ERBA Thyrokit® T3

REF **E-TT3-1P**

IVD

CE

This package insert must be read carefully before product use.
Package insert instructions must be carefully followed.
Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package.

Manufacturer:
Erba Lachema s.r.o.
Karásek 2219/1d, 621 00 Brno, Czech Republic
Telephone: +420 517 077 111
Website: www.erbamannheim.com
Email: diagnostics@erbamannheim.com

en

Symbols used on labels

				
In Vitro Diagnostic Medical Device	**Catalogue Number**	**Lot Number**	**See Instructions for Use**	**Use by**
				
Manufacturer	**Date of Manufacture**	**Temperature Limitation**	**Keep away from Sunlight**	**For Single Use Only**

EN *ENGLISH*

1.0 INTENDED USE
ERBA Thyrokit® T3 is a direct solid phase enzyme immunoassay for the quantitative measurement of total 3,5,3'-triiodothyronine (T3) in human serum or plasma.

2.0 SUMMARY AND EXPLANATION OF THE TEST
Thyroxine (T4) and 3,5,3'-triiodothyronine (T3) are the two principal thyroid hormones. In contrast to T4, which is exclusively a product of the thyroid gland, T3 is mainly produced in the peripheral tissues by 5'-monodeiodination of circulating T4. In normal adults about 80% of T3 is produced by peripheral conversion and about 20% is secreted directly by the thyroid. A small amount of T4 is 5-monodeiodinated to form 3,3',5'-triiodothyronine (reverse T3); this is probably biologically inactive. The proportion of T4 converted to reverse T3 may be increased under certain circumstances, such as severe illness or medication with Amodiarone or radiographic contrast agents. This reduces the production and hence the circulating level of T3. Considerable evidence exists to show that T3 is the more active thyroid hormone and some authors have suggested that T4 has no intrinsic activity. Both T3 and T4 circulate in association with serum proteins, the most important of which is thyroxine binding globulin (TBG). This has a higher affinity for T4 than for T3. Approximately 80% of circulating T3 is bound to TBG. Most of the rest is bound to prealbumin and albumin; only about 0.5% of T3 is "free" in serum (i.e. not bound to serum proteins). The concentration of T3 in serum can be alterated by a change in the activity of the thyroid gland or by alteration in the peripheral metabolism of T4. The thyroid is under the control of thyroid stimulating hormone (TSH) which in turn is regulated by hypothalamic thyrotrophin-releasing hormone (TRH). T3 exerts a negative feedback control on the release of TSH which is principally at the level of the pituitary. A slight elevation in T3 levels above normal will block TSH release in normal subjects; this block cannot be overcome by increasing doses of TRH. Measurement of the concentration of T3 in serum is most useful in the diagosis of hyperthyroidism. In the majority of patients with this condition, circulating total T4 and T3 are both elevated. In some cases, however, the hyperthyroidism results solely from an increased production of T3, a condition referred to as "T3 thyrotoxicosis". Elevated T3 levels with normal T4 may also be found in euthyroid partients with autonomous thyroid function (ophtalmic Graves' disease, autonomous nodules). It may be seen also as a compensatory phenomenon in patients with subclinical hypothyroidism and elevated TSH secretion. In severe hypothyroidism, total T3 levels are usually low, but in moderate hypothyroidism they may be normal. In severe non-thyroidal illness or after surgery, T3 levels are often low, with elevated production of reverse T3. T3 levels are generally higher in the newborn and infants and may be lower in older people.
Serum T3 values also vary with TBG concentration, being lower when TBG levels are low (as in liver disease, or congenital TBG deficiency) and higher during pregnancy or estrogen therapy.

3.0 PRINCIPLE OF THE METHOD
Sample of serum or plasma is pipetted into the well coated with Streptavidin. The addition of the Horseradish peroxidase-T3 conjugate (HRP-T3) and of the immunological reaction starter Anti-T3-Biotin conjugate initiates the competitive assay. During the immunological incubation, the T3 of the sample competes with the HRP-T3 conjugate for the anti-T3 binding sites of the biotinilated antibody which, in turn, is bound by the streptavidin coated wells through the biotin moiety. After washing off the non-reacted species, the amount of the immunological complex remained bound to the wells

streptavidin **:** Biotin-Anti-T3 **:** T3-HRP

is revealed by the incubation with the chromogen/substrate. The blue colour development is then stopped with sulphuric acid, turning the final solution to a yellow colour which is measured photometrically at 450 nm. The intensity of the colour is proportional to the bound HRP-T3 conjugate, and therefore inversely related to the amount of T3 in the sample.
By reference to a series of T3 standards, assayed in the same way, the concentration of T3 in the unknown sample is quantified.

