Radioimmunoassay kits for thyroid hormones

P. JACKSON, S. LEES, M. POW, G. W. WILLING, AND D. WATSON

From Area Laboratory, King Edward VII Hospital, Windsor, Berkshire

SYNOPSIS Characteristics of the T4 and T3 radioimmunoassay kits that are commercially available in the UK are described. Useful comparative data and criticism are given about many of these kits. Some unacceptable variation in accuracy is demonstrated.

An expanding variety of basically similar reagents for radioassay procedures is becoming commercially available. It seems that this profusion of manufacturing sources is causing some clinical scientists to doubt the reliability of certain 'kit' procedures. However, we believe that selected immunoassay reagents manufactured outside the district hospital or area laboratory will have a useful and fairly permanent place in the laboratory, exactly as bulk reagents for automated analysis have at present.

A recent World Health Organisation report on Biological Standardisation states 'it would be desirable to adopt a national scheme for the licensing of all reagents used . . . this would include the registration of kits and reagents, and release of batches only on the recommendation of the national

centre . . .'. The report (World Health Organisation, 1975) goes on to say that while peripheral laboratories would do the more 'frequently needed assays', eg, h-PL, some steroids, T4 . . . , a peripheral laboratory would only rarely do its own labelling of antigens'. We doubt whether it would be wise or practical to single out reagents for binding assay and cytochemical bioassay systems from all the other reagents used in medical diagnosis—for registration and approval. However, there will always be a need for information about the purity of reagents, and ease and comparability of analyses performed with them, to suggest the better and best 'buys'.

The T4 and T3 RIA kits

Complete lists of T4 and T3 RIA kits and reagents currently available in the UK are given in tables

Received for publication 20 April 1976

Test	Supplier and source	Cost ¹
<i>Kits of approx 100 tests</i>		
T4 RIA	Abbott	£85 per 100
T4 Single label	Beckman	£60 per 144
Quantimune T4	Bio-Rad Labs	£72 per 100
Tetra-Tab	Chem. Lab. Instr. (Nuclear Medical Labs)	£150 per 200
Immophase T4	Corning	£85 per 120
T4 feg	Diamed Diagnostics (Biodata)	£49 per 125
T4	Hoechst	£60 per 100
Mat T4	Mallinckrodt	£126 per 250
T4	Micro-Bio Labs (Diagnostics Biochem)	£35 per 100
T4 RIA	Precision Assays	£40 per 120
T4 RIA	Radiochemical Centre	£52 per 100
Immunotube T4	Smith Kline Instruments	£45 per 100
T4	Micro-Bio Labs (Wien)	£80 per 200
Tetralute ²	Ames	£102 per 112
<i>Reagents separately available</i>		
T4 Antiserum	Advanced Lab Techniques	£22 (500 tests)
T4 Antiserum	Corning	£50 (100 tests)
T4 Antiserum	Diamed Diagnostics (Serono)	£11 (125 tests)
T4 Antiserum	Micro-Bio Labs (Diagnostics Biochem)	£44 (500 tests)
T4 Label	Abbott	£93 (500 µCi; 450 µCi/µg)
T4 Label	Corning	£15 (120 tests)
T4 Label	Eurotope Services (Cea Ire Sorin)	£39 (500 µCi; 100 µCi/µg)
T4 Label	Radiochemical Centre	£35 (500 µCi; 50 µCi/µg)
T4 Label	New England Nuclear	£68 (500 µCi; 125 µCi/µg)

Table I *Commercial kits and reagents for T4 radioimmunoassays available in UK*

¹Cost of kit materials (exclusive of tax) for given number of tests including standards, controls, blanks, etc, April 1976

