Radioassay

Comparison of Certain Aspects of 24 Thyroxine I-125 Kits

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We compiled data comparing certain aspects of 24 T_4 kits: 18 RIA and 6 CPB. We found significant differences in reproducibility among kits, as well as variance in number of pipetting steps, incubation time, reagent preparation, and cost. This information may assist in selecting kits for user evaluation. Technologists may not depend solely on assurances by manufacturers' representatives when choosing a kit.

Radionuclide methods for determination of T_4 feature competition of radioactive thyroxine and patient thyroxine for either thyroid-binding protein (CPB or T_4D) or antithyroxine antibody (RIA). Such methods have largely supplanted earlier chemical procedures for measurement of T^4. A large number of manufacturers now offer radionuclide T_4 kits; inevitably, there are variations a-

TABLE 1. Comparison of T_4 CPB Kits

	C.V. (%)	Incubation time (min)	Centrifuge time (min)	Pipet steps	B/F antigen separation	Blocking agent or extraction	Tubes-per-kit	Cost-per-tube
Bio-Rad Tetra-Count II	8.8	20 (2 steps)	0	3	Resin column	Silicate	50	1.36
							100	1.05
							200	1.03
							500	1.01
Dade Data-tope	6.1	20 (2 steps)	10 (2 steps)	4	Resin tablet	Alcohol	10	1.80
							50	1.35
							100	1.10
Mallinckrodt ETR	5.2	60	5	3	Resin strip	Alcohol	15	3.25
							50	2.91
							250	2.21
Radx T-4 Tetrathyrofile	6.6	20	3 (2 steps)	2	Protein-coated kaolin	H_2SO_4	50	0.80
							100	0.70
Squibb Thyro-stat T_4	6.6	10 (2 steps)	8 (2 steps)	4	Resin tablet	Alcohol	25	2.10
							100	1.70
							500	1.60
Thyroid Diagnostic Sta-T_4	7.5	15	10 (2 steps)	3	$MgCO_3$	Ethanol and silica	30	1.65
							100	1.50
							300	1.30
							500	1.10

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mong manufacturers in various aspects of their kits and relatively little information concerning this is available in the literature (*1–9*). Because most technologists are not able to evaluate very many of these kits themselves before making a choice, we present data on certain features of 24 different I-125 kits for measurement of T_4. Information includes intra-assay reproducibility at the same normal range level, various parameters that affect ease of performance, and price. We evaluated 6 CPB methods and 18 RIA kits.

Materials and Methods

In most instances the data represent one or more evaluation kits supplied by a manufacturer. All kits except four were tested by the same technologist. Reproducibility (expressed as coefficient of variation, C.V.) was determined by assay of 20 aliquots in duplicate on the same day from a single pool of frozen serum, which we used for the St. Francis Hospital quality control program. The approximate thyroxine concentration was 8.0 μg/dl. In most cases the 20-determination series was performed on each of two successive days and the C.V. is the average of the two results. We were not able to obtain intra-assay reproducibility data at high and low T_4 levels because of the limited number of assays available in the kits we obtained. Likewise, accuracy studies were not performed in a sufficient number of kits to be included. Pipet variability of less than 2% was verified using the dilute isotope technique (*10*). We used either a Searle model 4222 automatic gamma counter (Searle Radiographics, Des Plaines, IL) or a Packard model 5210 automatic gamma counter (Packard Instrument Co., Inc., Downers Grove, IL). Both counters perform satisfactorally according to the chi-square test.

Results

Our findings are summarized in Tables 1 and 2; C.V. represents "within-run" or intra-assay reproducibility. Pipetting of standards and patient samples are included as one pipet step. If more than one size of pipet was needed to dispense the same reagent to different tubes, each pipet size was considered one additional pipetting step. Cost-per-tube is calculated on the basis of list price for a single kit and does not take into account the number of times a standard curve is generated, whether specimens are assayed singly or in duplicate, whether volume discounts are obtained, or whether special prices are available from local company representatives—all of which significantly influence reagent cost-per-tube.

Certain information cannot be conveniently included in Tables 1 and 2. For example:

□ Nuclear Medical Systems requires two incubation periods at different temperatures: 30 min at 40° C and 15 min at 4° C. Amersham T_4 (without PEG) requires three incubation periods; 60 min at 37° C, 60 min at room temperature, and 10 min at room temperature. Kits from Abbott T^4 (without PEG), Curtis Nuclear (now called DCA Corp.), Diagnostic Products, Meloy, Bio-Rad Tetra-Count, Dade CPB, Radx, and Squibb all require two incubation periods at room temperature.

□ Abbott, Antibodies Inc., Dade, Kallestad, Mallinckrodt SPAC, Meloy, Nuclear Medical Laboratories, Nuclear Medical Systems, Roche, and Smith-Kline kits are all supplied with ready-to-use standards. The remaining kits provide lyophilized standards, which must be reconstituted.

□ Curtis Nuclear (DCA), Meloy, Nuclear Medical Laboratories, and Nuclear Medical Systems require only a 10-μl patient sample. Beckman, Kallestad, and Smith-Kline use a 20-μl patient specimen; while Abbott T_4RIA (PEG), Antibodies Inc., Bio-Rad RIA, Corning,Dade RIA, Diagnostic Products, Mallinckrodt SPAC, and Roche need 25-μl of serum. The other kits use larger specimens.

