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A FULLY AUTOMATED TRIIODOTHYRONINE UPTAKE ASSAY UTILIZING A RECYCLING TRIIODOTHYRONINE ANTIBODY-SEPARATING AGENT ON ARIA II

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A 2-min fully automated serum triiodothyronine uptake test using recycling immunoadsorbent methodology and instrumentation is described. The immunoadsorbent is a specific antibody to triiodothyronine covalently attached to a solid support and contained in a Teflon chamber. The clinical serum sample is automatically aspirated, diluted, mixed with radioactive tracer and assayed for triiodothyronine uptake in conjunction with radioactive counting and data reduction. The comparison of values obtained by this method on 124 clinical serum samples to values obtained with a manual kit gave a 98% correlation. The inter- and intra-assay coefficients of variation were less than 5% as duplicates and less than 6% as singlet determinations through the hyper-, normal, and hypo-relative thyroid binding globulin capacity ranges.

INTRODUCTION

Triiodothyronine (T_3) uptake is a function of the degree of unsaturation of serum thyroid binding proteins, primarily thyroid binding globulin (TBG). Historically radioactive tracer was introduced into whole blood (1). Red blood cells were used to separate free from bound tracer requiring standardization of hematocrit. Later, noncellular separating agents such as charcoal, resins, and inorganic adsorbents (2–4) were employed. These separating agents have imparted greater ease to the T_3 uptake test, but not without adding their own unique problems (3,5). Recently, antibodies specific to T_3 have been used as separating agents by covalent or other attachment to solid supports such as particles or tube walls (3,6,7). The use of specific antibodies has minimized many of the inherent problems associated with noncellular separating agents, but in turn requires accurate dispensing or coating of tubes, long incubations, and high consumption of antisera when utilized as a throwaway item.

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The T_3 uptake test is currently gaining greater clinical importance due to its use as a reflection of free thyroxine (FT_4) serum levels. The FT_4 is metabolically active in regulating oxidative cellular processes (8), and it has been suggested that the level of serum FT_4 is directly related to the product of total thyroxine (TT_4) concentration and serum thyroxine binding protein saturation (9,10). Thus, with new understanding of thyroid function and the increased acceptance of the T_3 uptake relationship to FT_4, automation of the T_3 uptake test has become important. To date, the methods of automation have utilized current separating techniques, incorporating automatic dilution, centrifugation, radioactive counting, and tube mobilization.

An automated flow-through system ARIA II™, incorporating a unique reusable specific antibody as the separating agent, has been utilized for T_3 uptake. The separating agent, or immunoadsorbent, is a specific antibody covalently attached to a solid support (11). This system eliminates some weaknesses associated with T_3 uptake such as aliquoting precisely the separating agent, and tube manipulation, along with pipetting and mixing of reagents and antibody waste. All dilutions and mixing are done automatically. The counting and data reduction with printout are part of the automated system. Equilibration time is controlled and uniform from sample to sample, with a cycle time of 2 min from sample aspiration to printed uptake value. All samples are handled individually, yet identically, alleviating restricted batch sizes often found with manual assay.

MATERIALS

The *antibody chamber* contains the immunoadsorbent, which is used as the separating agent. Rabbit antisera produced against a T_3 bovine thyroglobulin conjugate (12) specific for T_3 is covalently attached to a solid support and placed in a Teflon chamber (11).

The *adsorption buffer* is 0.02 M sodium phosphate, pH 7.5, 0.05 M sodium chloride, 0.2 g/l sodium azide and 0.05 g/l gentamicin sulfate. This buffer is used to introduce the sample into the antibody chamber.

The *elution buffer* is 0.1 M glycinate, pH 10.5, and 0.1 g/l sodium azide, diluted with an equal volume of methanol. This buffer is used to elute the bound T_3 from the immunoadsorbent.

The *sample buffer* is 0.02 M sodium phosphate, pH 7.5, 0.05 M sodium chloride, 0.1 g/l bovine serum albumin, 0.2 g/l sodium azide and 0.05 g/l gentamicin sulfate, and ^{125}I-T_3 with a specific activity in the order of 3000 mCi/mg (13). This buffer is used to dilute the serum sample.

