AN ECONOMIC, SEMIAUTOMATIC METHOD FOR IN VITRO THYROID FUNCTION TESTS

P. Y. Wong, T. D. Cradduck, and D. E. Wood

Toronto General Hospital, Toronto, Ontario, Canada

Modified serum thyroxine and serum thyroid hormone binding-capacity assays are presented. These modifications permit significant economy without loss of accuracy or reproducibility. "Kits" are not purchased; rather specified quality control is imposed on all stages of the procedure from purchase and assay of the reagents through test performance to the analysis of the data produced. The resultant protocols permit a high-volume, low-cost laboratory operation that maintains appropriate laboratory proficiency.

Early uses for "saturation analysis" (*1*), "competitive protein-binding analysis" (*2–4*) or "radiotransient assay" (*5,6*) included the assay of endocrinologic, particularly thyroid, function. These applications persist to the present with the estimate of thyroxine and T_3 resin uptake being standard laboratory procedures and triiodothyronine, TSH, and TRF being commonly used research procedures.

In this communication, we wish to report our experience in a large-volume service laboratory. In addition to accuracy and reproducibility, it is essential that the cost per test be minimized. Both the technical and the reagent components must be carefully monitored. Applying our present workload statistics to catalog prices, it would cost on the order of $30,000 per annum to purchase T_3 and T_4 diagnostic kits. The technique described here, reducing the costs by approximately two-thirds, has resulted in a sensitive and reproducible method.

Significant technician time is saved by using a digital computer or programmable calculator. In our department, the digital computer assigned to the Anger gamma camera is made available for batch processing of results.

MATERIAL AND EQUIPMENT

Serum samples were obtained from patients and healthy laboratory workers at the Toronto General Hospital. Materials used included: thyroxine standard (Sigma Chemical, St. Louis, Mo.), ^{125}I-thyroxine (Abbott Laboratories, North Chicago, Ill.), barbital buffer (pH 8.6, 0.075 *M*), resin [Fisher, Rexyn 202 (Cl-SO_4), pH 8.6 20–40 mesh], Validate (Warner-Lambert Co., Morris Plains, N.J.), Trisorb Kit (Abbott Laboratories, North Chicago, Ill.), and Sephadex G-25: (Coarse, Pharmacia, Sweden). An LKB 300-sample automatic gamma counter with teletype printout and Digital PDP 11/40 computer with Gamma II operating system were also used.

METHODS

Preparation of ^{125}I-T_4-thyroxine-binding globulin. A 5 $\times$ 1.5-cm Sephadex G-25 column was used for the separation of ^{125}I-iodide from ^{125}I-thyroxine. A few grams of gel powder were put into 500 ml of distilled water in a beaker, mixed, and left to stand overnight. The column was packed and was washed with barbital buffer. One part ^{125}I-thyroxine solution (about 250 μCi in 50% propylene glycol) was mixed with four parts barbital buffer. The mixture was transferred onto the column. The column was drained and was subsequently washed with 2 $\times$ 5-ml barbital buffer. The eluate was collected and was diluted to 5,000 ml. The final ^{125}I-T_4-TBG solution was prepared to consist of 2% pooled serum and approximately 0.5 μCi/ml in barbital buffer.

Procedures for the determination of serum thyroxine and effective thyroxine ratio. Both serum

Received May 29, 1974; original accepted June 24, 1974.

For reprints contact: P. Y. Wong, Division of Nuclear Medicine, Toronto General Hospital, 101 College St., Toronto, Ontario, Canada.

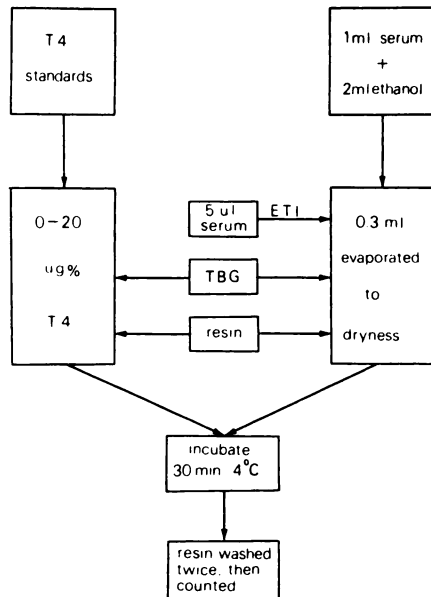

FIG. 1. Flow chart of procedure followed to determine serum T_4 and effective thyroxine index (ETI).