4.0 REAGENTS - STORAGE AND HANDLING
The ERBA Thyrokit® T3 contains sufficient reagents for 96 wells. All the reagents are ready to use. On receipt, store the kit and each reagent at 2...8°C, stable up to the expiration date on the labels.
If not otherwise specified, also, after the first opening all the reagent are also stable up to the expiry date printed on the labels, provided they are stored as indicated and no contaminations accur during the pipetting.

4.1 Streptavidin Microwell Plate
The bag contains a microplate of 12 strips x 8 wells.
Each well is coated with Streptavidin and it may be used individually. Allow microplate to warn to room temperature (18...25°C) before use. After the first opening the unused strips are stable for 2 months at 2...8°C, provided they are stored in the plastic bag with the dessicant.

4.2 Total T3 Anti HRP Enzyme Conjugate
1 vial contains HRP-T3 conjugate in TRIS buffer pH 7.8, a red dye, preservative and binding protein inhibitors**.** 8 ml.

4.3 Total T3 Biotin Conjugate
1 vial, contains monoclonal anti-T3 in TRIS buffer pH 7.8, preservative and a yellow dye**.** 8 ml.

4.4 Total T3 Standards
6 vials (1 ml each), containing 0- 0.5- 1- 2- 4- 8 ng/ml of T3 in stripped human serum.

4.5 TMB Substrate
1 vial of TMB Substrate containing 0.26 mg/ml of 3,3',5,5' Tetramethylbenzidin (TMB) and 0.01% w/v of Hydrogen peroxide (H_2O_2), in citrate buffer. 16 ml. Mix gently before use.

NOTES:

1. The TMB/H_2O_2 single solution is colourless or slightly yellow-blue. If accidental contamination occurs, the solution starts to develop a blue colour and must therefore be discarded.
2. The TMB/H_2O_2 single solution is not sensitive to light. Direct sunlight can however oxidize the solution to a blue colour. Such a colour disappears after 4 hours storage in the dark after which the solution can again be used.

4.6 Stop Solution

1 vial of Stop Solution containing Sulfuric acid (H_2SO_4) 0.3 mol/l. 15 ml. Mix gently before use.

4.7 Wash Solution 10X

The bottle contains 50 ml of borate citrate buffer and 2.5 µg/ml Amphotericin B. Dilute 1:10 with distilled or equivalent grade water.

5.0 MATERIAIS AND EQUIPMENT REQUIRED

5.1 Materials Provided

The kit contains reagents for 96 tests (code E-TT3-1P).

Material for 96 tests	Quantity
Streptavidin Microwell Plate	One bag
Total T3 Standard A	1x1 ml
Total T3 Standard B	1x1 ml
Total T3 Standard C	1x1 ml
Total T3 Standard D	1x1 ml
Total T3 Standard E	1x1 ml
Total T3 Standard F	1x1 ml
Total T3 Biotin Conjugate	1x8 ml
Total T3 Anti-HRP Enzyme Conjugate	1x8 ml
TMB Substrate	1x16 ml
Wash Solution (10x)	1x50 ml
Stop Solution	1x15 ml

5.2 Materials and Equipment Not Provided

- Distilled or deionized water
- 50 and 100 µl micropipettes
- 0.1 ml repeating dispenser or positive displacement pipettes for addition of Conjugate, Substrate and Stop Solution.
- Automatic plate washer.
- Microtiter incubator.
- Microtiter plate reader, equipped for the measurement of the absorbance at 450 nm (reference filter at 620 nm).
- Adsorbent pad or paper.
- Control sera (recommended).

6.0 WARNING, PRECAUTIONS AND IIMITATIONS

For *in vitro* diagnostic use.

Only experienced laboratory personnel should use this test and handling should be in agreement with GLP.

Do not interchange reagents from different lots. Do not use kit components beyond their expiration date.