METHODS

Automated Assay

The ARIA II instrumentation is readied by attaching the previously described materials and programming the pump rates, valve positions, and cycle time into the self-contained computer with a floppy disk (14). A minimum of 200 μl of serum is placed in the individual sample cups and the instrument is initiated to run. From this point, the sample is aspirated automatically and diluted fortyfold with sample buffer. A portion of the diluted sample is filtered and cooled just prior to entering the antibody chamber containing immunoadsorbent. The immunoadsorbent maintains near-maximum retention of tracer in the absence of serum proteins. The serum protein-bound tracer passes through the immunoadsorbent and into a gamma counting sodium iodide scintillation detecting flow cell for counting. This fraction represents the free peak. The tracer which was not bound

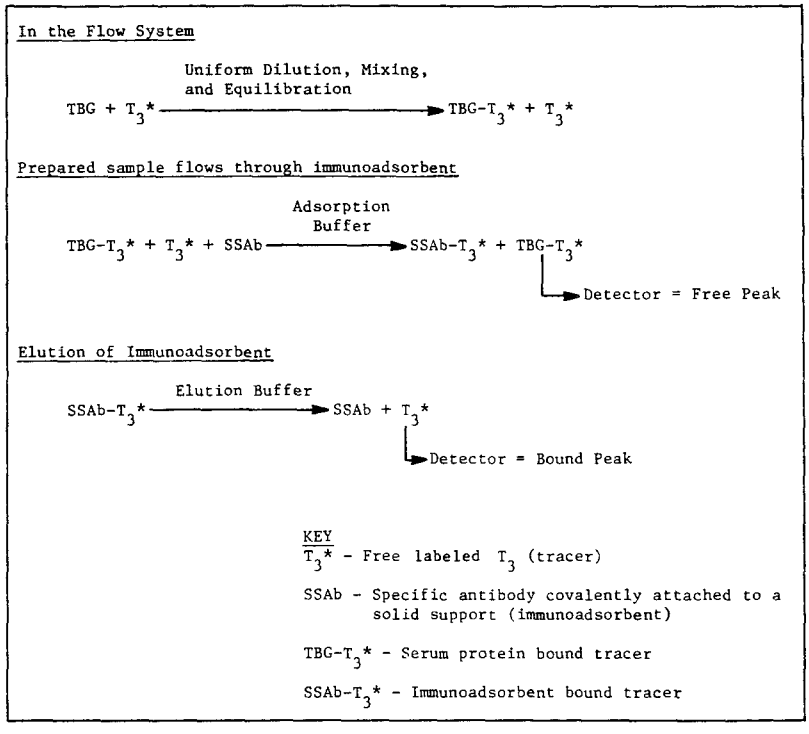

FIG. 1. Graphic representation of the incubation reaction and subsequent separation of the free and bound fractions using the automated T_3 uptake methodology.

by the serum proteins is retained in the antibody chamber and subsequently is quantitatively released from the immunoadsorbent by the elution buffer flowing over the immunoadsorbent. This previously retained tracer, in turn, enters the radiation counting flow cell for counting as the bound peak (Fig. 1). A typical 2-min cycle elution curve (Fig. 2) with free and bound peaks illustrates separation utilizing the recycled flow-through immunoadsorbent.

In summary, the assay sequence includes the sample aspiration and dilution, a free–bound separation using an immunoadsorbent, a counting

