Improved Spectrophotometric Vitamin A Assay

To the Editor:

Considerable controversy seems to still exist in the choice of a simple, quick, and reliable method for Vitamin A assay, especially for field studies (1). The International Vitamin A Consultative Group (2) recommends the use of the Carr–Price reaction (3) and either trifluoroacetic or trichloroacetic acid (4), in spite of the problems created by the use of highly corrosive reagents, the critical timing for the readings, and the interference of hemolysis, which may be quite frequent in field studies.

The spectrophotometric procedure of Bessey et al. (5) has been claimed to be disadvantageous because it requires two sets of readings, i.e., before and after irradiation. In fact, this may be advantageous, especially when working in the micro scale, because it minimizes errors due to optical and geometrical factors, thus demanding less skill from the operator.

In our experience, the spectrophotometric procedure as originally described presents the following disadvantages: (a) the high absorbance of the kerosene used to extract the retinol, a serious drawback when the method is scaled down to 50 or 100 μl; (b) the sensitivity of the method to hemolysis; (c) the increasing unavailability of the originally described reagents and equipment (kerosene and ultraviolet lamp).

We believe we have dealt satisfactorily with these problems by effecting the following modifications:

(a) The kerosene used is ordinary jet fuel (Turbo-fuel A-1 from the Esso Standard Oil Co.; obtainable at any airport) purified by distillation. From 300 ml of kerosene, the first 50 ml is discarded, and the next 200 ml is collected and kept in an amber-colored bottle, to be mixed with xylool just before use. The absorbance of the kerosene/xylool mixture at 328 nm, vs. water, is about 0.080.

(b) The ultraviolet lamp used for irradiation is an ordinary 250-W mercury-vapor lamp, from which the glass cover is removed by cutting it at the base. It is mounted on a suitable reflector. A potassium dichromate solution, 0.1 mg/liter, must be used as a filter between the lamp and the samples. This solution is kept in a glass container to provide a 5-cm light path.

We recommend that each laboratory determine the irradiation conditions, using as criteria (a) that blank values do not change upon irradiation, and (b) that destruction of standard solutions of retinol be complete after 20 to 60 min. Hepatic Vitamin A can be determined in a 50-volumes homogenate in glycerol water (1/1). We have established that the liver Vitamin A concentration is stable at room temperature (about 26°C) for at least 30 h after removal of the organ.

With the modifications described above, volumes of sample between 50 and 1500 μl give quite satisfactory results. Hemolysis has very little effect upon the final results: hemolysed samples give higher readings both before and after irradiation, and a two-tailed Student's t-test shows the difference between the mean values of hemolysed and the corresponding nonhemolysed samples of serum to be not significant (Table 1). The slight modifications described here of the method of Bessey et al. render it quite reliable, and the simplest, quickest, and most suitable for field studies.

Financed by grants 710-0328 from The Ford Foundation, and 236-CT from FINEP (Brazil).

References


Cirlene Ribeiro Correia de Araújo
Hernando Flores

Departamento de Nutrição do Centro de Ciências da Saúde e Departamento de Bioquímica do Centro de Ciências Biológicas
Universidade Federal de Pernambuco, Cidade Universitária, 50000 Recife, Pernambuco, Brazil.

Evaluation of a Glucose Method in Which a Hydrogen Peroxide Electrode Is Used

To the Editor:

Alpert (1) recently described the operation and features of the YSI 23A Glucose Analyzer (Yellow Spring Instrument Co., Yellow Springs, Ohio 45387) but his reports in this series do not include a comparison of results obtained on the 23A with those by other methods. Passey et al. (2) evaluated 10 glucose methods, but the 23A was not included in this study.

Here, we compare this instrument to the Beckman Glucose Analyzer and the Du Pont ac, in which a glucose oxidase method and a hexokinase method, respectively, are used. The YSI 23A analyzer is based on polarographic principles (3). Glucose oxidase (EC 1.1.3.4) is layered between two membranes of differing materials, premounted on an O ring. Glucose diffuses through a polycarbonate membrane to the site of the glucose oxidase, where it is oxidized to H₂O₂ and gluconic acid. The hydrogen peroxide formed is proportional to the glucose concentration; it then diffuses through the cellulose acetate membrane to the surface of the electrode, where the

---

Table 1. Effect of Hemolysis on the Spectrophotometric Determination of Serum Vitamin A

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>Vitamin A concn, μg/liter</th>
<th>Clear serum</th>
<th>Hemolysed aliquot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (16)</td>
<td>60.9 ± 9.7</td>
<td>67.1 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>Men (14)</td>
<td>72.8 ± 19.0</td>
<td>79.2 ± 18.3</td>
<td></td>
</tr>
<tr>
<td>Male rats (8)</td>
<td>37.8 ± 11.7</td>
<td>40.2 ± 10.0</td>
<td></td>
</tr>
<tr>
<td>Female rats (12)</td>
<td>31.8 ± 10.8</td>
<td>36.0 ± 12.3</td>
<td></td>
</tr>
</tbody>
</table>

* Samples of clear serum were obtained by centrifugation after clotting of the blood at room temperature. Sufficient erythrocytes were added to aliquots of the clear sera to give a hemoglobin concentration between 60 and 80 g/liter. Vitamin A was then assayed in the clear sera and the hemolysed aliquots by the procedure of Bessey et al. (5) with the modifications reported in the text. Values shown are means ± SD.
following reactions occur:

\[ \text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}^- \]
\[ 4\text{H}^+ + \text{O}_2 \rightarrow 2\text{H}_2\text{O} - 4\text{e}^- \]

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 ± 12.8 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’ results 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 23 A 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 23 A vs. Du Pont aca.** 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 23 A and those from either the Beckman or the Du Pont systems. We find the 23 A 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**


**Clinical Chemistry Training Program University of Dayton, Dayton, Ohio 45409**

**Gilbert H. Nelson**

Miami Valley Hospital
Dayton, Ohio 45409

**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 Multiplicated 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, 136 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.

**Control**

<table>
<thead>
<tr>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubator</td>
</tr>
<tr>
<td>Mode</td>
</tr>
<tr>
<td>Reaction dir.</td>
</tr>
<tr>
<td>Analysis time</td>
</tr>
<tr>
<td>Revolutions</td>
</tr>
<tr>
<td>Filter</td>
</tr>
<tr>
<td>Syringe plate</td>
</tr>
<tr>
<td>Decimal</td>
</tr>
<tr>
<td>Zero</td>
</tr>
<tr>
<td>Calibration factor</td>
</tr>
<tr>
<td>Aug. dispenser</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 0, with an air-piston pipet. With a repeating dispensing syringe, 20 μl of serum-pretreatment 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

**CLINICAL CHEMISTRY, Vol. 24, No. 2, 1978 387**