thyroxine (T_4) and effective thyroxine index (ETI) were modified from the work of Murphy and Pattee (*3,4*) and Mincey, et al (*5*). The experimental procedures are summarized in Fig. 1. Generally 1.0 ml of serum was added to 2.0 ml of 95% ethanol in a 12 × 75-mm polystyrene tube, mixed with a vortex mixer, and centrifuged for 10 min at 3,000 rpm. Three 0.3-ml aliquots of the supernatant were transferred into each of three tubes (two tubes for T_4 and one tube for ETI). The ethanol extracts were evaporated to dryness under nitrogen at 45°C. After 5 μl patient's serum had been pipetted into the corresponding ETI tube, 1.0 ml ^{125}I-T_4-TBG solution was added to the bottom of each tube. The initial radioactivity of T_4 standards was determined. All T_4 and ETI tubes were placed in a water bath at 45°C for 5 min to ensure solution of the thyroxine and equilibration with the isotope. The tubes were placed in an ice bath for at least 10 min. Resin (~150 ± 1.5 mg) was added to each tube using a dispenser (Fig. 2). The test tubes were incubated for 30 min in an ice bath and were shaken for 30 sec at 5-min intervals. At the end of the incubation period, the resin was washed twice with cold distilled water. The radioactivity held by the resin was determined.

Calculations. A punched paper tape is produced by the automatic sample changer and this is used as input to a DEC Gamma 11 system (PDP 11/40) through a fast paper tape reader.

ETIs require only a simple ratio calculation. Consequently, a tape can be read and the results for 40 samples printed in a little under 3 min. For T_4s a curve-fitting program fits a fifth-order polynomial to the nine standard values and then tabulates the results for duplicate samples by interpolating from that standard curve. The results for 50 duplicate samples are tabulated in 6 min.

RESULTS

Table 1 shows the purity of several lots of commercial ^{125}I-thyroxine. All but one (Abbott's Lot #320) contained less than 3% ^{125}I-iodide.

The reduction of the sensitivity of the T_4 standard curve by about 10% ^{125}I-iodide in ^{125}I-T_4-TBG is shown in Fig. 3 (Curve A). After ^{125}I-iodide had been removed from the ^{125}I-T_4, the sensitivity of the T_4 standard curve B was comparable to that of the commercial curve C (Abbott's Tetrasorb).

Figure 4 shows the change of sensitivity of T_4 standard curves upon the dilution of serum proteins.

Of 269 patients selected for this study, 83 were euthyroid, 86 were euthyroid on oral contraceptives, 54 were hyperthyroid, and 46 were hypothyroid. The mean ± s.d. values of T_3 uptake, serum T_4 concentration, and ETI are shown in Table 2.

Table 3 shows the day-to-day variations of the procedure in our laboratory with the method of T_4

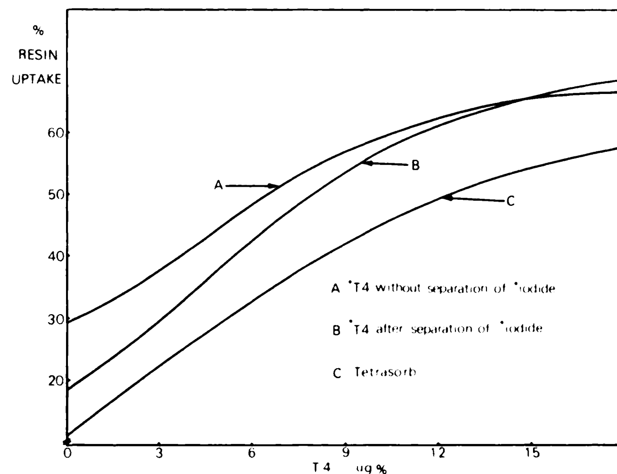

FIG. 2. Resin dispenser for serum T_4 and ETI determinations. Mean ± s.d. for dispensing is 156.4 ± 1.4 mg (n = 35).

TABLE 1. PURITY OF COMMERCIAL ^{125}I-THYROXINE

Company	Lot no.	% Free ^{125}I
Abbott	315	1.6
Abbott	316	1.7
Abbott	320	5.1
Frosst	23,470	2.7
Amersham/Searle	182	1.9

TABLE 2. VALUES OF T_3 UPTAKE, SERUM T_4, AND EFFECTIVE THYROXINE INDEX (ETI) (MEAN $\pm$ s.d.)