Attention

Stop Solution contains sulphuric acid <5% and is classified as Skin Corr. 1A, H314

Danger

Hazard statements:

H314 Causes severe skin burns and eye damage.

Precautionary statements:

P260 Do not breathe vapours/spray.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501 Dispose of contents/container in accordance with local regulations.

Standards and Total T3 Biotin Conjugate contain Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) and are classified as Skin Sens. 1, H317

Warning

Hazard statements:

H317 May cause an allergic skin reaction.

Precautionary statements:

P261Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P302+P352 IF ON SKIN: Wash with plenty of water.
P501 Dispose of contents/container in accordance with local regulations.

6.1 Safety Precautions

- Do not pipet by mouth.
- Do not smoke, eat or apply cosmetics in areas in which patients samples or kit reagents are handled.
- Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing.
- Take care to avoid self-inoculation, splashing of mucous membranes or generation of aerosols.
- Laboratory gloves should be worn while handling patient samples or disposing of solid or liquid wastes.
- Avoid microbial contamination of standards during pipetting by using disposable pipet tips.
- Disposal of all waste should be in accordance with local regulations.
- Read carefully the Safety Data Sheet (SDS) before product use.

6.2 Potential Biohazard Warning - Human Serum

Some reagents used may have been prepared from pools of human serum. Each unit of blood used to prepare these pools were tested and found non reactive for syphilis, for the presence of Hepatitis B Surface Antigen (HBsAG) and for antibodies to Human Immunodeficiency Virus (HIV 1 and 2) using an FDA approved method. Because no test can offer complete assurance that Hepatitis B virus, HIV or other infectious agents are absent, these reagents should be considered as potentially biohazardous and handled with the same precautions as applied to any serum or plasma samples. Some reagents such as calibration standards and control may contain materials of human tissue origin. At present there is no standard test method for the presence of HIV in such material. It is therefore recommended that these reagents are also considered as potentially biohazardous.

Such materials should be handled according to good laboratory practices, as described in CDC (Center for Disease Control, Atlanta U.S.) document: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood bourne pathogens in healthcare setting "MMWR" 37:377-387, 1999.

6.3 Sodium Azide (NaN_3) Warning

Sodium azide is present as a preservative in the standard matrix at a concentration of no more than 0.09% w/w. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Liquid and solid wastes should be disposed of safety, in accordance with local regulations. Azide at concentration higher than 0.1% w/w interfere in this assay, therefore the assay of control sera or samples containing the above compound may give overestimated results.

6.4 Sulphuric Acid (H_2SO_4) Warning

Sulphuric Acid is present in the Stop Solution at a concentration of no more than 0.3 mol/l.

Do not pipette by mouth.

6.5 3-3'-5-5' Tetramethylbenzidine (TMB) Warning

TMB (3-3'-5-5' Tetramethylbenzidine) is present in the TMB Substrate. Avoid contact of this reagent with skin and mucous membranes. Should this occur, wash thoroughly with cold tap water. Do not pipette by mouth.

6.6 Limitations

For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Procedural directions must be followed exactly as any modification of the procedure may change the results.

Use of reagents, disposables or spare parts other than those supplied by authorized distributor may produce incorrect results.

6.7 Indications of Substrate Deterioration

- The Substrate single solution is colourless or slightly yellow-blue. If accidental contamination occur, the solution starts to develop a blu colour and must therefore be discarded.
- The Substrate single solution is not sensitive to light. Direct sunlight can however oxidize the solution to a blue colour. Such a colour disappears after 4 hours storage in the dark after which the solution can again be used.
- On aging the substrate may became of slight yellow-orange colour. This does not affect its performances.
- Should only part of the Substrate vial content be used, in order to avoid contamination, transfer the volume needed into a clean plastic container which has previously been washed with ethanol and rinsed with high-quality distilled water.

7.0 SPECIMEN COLLECTION AND STORAGE

7.1 Serum

Collect 5 ml of venous blood in a glass tube without additives. Allow to clot at room temperature. Centrifuge, separate the serum fraction, and store.

7.2 Plasma

Collect 5 ml of venous blood in a glass or plastic tube containing heparin or citrate as an anticoagulant. Centrifuge, separate the serum fraction, and store.

7.3 Storage

Serum and plasma specimens are stable for up to 24 hours at 2...8°C. For longer storage, aliquot and store at -20°C for up to 90 days.