²Simplest CPB kit given for comparison

Test	Supplier and source	Cost ¹
Test kits		
T3 RIA kit	Abbott	£114 per 100
Seralute T3	Ames	£54 per 100
Quantimune T3	Bio-Rad Labs	£67 per 100
TRIK T3	Eurotope Services (Cea Ire Sorin)	£45 per 100
Immophase T3	Corning	£50 per 120
T3 peg	Diamed Diagnostics (Biodata)	£59 per 125
Total serum T3	Dow-Lepetit	£46 per 100
T3 RIA	Hoechst	£50 per 50
Mat T3	Mallinckrodt	£92 per 100
T3	Micro-Bio Labs (Diagnostics Biochem)	£86 per 250
Phadebas T3	Pharmacia	£41 per 100
T3 RIA	Radiochemical Centre	£25 per 50
Reagents separately available		
T3 Antiserum	Corning	£32 (120 tests)
T3 Antiserum	Diamed Diagnostics (Biodata)	£11 (125 tests)
T3 Antiserum	Micro-Bio Labs (Diagnostics Biochem)	£43 (500 tests)
T3 Label	Abbott	£93 (500 µCi; 450 µCi/µg)
T3 Label	Corning	£10 (120 tests)
T3 Label	Eurotope Services (Cea Ire Sorin)	£60 (100 µCi; 100 µCi/µg)
T3 Label	New England Nuclear	£68 (500 µCi; 125 µCi/µg)

Table II Commercial kits and reagents for T3 radioimmunoassays available in UK

¹Cost of kit materials (exclusive of tax) for given number of tests including standards, controls, blanks, etc, April 1976

and II. Because of their greater specificity and accuracy, only true radioimmunoassays are considered here. Because of their very limited clinical application, kits for serum rT3—available from Diamed Diagnostics (Biodata) and Micro-Bio Labs (Wien) have not been studied. Some experiences with the earlier competitive protein-binding T4 kits and T3 uptake test kits in this laboratory have previously been reported (Watson, 1974; Watson *et al*, 1974). The kits have previously been classified into so-called liquid phase and solid phase procedures, but this division is less appropriate today. The majority of methods utilize a solid phase step which may occur (1) at the end of the assay to obtain instant or rapid fixation of bound T4-antibody, (2) during the assay, in which the suspended solid is continuously agitated or rotated with the attendant step of stoppering, fixing, and unstoppering tubes, (3) at the start of the determination in which the T4-antibody

is itself coupled to a very fine solid suspension such as resin or glass. With the exception of de-ionized distilled water, all the reagents needed for the determination are supplied with each RIA kit. Most T4 and T3 kits are supplied with four to six dry 'standards'. The standards for Corning are in protein-based solution and for Bio-Rad composition not stated; all others are in human serum. Those of RCC are prepared in human serum 'maintained at uniform concentration'.

Reproducibility of results with individual kits

Some of the important features of selected T4 and T3 kits are given in tables III and IV. These reveal considerable variation in properties which might influence precision capability, for example, number of pipettings. Available information collected in this laboratory (table V) indicates that most (though not

Supplier and test	Time (h) required for 40-50 tests ¹	Special equipment needed ²	No. of precision pipettings	Separating agent for bound and unbound antigen	Slope ³ of linear RIA curve
Abott, T4 RIA	4-5	Ice bath; centrifuge	3	Resin sponge	1.4
Bio-Rad, Quantimune T4	3-5	Centrifuge	6	Anion-exchange columns	1.4
Corning Immophase	3-3.5	Centrifuge	2	None	3.4
Diamed Diagnostics T4 peg	5	Centrifuge	7	Polyethylene glycol solution	2.0
Hoechst, Insert T4	4-5	Inversion-mixer	2	Anion-exchange-resin stopper	1.7
Mallinckrodt, Mat	3-5	37°C bath; rotary mixer	2	Resin strip	1.7
Nuclear Medical Labs, Tetratab	3-5	Centrifuge	4	Ammonium sulphate-albumin suspension	1.7
Precision Assays	4	Centrifuge	4	Polyethylene glycol solution	2.4
RCC T4 RIA	3-3.5	37°C bath; rotary mixer	4	Sephadex suspension	2.2
Ames, Tetralute ⁴	3-3.5	None	3	Sephadex column	2.9
SKI Immotube	3	None	2	None	1.7

Table III Comparison of some RIA kits for T4 assay

¹From start of assay to reporting result

²Other than a gamma counter; all methods except Ames require a vortex mixer

³Ratio of high to low counts, ie, counts for 2 µg divided by those for 12 µg T4/dl or vice versa