□ The Abbott T_4 without PEG separates free radioactive T_4 from bound complex by means of a resin sponge, which requires three washes.

□ Beckman literature stated that the kit reagents were only useful for seven days after reconstitution. We have been told that the allowable time period has been extended to 14 days, but this is still much shorter than the reconstituted reagent life of other kits.

□ Smith-Kline offers a choice of a 30-min incubation at 37° or 60 min at room temperature.

□ Corning requires a 5-min pre-test centrifugation step in order to insure that all of the antibody-coated glass beads in each tube are located in the bottom of the tube. This step has been included in the centrifugation time of Table 2. All of the tubes can be centrifuged together when the kit is received, providing that the tubes are then stored upright.

□ Corning manufactures the only kit among those tested that is supplied with controls as well as standards.

□ Amersham T_4 (without PEG) and Mallinckrodt ETR need a special rotator during incubation time.

□ Mallinckrodt ETR does not use a standard curve; instead, patient specimens are compared to a normal "standard" serum and a ratio is obtained. Most other kits supply at least five standards. Radx and Squibb use only four (zero, hypo, normal, and hyper range); Bio-Rad Tetra-Count and Dade CPB have only three (hypo, normal, and hyper range); and Thyroid Diagnostics provides only two (normal and elevated).

Discussion

Our results demonstrate that significant differences do exist among T_4 kits. In general, the T_4 RIA procedures seemed to provide better reproducibility than the CPB kits. The RIA kits were no more expensive than CPB; in some cases they cost less.

Other theoretical advantages of RIA over CPB are elimination of sample extraction and use of a smaller patient specimen. A practical RIA advantage is comparative insensitivity to serum fatty acid concentration, which be-

TABLE 2. Comparison of T_4 RIA Kits

	C.V. (%)	Incubation time (min)	Centrifuge time (min)	Pipet steps	B/F antigen separation	Blocking agent or extraction	Tubes-per-kit	Cost-per-tube
Abbott T-4 RIA	5.3	90 (2 steps)	0	4	Resin sponge	Sodium trichloroacetate	25 100 500	1.85 1.72 1.28
Abbott T-4 RIA (PEG)	6.6	60 (37°)	10	4	PEG*	ANS‡	50 100 500	1.86 1.72 1.29
Amersham T-4	3.2	130 (3 steps)	0	5	Talc powder	Thiomersalate	50 100	1.50 1.35
Amersham T-4 RIA (PEG)	4.0	45	15	4	PEG	Thiomersalate	50 100	1.20 1.10
Antibodies, Inc. T_4 RIA	5.1	60	10	3	Double Ab+	ANS	100	0.50
Beckman "Single Label" T_4	5.7	60 (37°)	15	6	Double Ab	ANS	50 100	0.70 0.65
Bio-Rad Quantimune T_4 RIA	3.3	60	10	3	Solid phase	ANS	100 200 500	0.80 0.70 0.60
Corning Immunophase T_4 RIA	2.3	60	15 (2 steps)	2	Solid phase	Thiomerosal	120	1.46
Curtis-Nuclear (DCA) Liqua T_4	7.1	65 (2 steps)	10	4	Double Ab + PEG	Sodium salicylate	100 200	0.93 0.83
Dade Data-tope T_4 RIA	2.8	30 (37°)	15	4	Double Ab + PEG	ANS	50 100	0.82 0.65
Diagnostic Products	7.0	45 (2 steps)	15	5	Double Ab	ANS	100 500	0.60 0.45
Kallestad Quantitope I-125 T_4	6.0	30 (50°)	20	3	Double Ab	ANS	100 200	0.85 0.77
Mallinckrodt SPAC T_4 RIA	3.5	60 (37°)	0	2	Solid phase	ANS	50 100	2.20 2.14
Meloy Immunostat T_4	3.5	35 (2 steps)	10	4	Ammonium sulfate	ANS	120 500	0.96 0.65
Nuclear Medical Lab Tetra-Tab RIA	5.4	30	10	5	Ammonium sulfate	HCl	40 200	1.79 0.90
Nuclear Medical Systems Tetra-RIA	5.6	45 (2 steps)	15	4	Double Ab	ANS	40 80	1.10 1.05
Roche T-4 RIA	3.3	45	20	4	PEG	ANS and sodium salicylate	100	1.55
SKI Immunotube T-4	7.2	30 (37°)	0	2	Solid phase	ANS and salicylate	25 100	1.50 0.95

* PEG = polyethylene glycol.
+ Ab = antibody.
‡ ANS = 8-anilino-1-naphthalene sulfonic acid.

comes elevated if a sample is allowed to remain at room temperature for long periods of time (as might occur in shipment by mail) and which could falsely elevate CPB methods (*11*,*12*).

Summary

We surveyed certain features of T_4 methods from a large number of manufacturers in order to demonstrate some of the variables that exist among kits. This information may provide a framework for selecting kits, which then may be further evaluated under the conditions of the technologist's own laboratory. It is imperative that every laboratory perform its own in-depth reproducibility and accuracy studies—and not rely on assurances of others, statements from manufacturers representatives, or comparison of assay values with another kit involving only a few controls or patient specimens.

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