Automated Measurement of Serum Thyroxine with the “ARIA II,” as Compared with Competitive Protein Binding and Radioimmunoassay

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Two conventional serum thyroxine assays, run in separate laboratories, one by competitive protein binding and one by radioimmunoassay, were used to evaluate the automated ARIA II (Becton Dickinson Immunodiagnostics) serum thyroxine assay. Competitive protein binding as compared to ARIA II with 111 clinical serum samples gave a slope of 1.04 and a correlation coefficient of 0.94. The radioimmunoassay comparison to ARIA II with 53 clinical serum samples gave a slope of 1.05 and a correlation coefficient of 0.92. The ARIA II inter-assay coefficient of variation for 10 replicates of low, medium, and high thyroxine serum samples was 6.2, 6.0, and 2.9%, respectively, with an inter-assay coefficient of variation among 15 different assays of 15.5, 10.1, and 7.9%. The automated ARIA II, with a 2.2-min cycle per sample, gives results that compare well with those by manual methodology.

In the ARIA II serum thyroxine assay, a specific antibody is used that is covalently attached to a solid support, providing an immobilized antibody system (1). The sample or standard flows over the immobilized antibody, followed sequentially by a known volume of [125I]thyroxine from a separate reservoir. Sample and tracer are not mixed. The labeled antigen attaches to the remaining antibody binding sites in the antibody chamber. The unbound thyroxine, including the free labeled thyroxine, enters a gamma-counting flow cell within a sodium iodine detector, where its radioactivity is counted. An eluting agent then passes over the immobilized antibody and releases the bound thyroxine, which enters the flow cell and its radioactivity is counted. The elution curve with the bound and free peaks in Figure 1 represents a typical cycle, illustrating the binding and releasing phenomenon. Residual elution buffer is washed from the antibody chamber with adsorption buffer and the next sample is introduced. The serum thyroxine radioimmunoassay as run by the ARIA II is presently a 2.2-min cycle for each sample, with no incubation or waiting required.

Materials

The ARIA II serum thyroxine radioimmunoassay kit provides the following materials:

Thyroxine standards, which are prediluted in sample buffer containing thyroxine-free serum equivalent to the final clinical sample dilution of 41-fold. The standards are poured in the sample cup and are run directly. The nominal concentrations of the standards are 0, 2.5, 5, 10, 20, 40 µg/dl, with actual 41-fold smaller concentrations of 0, 0.06, 0.12, 0.24, 0.49, 0.98 µg/dl.

[125I]Thyroxine, monolabeled, with a specific activity of 250 Ci/g (2) and diluted in sample buffer.

Thyroxine antibody chamber: This is rabbit antiserum covalently attached to a solid support, and placed in a Teflon chamber.

Adsorption buffer: 0.1 mol/liter glycylglycine, pH 10.5; bovine serum albumin, 100 mg/liter; ethanol, 50 ml/liter; 8-anilino-1-naphthalene sulfonic acid, 300 mg/liter sodium azide, 200 mg/liter.

Elution buffer: An equivalence mixture of methanol and adsorption buffer lacking the bovine serum albumin.

Gamma cocktail: Dimethylammonium lineardodecylbenzenesulfonate in water, 10 g/liter.

Methods

Automated Assay

The ARIA II pump rates, valves, and timing are controlled by a self-contained computer that is programmed with a floppy disc (3). The reagents are attached to the instrument, which is then ready for operation. The individual serum

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Received Oct. 3, 1977; accepted Nov. 30, 1977.
Fig. 1. Typical ARIA II elution curve, showing the bound and free peaks, the 2.2-min cycle, the percent bound, and the regions of count accumulation.

Fig. 2. Correlation between thyroxine values as measured by competitive protein binding and ARIA II.

Manual Assays

The clinical sera and the thyroxine results by a modified competitive protein binding assay (5) using alkaline Sephadex columns and thyroxine-binding globulin were obtained from the L.D.S. Hospital, Salt Lake City, Utah. The clinical sera and the thyroxine results by a conventional radioimmunoassay (6) using a polyethylene glycol separation were obtained from Holy Cross Hospital, Salt Lake City, Utah.

Results

ARIA II serum thyroxine assay, on the ordinate, was compared with a competitive protein binding assay, on the abscissa, for 111 individual sera. Figure 2 summarizes the results. Comparison of ARIA II thyroxine assay with a conventional tube-type radioimmunoassay (n = 53) gave the results shown in Figure 3.

The ARIA II intra-assay coefficient of variation for 10 replicates of sera with low, medium, and high thyroxine concentrations was 6.2, 6.0, and 2.9%, respectively; the interassay coefficient of variation among 15 different assays was 15.5, 10.1, and 7.9%, respectively.

Linearity was shown by diluting individual serum samples 41-fold and further dilution to 51-, 67-, 101-, 201-fold. The straight line is verification of linearity (Figure 4).

The cross-reaction of the antibody used with the ARIA II system was determined against eight potentially cross-reacting substances. The D- and L- isomers of thyroxine showed 100% cross-reaction, and the D- and L- isomers of triiodothyronine showed less than 0.27% cross-reaction, while salicylic acid, phenylbutazone, phenytoin, iodotyrosine, and diiodotyrosine all cross-reacted by less than 0.01% (Table 1).

Sensitivity was adequate to discriminate between a 1 μg/dl (10 μg/liter) thyroxine serum concentration and thyroxine-free serum.

Discussion

Comparison of competitive protein binding and conventional tube-type radioimmunoassay with the automated ARIA II results shows excellent results with individual serum samples. The ARIA II has shown inter- and intra-assay coefficients of variation that compare well with those for manual assays (6-8). Other radioimmunoassay variables—sensitivity, specificity, and linearity—are all more than adequate for a reliable serum thyroxine test system (9, 10).

The automation of the serum thyroxine assay by ARIA II.

FIG. 3. Correlation between thyroxine values as measured by conventional tube-type radioimmunoassay and ARIA II. Slope, 1.05; intercept, -0.45; r, 0.92; N, 53

FIG. 4. Linearity of thyroxine values measured by ARIA II serum thyroxine assay, as shown by dilution of individual serum samples.

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allows thyroxine assay in 2.2 min, with a minimum of sample preparation. Total thyroxine is measured in a serum sample and the results are printed out with no operator or additional computing intervention.

References
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CLIN. CHEM. 24/2, 344–347 (1978)

High-Speed Automated Atomic Absorption Spectrophotometry

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We describe an integrated automated system for the atomic absorption spectrophotometer. The system makes use of a light-dependent resistor to detect the presence of a marker ion which is present along with the sample ion in the flame. An electronic circuit has been developed which enables the marker ion to trigger the read signal of the spectrophotometer. The system has been tested by determining magnesium and calcium in serum. Alternative modes of operation and different potential applications are discussed.

Sometimes the clinical chemistry laboratory may have to determine certain cations in large numbers of samples. It is therefore of interest in such situations to develop techniques that make use of the inherent high speed capability of the atomic absorption spectrophotometer (AAS). Of particular relevance is the determination of magnesium and calcium in samples of serum and plasma.

The estimation of these metals has been automated to various degrees previously (1–3). With conventional contin-

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Received Sept. 1, 1977; accepted Dec. 5, 1977.