⁴Data from the simplest CPB kit are given for comparison purposes

Supplier and test	Time (h) required for 40-50 tests ¹	Special equipment needed	Displacing agent	No. of precision pipettings	Separating agent for bound and unbound antigen	Slope ² of linear RIA curve
Abbott, T3 Riakit	about 25	Refrigerator centrifuge	Anilino-naphthalene sulphonate	4	Dextran-charcoal suspension	3.2
Ames, Seralute	5.5	None	None stated	3	Sephadex column	1.7
Bio-Rad, Quantimune	5.5	Incubator	Thyroxine	5	Anion-exchange columns	1.3
C-I-S, TRIKT3	5	Ice bath; centrifuge	Thiomersalate	5	Dextran-charcoal suspension	2.2
Dow-Lepetit, T3	7	Refrigerator	Anilino-naphthalene sulphonate	4	Dextran-charcoal suspension	1.7
Hoechst, T3	7	Inversion mixer	Anilino-naphthalene sulphonate	2	Anion-exchange resin stopper	1.4
Mallinkrodt, Mat T3	5	Mixer 37°C bath	Magnesium ANS	4	Resin strip	1.3
Pharmacia T3	10	Centrifuge	Aniline-naphthalene sulphonate	6	Sephadex-anti T3 complex	1.7
RCC T3	about 21	Rotary mixer	Thiomersalate	5	Sephadex powder suspension	1.5

Table IV Some RIA kits for T3 compared

¹From start of assay to reporting result²Ratio of high to low counts, ie, counts for 0.5 µgT3/1 divided by those for 2.5 µgT3/1 or vice versa

	Coefficient of variation found ¹		Suppliers' statement of precision ² and replicates recommended
	Within batch	Between batch	
<i>Thyroxine</i>			
Abbott, T4 RIA	4-8%	10-18%	CV 5-16%; duplicate
Ames, Tetralute	8-10%	20-26%	SD 0.75 µg/dl; NS
Bio-Rad, Quantimune	12-20%	—	NS; duplicate
Corning, Immophase	—	—	CV 5-7%; duplicate until experienced
Hoechst	8-10%	—	
Mallinckrodt, RIA-Mat	—	—	CV 3-10%; standards in duplicate
NML, Tetra-Tab	3-8%	5-12%	CV 3-8%; NS
Precision Assays	3-8%	—	Reject duplicates >5% mean; duplicate
SKI Immunotube	12-20%	—	CV 8-13%; duplicate until familiar
<i>Tri-iodothyronine</i>			
Abbott, Riakit	15-18%	—	NS NS
Ames, Seralute	8-10%	—	CV 5-10%; duplicate
Dow-Lepetit	10-15%	22%	8%; at least duplicate
Mallinckrodt RIA-Mat	8-10%	—	about 9%; at least duplicate
RCC T3	8-10%	—	CV 5-10%; duplicate

Table V Reproducibility of some T4 and T3 kits

¹Euthyroid serum; minimum of 15 assays²Precision performance of kit obtainable within the dating period

NS = not stated

all) of the kits have an acceptable precision both 'within' and 'between' laboratory batches when precision is a measure of repeatability of value (themselves the mean of two results) almost all of which fall within the 'normal range'. We have insufficient data to assess the extent of the non-linearity of variance. Most kit methods produce fairly straight line curves extending from very low hormone concentrations to values well into the hyperthyroid range. A few assays yield relatively flat insensitive straight line graphs (see tables III and IV). Yet others, not detailed in the tables, produce unacceptable discrimination—due to curve flattening—at and above moderate hyperthyroid levels. In these cases, approaching antibody saturation introduces inaccuracy and imprecision so that although the assay may be reliable in the low concentrations it is not in the high range. It is interesting to note that the slope of the RIA curve is not particularly related to precision or accuracy (tables III and IV).