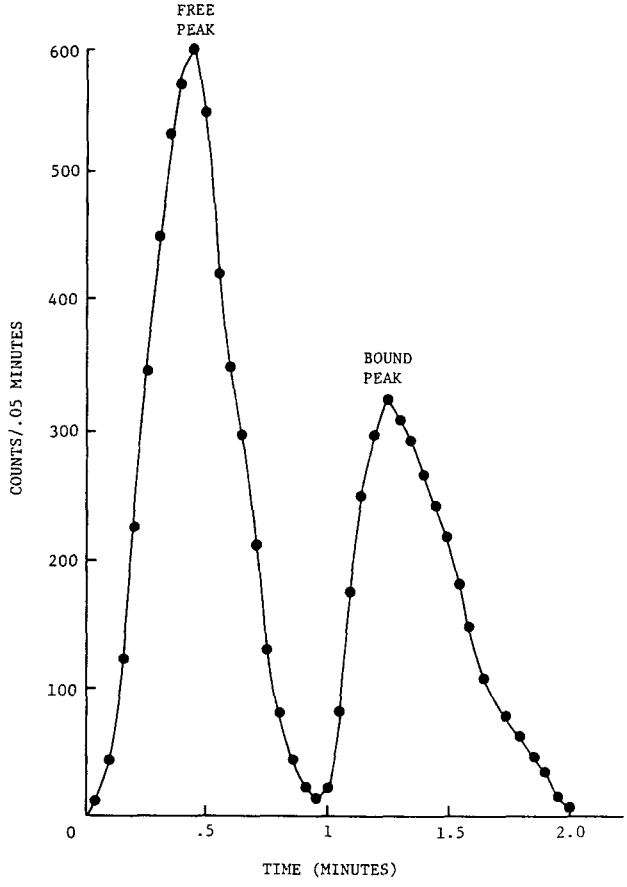

FIG. 2. Elution curve of the automated T_3 uptake test on the ARIA II instrumentation of a clinical serum sample showing the free peak, the bound peak, and the 2.0-min cycle time.

$$\%T_3 \text{ Uptake} = \frac{(\%B(s))\ (\%\text{ Uptake }(R))}{\%B(R)}$$

Where: $\%B(s) = \frac{B(s)}{F(s) + B(s)} \times 100$

$$\%B(R) = \frac{B(R)}{F(R) + B(R)} \times 100$$

and B(s) = Bound $^{125}I\text{-}T_3$ eluted from the immunoadsorbent of the unknown sample.

F(s) = Serum protein bound $^{125}I\text{-}T_3$ not retained by the immunoadsorbent of the unknown sample.

B(R) = Bound $^{125}I\text{-}T_3$ eluted from the immunoadsorbent of the reference pool.

F(R) = Serum protein bound $^{125}I\text{-}T_3$ not retained by the immunoadsorbent of the reference pool.

%Uptake (R) = Percent uptake value of the reference as verified by independent reference methods.

$$T_3 \text{ Ratio} = \frac{\%T_3 \text{ Uptake}}{\text{Average } \%T_3 \text{ Uptake}}$$

Where: Average $\%T_3$ Uptake = The percent uptake value at the midpoint of the normal range.

FIG. 3. Data reduction formula utilized by the ARIA II to automatically calculate percent T_3 uptake and T_3 ratio in the T_3 uptake assay.

step, and finally data reduction, which is accomplished automatically at the completion of each cycle. The data are displayed on a video screen and printed as hard copy giving percent T_3 uptake and T_3 ratio (Fig. 3). The various thyroid parameters such as free thyroxine index (FTI) and T_7 are easily computed using the T_3 uptake and a TT_4 value. Standardization of the ARIA II T_3 uptake assay is accomplished using a reference serum with a known T_3 uptake value as verified by independent reference methods. All clinical samples are calculated against this reference, which gives continuity of inter- and intra-assay values, as well as some degree of correlation to other T_3 uptake methods.

Manual Assay

Clinical sera as well as T_3 uptake values used for the comparison method were obtained from the LDS Hospital, Salt Lake City, Utah. This hospital determines T_3 uptake using a kit from Diagnostics Corporation of America, which employs an insoluble colloidal suspension of bovine serum albumin as the free–bound separating agent.

RESULTS

A comparison of the ARIA II T_3 uptake assay (ordinate) with the manual tube kit (abscissa) was evaluated using 124 individual clinical serum samples. The T_3 uptake values from one assay method to another are not quantitatively comparable in the hyper- and hypo-TBG ranges; however, the actual comparative data points are displayed by this presentation (Fig. 4). The range for normal TBG relative capacity in the ARIA II T_3 uptake assay is 24–34% uptake, or a T_3 ratio of 0.83 to 1.17. The hyperthyroid range is less than 24% with the hypothyroid greater than 34%. Results of