Avoid repeated freezing and thawing.

7.4 Known Interference

Avoid using the following types of serum or plasma samples as these may give incorrect results:

Grossly hemolyzed samples;

Grossly lipemic samples:

Grossly icteric samples;

EDTA anticoagulated plasma samples with EDTA concentration higher than 5 g/l should not be used.

8.0 ASSAY PROCEDURE

8.1 Preparation for Assay

Bring all reagents and specimens to room temperature (20°C - 30°C) before beginning the assay. Swirl gently before use.

8.2 Pipetting and Incubation steps.

1. Pipette in duplicate 50 µl of each standard and 50 µl of each sample into the appointed wells.
2. Pipette 50 µl of the HRP-Conjugate (HRP-T3 conjugate) into all the wells
3. Pipette 50 µl of the Anti-T3-Biotin Conjugate into all the wells
4. Shake the plate for 10 seconds on an orbital shaker or manually, by gently hitting the side of the microplate against your index finger; the movement must be sideways to avoid spilling the well content.
5. Incubate the plate at room temperature for 1 hour.
6. At the end of the incubation period, wash the strips 3 times as described in par. 8.3, section 8.
7. Add 100 µl of TMB Substrate.
8. Incubate at room temperature for 15 min.
9. Stop the reaction by adding 100 µl of stop solution to each well in the same order followed for dispensing the substrate.
10. Measure the absorbance within 30 min. with microtiter reader at the wavelength of 450 nm (reference filter at 620 nm)

8.3 Procedural Notes

1. Room temperature is defined between 18° and 25°C.
2. A standard curve must be run in each assay to assure valid results.
3. Reagents from different kits and lots should not be mixed.
4. Add the reagents in the same order as the standards and samples.
5. It is recommended to time the addition of the chromogen/substrate solution and stop solution until familiar with the method (i.e. if the chromogen/substrate solution is dispensed into the wells every 3 seconds one from each other, the stop solution should also be dispensed in the same order and at the same frequency).
6. The total dispensing time of standards, controls and speciments for a whole plate should not exceed 15 minutes.
7. **The shaking step after the addition of the conjugate reagents is critical and must be performed correctly.**
8. **Washing procedure:** For the washing procedure, the use of an automatic plate wash equipment is recommended. After the washing, tap the inverted plate on absorbent paper to remove any residual from the wells. Three washings are required.
   If an automatic plate washer is not available, the washing procedure can be carried out manually using a simple wash-bottle filled with the washing solution:
   - Empty the content of the wells by keeping the plate tight in the middle and turning it firmly upside-down.
   - Fill the wells with 300 µl/each of the washing solution contained in the wash-bottle and empty them as aforesaid.
   - Repeat this procedure twice more.
   - Firmly tap the inverted plate on absorbent paper to remove any residual from the wells.

9.0 QUALITY CONTROL

It is recommended that each laboratory routinely use quality control materials and establish its own control ranges. Multi-level controls should be used in each Total T3 run.
The Total T3 values obtained for the quality control material should not repeatedly fall outside the control ranges established in each laboratory.

10.0 CALCULATION OF RESULTS

10.1 Data reduction - Manual Method

Plot the ODs of standards versus the respective T3 concentration (use a linear or a semilogarithmic scale). Determine the T3 concentration of the sample by interpolation of the sample ODs on the calibration curve.

10.2 Data Reduction - Automated Method

Use the 4 parameters logistic (preferred method) or the smoothed cubic spline function as calculation algorithm.

11.0 EXPECTED VALUES

Serum samples from normal (i.e. blood donors) and clinically euthyroid subjects have been assayed by manual method and automated instruments Labotech and Personal Lab. The median values, 2.5 and 97.5 percentiles were calculated.

	T3 conc. (ng/ml)		
Method	**n**	**median**	**Range (*)**
Manual	122	1.00	0.51-1.58
Labotech	123	1.00	0.67-1.64
Personal Lab	121	1.13	0.74-1.79

(*) 2.5 and 97.5 percentile

11.1 Factors Associated with increased values

Physiological:
Childhood, pregnancy, medication with estrogens (e.g. oral contrapcetives), congenitally elevated TBG.
Pathological:
Hyperthyroidism (primary or secondary), Graves' disease, over-administration of T3, T3 thyrotoxicosis.