It would be desirable for the component steps in these kit methods to have a wide resilience in relation to variation in temperature, time, light intensity, and pH and from gross excesses or deficiencies in serum of, for example, protein and lipids. All the kit methods worked satisfactorily with plasma and with turbid lipaemic sera. Although not rigorously tested, accumulated data suggested that most T3 and T4 kit results were unaffected by the hot and cold weather experienced in Windsor. However, once a kit had been partly used, large differences between the kits were found in the length of time which they could be kept—correctly stored—before failing to produce acceptable results.

Accuracy of kit results

THYROXINE

Several classes of blood serum have been analysed with different kits for T4. Serum from patients with hypo- and hyper-proteinaemia and from late preg-

Serum		Value (nmol/l) obtained by method						
Source	Type	RCC	Precision	SKI	Ames ¹	NML	Abbott	Corning
IDA	Control	94	89	97	72	88	79	74
Hyland	Control	200	200		175	191	179	165
Corning	Control	52			31	52	58	39
Corning	Control	196			141	182	203	150
WRI	Hyper-lipaeamic	181	0	168	136	168	147	129
Bry	Pregnant patients	190			107	137	151	157
Wella		166	142	123	121	154	150	155
Hard		157	152	143	114	155	155	125
Mott	Hyper-thyroid patients	196	191	208	157	188	186	184
Hick		205			145	178	184	142
Mich		231			139	155	156	170
Si	Hypo-thyroid patients	44			26	30	26	28
Sz		40			28	31	32	31
Bro		35			25	35	27	30
C	Hyper-protein-aemia	156			86	102	104	111
Cl		41			23	27	25	26
Hor	Hypo-protein-aemia	152			90	103	115	108
Sm		155			94	105	111	108
Wells		49			23	41	25	35
Reference range quoted (µg/100 ml) (nmol/l)		4.9-13.5 63-173	4.5-14.5 58-187	4.5-13.0 58-167	4.7-10.7 60-138	4.5-11.5 58-148	5.0-13.4 64-172	4.5-13.0 58-167

Table VI Comparison of thyroxine results obtained on 'control sera' using different sets of RIA reagents and standards

¹The simplest competitive-protein binding kit method

nancy patients showed no detectable interference with any kit assay (table VI). T4 was not measurable with one liquid phase assay for T4 in the case of a milk-white serum from a patient with marked hyper-pre-β-lipoproteinaemia.

Part of the problem of accuracy resides in the standard supplied. We have observed with some kits that a change in the batch of the base serum in which the T4 standards are made up can lead to an appreciable deviation in the assay results. When each

available standard for thyroxine analysis is carefully and repeatedly assayed by the different kit methods, a scatter of results is found (table VII). This is so wide that it is impossible for every standard to have acceptable accuracy. It may therefore be claimed that some kit methods are clinically unacceptable owing not only to their being incorrect but to the fact that patients for thyroid screening are likely to attend different clinics or centres where different assay procedures are used.

Source of 'standard'	Stated value of standard		Value ¹ found (nmol/l)							Likely value (mean)
	µg/dl	nmol/l	Meth. 1	Meth. 2	Meth. 3	Meth. 4	Meth. 5	Meth. 6	Meth. 7	
			NML	Abbott	Corning	RCC	Precision Assays	Ames	Hoechst	
NML	6.0	77	—	81	75	93	77	70	66	77
NML	12.0	155	—	166	142	182	153	138	159	155
Abbott	5.0	64	67	—	67	81	66	36	—	67
Abbott	15.0	193	179	—	146	200	184	128	204	189
Corning	5.0	64	81	111	—	71	52	57	—	81
Corning	10.0	129	157	181	—	143	173	119	—	170
RCC	4.9	63	53	57	53	—	63	57	46	57
RCC	12.0	155	155	155	134	—	156	137	166	155
Precision assays	7.5	97	—	—	—	90	—	77	83	—
Ames	7.4	95	106	94	85	117	116	—	116	106
Hoechst	5.0	64	—	—	52	76	70	48	—	—
Hoechst	10.0	129	—	—	—	143	132	—	—	—

Table VII Comparison of some commercially available 'standard thyroxine sera'

¹Each result is the mean result from at least two assays carried out on different days. On the second day the reagents used were from a new batch. Each assay was performed in duplicate