Patient category	T_3 resin uptake (%)	T_4 (μg%)*	ETI
Euthyroid (83)	29.6 ± 2.0	7.3 ± 1.3	0.98 ± 0.14
Euthyroid on estrogens (86)	23.1 ± 2.1	9.6 ± 1.9	1.09 ± 0.11
Hyperthyroid (54)	38.8 ± 4.7	14.9 ± 2.8	1.48 ± 0.13
Hypothyroid (46)	23.0 ± 2.3	2.5 ± 1.0	0.45 ± 0.09

* Without correction of extraction efficiency.

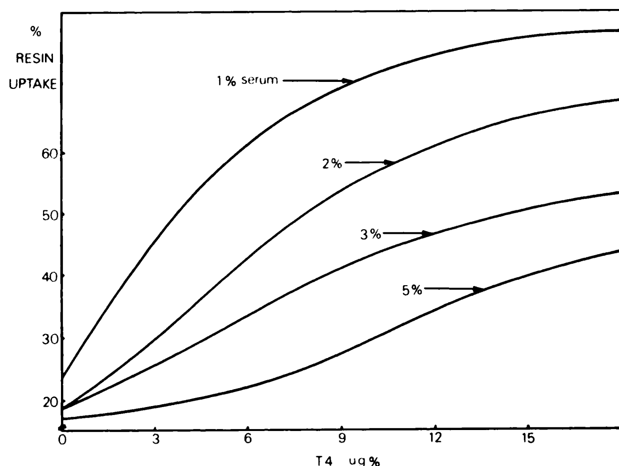

FIG. 3. Comparison of sensitivity of T_4 standard curves using purified and unpurified ^{125}I-T_4.

and ETI assays using Validate Lot #0193022 throughout May to August 1973.

Table 4 shows the results of external quality control organized by the College of American Pathologists.

DISCUSSION

Whereas the purity of commercially available ^{125}I-thyroxine could not be confirmed in our laboratory, the sample that contained the largest quantity of ^{125}I-iodide was shipped midsummer in temperatures over 90°F. The high percentage of ^{125}I-iodide may be due to heat decomposition during transportation.

The sensitivity and reproducibility of serum thyroxine and ETI values depends upon the quality of ^{125}I-T_4-TBG. The latter depends upon the purity of ^{125}I-T_4 and the serum protein concentration. As shown in Figure 3, the sensitivity of T_4 standard curve is increased upon the removal of ^{125}I-iodide. Figure 4 shows that the optimum serum protein concentration to cover 0–18 μg% range was about 2%. Any decrease leads to the reduction of sensitivity and reproducibility of the assay. Iodine-125-T_4-TBG prepared by this method was stored up to 2 months at 4°C. Separate experiments showed that standard curves could be reproduced from day to day, from period to period. The results shown in Table 2 agree with the results of Thorson, et al (7) that the ETR provides "a precise measure of thyroid status irrespective of serum protein-binding concentration."

Although T_3 uptake and serum T_4 assay are the most common laboratory aids in assessing thyroid function, the well-known influence of hormonal therapy, oral contraceptives, pregnancy, and changes in the serum proteins on these two tests occasionally presents a diagnostic dilemma that may be avoided by the ETI test. However, serum thyroxine still reflects a true hormone level in the circulation and is extremely valuable for both diagnosis and followup of thyroid disease. At the Toronto General Hospital we have performed both serum T_4 and ETI assays since April 1973. By using the method described above, we have handled over 16,000 T_4 and ETI determinations in the past 12 months at an average cost of approximately 30 cents per test (excluding costs for floor space, utilities, or professional services). A significant saving has been achieved as compared with the use of commercial "kits".

The normal range of our ETI is wider (0.70–1.30) than the ETR range (0.86–1.13). This may be due to two different formulas being used to obtain the ETI and the ETR, i.e., ETI is resin counts of sample/resin counts of reference serum and ETR is supernatant counts of reference serum/supernatant counts of sample.

These two methods were compared and no significant difference was found. In general, reproducibility would depend upon many factors including accuracy of pipetting, temperature control, timing, proper storage of reagents, and the technicians' work performance.

