (gift of Dr. S. Waxman, Mt. Sinai School of Medicine, New York; 1975) are, respectively, 2.9- and 5.6-fold more reactive than the monoglutamates (2). In view of the reactivities of these oligoglutamates and the increased re-