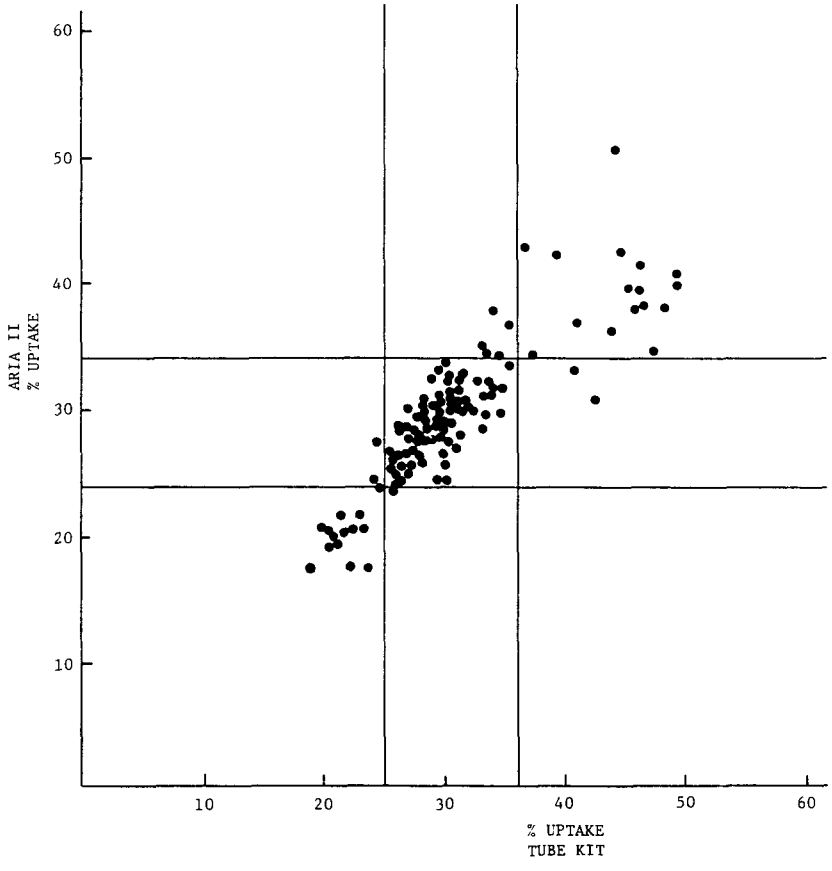

FIG. 4. Comparative data results of 124 clinical samples evaluated by the ARIA II T_3 uptake assay (ordinate) and a commercial tube assay (abscissa) showing the normal TBG relative capacity range of both assays.

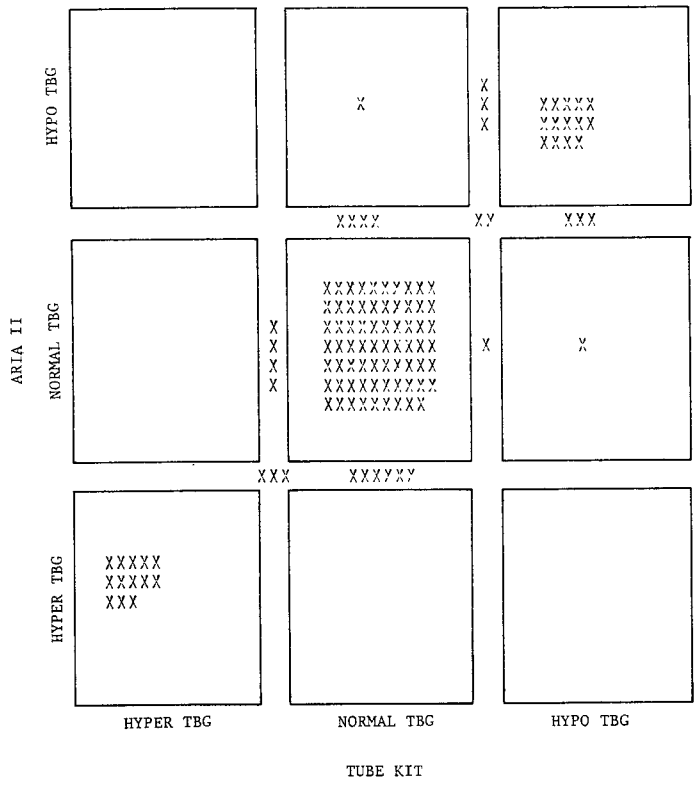

FIG. 5. Qualitative T_3 uptake results of ARIA II as compared to a commercial tube assay for 124 clinical samples showing results in the hyper-, normal, and hypo-TBG relative capacity ranges. Marginal ×'s represent values within one standard deviation of the boundaries as determined by intra-assay reproducibility of a normal reference serum.