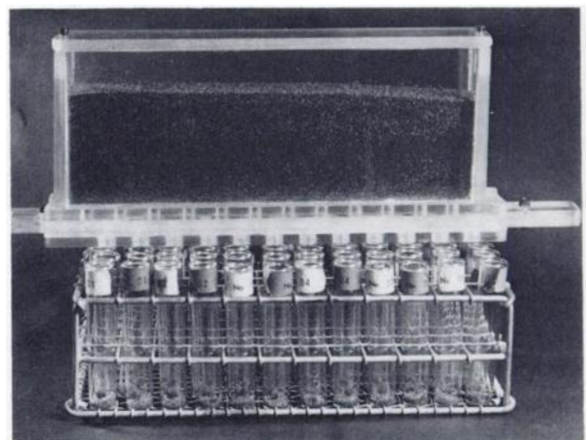

FIG. 4. Comparison of sensitivity of T_4 standard curves upon change in serum protein concentrations.

TABLE 3. DAY-TO-DAY VARIATIONS OF T_4 AND ETI (VALIDATE LOT 0193022)

	T_4				ETI			
	May 1973	June 1973	July 1973	August 1973	May 1973	June 1973	July 1973	August 1973
Total no.	23	60	59	65	23	69	61	64
Mean ± s.d.	7.2 ± 0.4	6.9 ± 0.4	7.1 ± 0.6	7.2 ± 0.6	1.09 ± 0.04	1.07 ± 0.05	1.07 ± 0.05	1.06 ± 0.04
C.V. (%)	5.6	5.8	8.5	8.3	3.7	4.7	4.7	3.8

TABLE 4. CAP QUALITY CONTROL 1973—SERUM THYROXINE

	N-01	N-02	N-03	N-04	N-05	N-06	N-07	N-08
Our result	8.1	29.1	3.6	25.5	18.6	14.7	8.9	3.3
CAP Mean ± s.d.	7.4 ± 1.4	26.7 ± 9.7	3.6 ± 1.3	26.0 ± 6.6	16.7 ± 1.7	16.0 ± 1.9	7.9 ± 0.9	2.9 ± 0.8

Significant saving in technician time is achieved by using the digital computer to perform the necessary calculations and tabulate the results. Additionally, a greater degree of accuracy and reliability is attained by eliminating the sources of human error.

Although the Gamma 11 system is specifically designed for gamma camera imaging, it can be used for batch processing under a FOCAL interpreter. The in vitro calculations can be made between collections of patient data without disconnecting the gamma camera on-line feature.

QUALITY CONTROL

The LKB automatic gamma counter is standardized every day by a senior technologist using a ^{129}I counting standard.

Validate, supplied by Warner-Lambert, is used for internal quality control. Three control sera are employed in each run of determinations. Between May and August 1973, six registered technologists handled the T_4 and ETI assays using the method described above. The results are highly reproducible as shown in Table 3.

External quality control is the proficiency testing in the laboratory through unknown samples obtained from the College of American Pathologists, U.S. Department of Health, Education, and Welfare, and Canadian Society of Clinical Chemists. We participated in the CAP quality evaluation program in 1973 and the results are summarized in Table 4.

ACKNOWLEDGMENTS

The authors wish to thank the technologists in the Division of Nuclear Medicine at the Toronto General Hospital for performing T_4 and ETI determinations, and Susan Slattery for typing the manuscripts. This work was originally presented at the 17th annual meeting of the Canadian Society of Clinical Chemists in August, 1973.

REFERENCES

1. Ekins RP: The estimation of thyroxine in human plasma by an electrophoretic technique. *Clin Chim Acta* 5: 453–459, 1960

2. Murphy BEP: Application of the property of protein-binding to the assay of minute quantities of hormones and other substances. *Nature* 201: 679–682, 1964

3. Murphy BEP, Pattee CJ: Determination of thyroxine utilizing the property of protein-binding. *J Clin Endocrinol* 24: 187–196, 1964

4. Murphy BEP: The determination of thyroxine by competitive protein-binding analysis employing an anion-exchange resin and radiothyroxine. *J Lab Clin Med* 66: 161–167, 1965

5. Mincey EK, Thorson SC, Brown JL: A new in-vitro blood test for determining thyroid status—the effective thyroxine ratio. *Clin Biochem* 4: 216–221, 1971

6. Mincey EK, Thorson SC, Brown JL, et al: A new parameter of thyroid function—the effective thyroxine ratio. *J Nucl Med* 13: 165–168, 1972

7. Thorson SC, Mincey EK, McIntosh HW, et al: Evaluation of a new in vitro blood test for determining thyroid status: The effective thyroxine ratio. *Br Med J* 2: 67–71, 1972