Serum	Biorad	Lepetit	Mallinkrodt	Ames	CIS	RCC	Windsor Lab ¹
IDA control		3.4	1.5		4.8	1.4	1.2
Hoechst control	1.8	3.4	1.8				
Bio-Rad standard		1.1	1.0	1.4		0.8	0.9
Lepetit standard				3.1	6.6		
Ames standard		4.3	4.0			4.4	4.4
Normal (Bon)	3.4	2.3	1.1	2.4		2.6	2.5
Normal (Wat)	3.2		0.8		5.4	1.3	1.4
Hyper (McC)		9.0	3.1	6.5	10.5	7.0	6.8
Reference range quoted							
($\mu\text{g/l}$)	0.7-2.0	0.8-1.8	0.6-2.1	0.7-2.2	NS	1.0-1.9	0.7-1.8
(nmol/l)	1.1-3.1	1.2-2.8	0.9-3.2	1.15-3.4	NS	1.5-2.9	1.1-2.7

Table VIII Comparison of tri-iodothyronine results (nmol/l) obtained on miscellaneous sera using different T3 RIA kit methods

¹Stafford, J. E. H., *et al* (1976).

The method of Ames gives consistently and significantly lower T4 values than those stated—very probably because the Ames 'standard' contains more T4 than stated. The method of the RCC gives consistently and significantly higher T4 values than those stated—possibly because the RCC 'standard' contains less T4 than stated.

TRI-IODOTHYRONINE

Some sera have been analysed with different kits for T3. The results are shown in table VIII. Apart from the CIS kit and some outliers, fairly close agreement in the various T3 concentrations was found. We have not tested for specificity, and note that the maximum T4 cross-reactivity of 0.2% quoted in three of the package leaflets represents a positive error of 10-20% in the T3 value of a euthyroid serum.

Comments

Criteria by which kit performances should be judged have been proposed in detail by Barnett and Youden (1970), and Logan (1972). A central matter is their comparison with a carefully chosen and agreed accurate reference method. However, choice of a reference method is difficult even with simple substances and increasingly so with steroid hormones, peptide hormones, proteins, enzymes, etc. It would be desirable in future kit evaluations to attempt a comparison of values from the best reference method against the kit procedure with sera from patients. In the absence of such reference, a 'best buy' analysis for T4 might be assisted by calculating the per cent deviation from a likely value for each kit to obtain a mean for the number of T4 levels tested. If a score is rated as 1/number of observations $\times$ sum of (% deviations) then the data in table VII give the best (lowest) scores with NML Tetra-Tab kit (3) and with Precision Assays kit (7). When precision is examined as well (table V), these two kits also rate well.

All the T4 kits and most of the T3 kits are capable of giving same-day results. A threefold difference in the specific activity of the freshly supplied labelled hormone was found among the kits. High activity label is important if counting is to be performed manually when counting times in excess of 20 or 30 seconds are inconvenient and tedious.

Other simple and obvious improvements could be suggested to the manufacturers of these kits. The enclosed literature is extremely variable in quality and quantity. Some data (for example, recovery experiments, what A, B, and C found, etc) would be better not included in the package insert but a statement might be given that these were available on request. The origin, constitution, and concentration of all reagents should always be stated. This was not the case in several T3 and T4 kits.

It is internationally agreed that T3 and T4 should be expressed in molar terms. At the time of writing (April 1976) Only one (Pharmacia) of the 26 kits examined supply SI-calibrated standards. Only one (RCC) presents a nonogram to convert T4 into SI units. In some cases graph paper is supplied in a format that actually discourages the reporting of molar (SI) units. This is to be deprecated.

Circumstances may determine when it is desirable to include polythene tubes in each individual kit package. However, when desirable, a welcome improvement would be to have them serially pre-numbered. A current conclusion is that only a few of the kits evaluated here are sufficiently accurate, reliable, and economical to warrant a recommendation for their use in health screening in a community hospital or clinic in the UK.

We are grateful to the companies that provided the kits free of charge and thank Mrs J. Hawkins for her help in compilation.