the clinical evaluation by a nine-box presentation (Fig. 5) indicate good agreement with regard to thyroid condition. Using boundaries to include the intra-assay normal TBG pool standard deviation (0.9), the ARIA II samples in the hyper-, normal, and hypo-TBG relative capacity ranges agreed at 87%, 99% and 89%, respectively, with an overall agreement of 98%. Pooled sera to represent the three ranges of hyper-, normal, and hypo-TBG were used to determine the inter- and intra-assay variability. Inter-assay variability of seven individual runs on the ARIA II was determined for samples run as singlets and as consecutive duplicates. Coefficients of variation for single samples were 5.7%, 2.7%, and 2.0% for the hyper-, normal, and hypo-TBG range, respectively, and duplicate determinations gave coefficients of variation of 4.0%, 1.5%, and 2.4% (Table 1). These data

TABLE 1. **Inter- and Intra-Assay Reproducibility Giving Percent Uptake and Coefficients of Variation for Hyper-, Normal, and Hypo-TBG Serum Pools**

		Inter-Assay		
	% Uptake	S.D.	%C.V.	N
Hyper-TGB pool				
Singlet	19.7	1.1	5.7	7
Duplicates	19.3	0.8	4.0	7
Normal TBG pool				
Singlet	29.5	0.8	2.7	7
Duplicates	29.4	0.4	1.5	7
Hypo-TBG pool				
Singlet	39.2	0.8	2.0	7
Duplicates	39.2	1.0	2.4	7
		Intra-Assay		
	Mean (% Uptake)	S.D.	%C.V.	N
Hyper-TBG pool	18.4	0.4	2.1	10
Normal TBG pool	29.3	0.9	3.2	10
Hypo-TBG pool	38.8	0.5	1.2	10

indicate that a singlet determination is satisfactory for most clinical use. The intra-assay variability as groups of ten gave coefficients of variation of 2.1%, 3.2%, and 1.2%, respectively (Table 1).

DISCUSSION

The T_3 uptake test does not measure T_3 but in fact measures predominantly the relative TBG saturation and capacity (15). Total T_4 and T_3, as well as serum binding proteins, are the variables affecting the T_3 uptake test. There is ambiguity and a lack of uniformity to the definition of percent uptake. That is to say, the percent tracer bound by the separating agent is expressed as the percent uptake, or the percent of the tracer bound to TBG is expressed as the percent uptake (16). Also, the normal ranges expressed as percent uptake will vary from one method to another with some at 25–35% uptake and others at 40–60% uptake. These inconsistencies contribute to a certain amount of confusion regarding the T_3 uptake test.

Various mathematical combinations of the percent T_3 uptake and TT_4 are used, such as FTI and T_7, to estimate FT_4 concentration. The ARIA II gives results as percent T_3 uptake and the T_3 ratio, which can be used to

calculate a T_7 and an FTI, respectively. It is noteworthy to mention that the FTI is a more transferable number between different T_3 uptake methodologies because it utilizes the ratio which places the normal range around one. In fact, most T_3 uptake tests in common use give similar limits to the normal TBG capacity range when expressed as a ratio, while being very dissimilar as a percent uptake. Since a preferred quantitation has not yet emerged. The ARIA II presents the data as a T_3 ratio and as percent T_3 uptake.

The T_3 uptake test using the ARIA II system has shown good correlation to a manual tube type assay. The inter- and intra-assay variability are very acceptable. Carryover from sample to sample often associated with flowing systems (17) has presented no problem as demonstrated by inter-assay coefficients of variation less than 6% using either singlet or duplicate determinations. The complete automation from direct serum aspiration to T_3 uptake data presentation at 2-min intervals has been provided as *STAT* T_3 uptake test.

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