References

- Barnett, R. N. and Youden, W. J. (1970). A revised scheme for the comparison of quantitative methods. *Amer. J. clin. Path.*, **54**, 454-462.
- Logan, J. E. (1972). Evaluation of commercial kits. *Crit. Rev. clin. Lab. Sci.*, Chemical Rubber Co, Ohio, September 1972.
- Stafford, J. E. H., Lees, S., and Watson, D. (1976). Serum tri-iodothyronine determination in clinical use. *J. clin. Path.*, **29**, 642-647.
- Watson, D. (1974). News Sheet No. 130, Association of Clinical Biochemists.
- Watson, D., Lees, S., and Stafford, J. E. H. (1974). Accuracy and standardisation of serum thyroxine assays. *Ann. clin. Biochem.*, **11**, 1.
- World Health Organisation (1975). Expert Committee on Biological Standardisation, Twenty-Sixth Report. WHO, Geneva.

The September 1976 Issue

THE SEPTEMBER 1976 ISSUE CONTAINS THE FOLLOWING PAPERS

Immune potential in human uraemia

1 Relationship of glomerular filtration rate to depression of immune potential P. R. BYRON, N. P. MALLICK, AND GEOFFREY TAYLOR

Immune potential in human uraemia

2 Changes after regular haemodialysis therapy P. R. BYRON, N. P. MALLICK, AND GEOFFREY TAYLOR

Severe selective IgM deficiency I. N. ROSS AND R. A. THOMPSON

Rosette inhibition tests in chronic liver disease F. SALERNO, S. R. FARGION, M. D. CAPPELLINI, G. FIORELLI, AND N. DIOGUARDI

Polyacrylamide gel disc electrophoresis of alkaline phosphatase isoenzymes in bone and liver disease T. W. WARNES, P. HINE, AND G. KAY

A case of veno-occlusive disease of the liver in Britain associated with herbal tea consumption J. O'D. MCGEE, R. S. PATRICK, C. B. WOOD, AND L. H. BLUMGART

Papillary tumours of the minor salivary glands J. S. WHITTAKER AND E. P. TURNER

Pseudotumour of the lung caused by infection with *Bacillus sphaericus* P. ISAACSON, P. H. JACOBS, A. M. R. MACKENZIE, AND A. W. MATHEWS

Haemophilus aphrophilus endocarditis in pregnancy C. M. ANAND, A. D. MACKAY, AND J. I. EVANS

Sensitivity of Providence to antiseptics and disinfectants D. J. STICKLER AND B. THOMAS

Flavobacterium meningosepticum in the hospital environment MARGARET M. COYLE-GILCHRIST, PATRICIA CREWE, AND G. ROBERTS

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, WC1H 9JR, price £2.00, including postage

An assessment of inositol fermentation by *Klebsiellae* and its implication in screening media J. G. BARR AND REBECCA J. MAHOOD

Detection of staphylococcal enterotoxin in gastric juice N. DICKIE, C. E. PARK, AND H. ROBERN

Serological diagnosis of infection of the urinary tract by yeasts D. W. WARNOCK, D. C. E. SPELLER, J. A. MORRIS, AND P. H. MACKIE

Measurement of heterophil antibody and antibodies to EB viral capsid antigen IgG and IgM in suspected cases of infectious mononucleosis J. M. BLAKE, J. M. B. EDWARDS, W. FLETCHER, D. A. MCSWIGGAN, AND M. S. PEREIRA

A case of small-cell Sézary's syndrome with null-cell features A. H. GOLDSTONE, J. C. CAWLEY, S. O. ROBERTS, A. LEVENTINE, AND C. R. BARKER

Rate of clearance by the spleen of heat-damaged erythrocytes C. S. BOWRING, H. I. GLASS, AND S. M. LEWIS

Technical methods

A simple method for measuring erythrocyte deformability H. L. REID, A. J. BARNES, P. J. LOCK, J. A. DORMANDY, AND T. L. DORMANDY

A rapid microbiological procedure using *Bacillus stearothermophilus* for the assay of antibacterial drugs H. WAHLIG AND R. J. HOLT

Autoclavable plastic dip-slide E. H. SNG, Y. A. TAN, AND H. K. TEO

Letters to the Editor

Book reviews