11.2 Factors associated with decreased values

Physiological:
Old age, medication with diphenylhydantoin, fenclofenac, aspirin, androgens, glucocorticoids (in large doses), congenital TBG deficiency.
Pathological:
Hypothyroidism (primary or secondary), endemic goitre, lesions of the thyroid gland, severe non-thyroidal illness.

12.0 PERFORMANCES

12.1 Sensitivity

The sensitivity limit of the assay, defined as the concentration of T3 equivalent to the mean absorbance of zero standard, assayed in 20 replicates, minus two standard deviations, is 0.19 ng/ml.

12.2 Precision

The precision of the assay was assessed by the manual method and by the automated instruments Labotech and Personal Lab.
The same control sera were assayed in several replicates and on some occasions, with both manual and automated methods.

Within Assay

	Sample 1	Sample 2	Sample 3
Manual method			
n	22	22	22
mean (ng/ml)	1.26	2.56	3.77
s.d. (ng/ml)	0.115	0.20	0.24
C.V.%	7.4	7.8	6.4
Labotech	**Sample 1**	**Sample 2**	**Sample 3**
n	22	22	22
mean (ng/ml)	1.08	2.33	3.51
s.d. (ng/ml)	0.076	0.147	0.228
C.V.%	7.0	6.3	6.5
Personal Lab	**Sample 1**	**Sample 2**	**Sample 3**
n	22	22	22
mean (ng/ml)	1.18	2.33	3.42
s.d. (ng/ml)	0.083	0.172	0.335
C.V.%	7.0	7.4	9.8

Between Assay

	Sample 1	Sample 2	Sample 3
Manual method			
n	10	10	10
mean (ng/ml)	1.31	2.57	3.82
s.d. (ng/ml)	0.161	0.203	0.336
C.V.%	12.3	7.9	8.8
Labotech	**Sample 1**	**Sample 2**	**Sample 3**
n	10	10	10
mean (ng/ml)	1.09	2.30	3.46
s.d. (ng/ml)	0.128	0.186	0.318
C.V.%	11.7	8.1	9.2
Personal Lab	**Sample 1**	**Sample 2**	**Sample 3**
n	10	10	10
mean (ng/ml)	1.21	2.35	3.40
s.d. (ng/ml)	0.156	0.207	0.377
C.V.%	12.9	8.8	11.1

12.3Accuracy

Dilution test

Three samples were tested after dilution with Zero Standard. Results are shown on the following table:

Sample	Dilution	Measured (ng/ml)	Recovery
	0	2.01	-
42A	1:2	0.98	97.5
	1:4	0.49	97.5
	0	2.22	-
59A	1:2	1.23	110.8
	1:4	0.61	109.9
	0	4.04	-
47.19	1:2	1.96	97.0
	1:4	0.96	95.5
	1:8	0.42	83.2

Mean recovery: 98.8 ± 9.4

Recovery

Three samples were spiked with pure T3 and assayed: results are reported in the following table:

Sample	Added (ng/ml)	Measured (ng/ml)	Recovery %
	0	0.79	-
1	0.82	1.64	103.7
	1.64	2.16	83.5
	0	0.80	-
2	0.82	1.72	112.2
	1.64	2.24	87.8
	0	0.57	-
3	0.82	1.32	91.5
	1.64	2.13	95.1

Mean recovery: 90.6 ± 16.4

12.4 Specificity

Cross-Reactivity

Potential cross-reagents have been tested and the results are expressed as the ratio of T3 concentration to the concentration of the cross-reagent that will displace 50% of the bound enzyme conjugate x 100 (Abraham's method).

Substance	Cross Reactivity %
L-3,3'-5-Triiodothyronine (T3)	100
3,3',5-Triiodothyroacetic acid	59.5
D-Thyroxine	0.33
L-Thyroxine	0.6
L-3,3',5'-Triiodothyronine (Rev. T3)	0.2
3,5-Diiodo-L-Thyrosine	< 0.1
Acetylsalicylic acid	not detectable
Salicylic Acid	not detectable

13.0 AUTOMATION

Application protocols for the proper automation on the microplate analyzers are available upon request at ERBA GmbH directly.

14.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors

ERROR	POSSIBLE CAUSES / SUGGESTIONS
OD very different (± 50%) from OD reported on QC	- incorrect dispensing volume of reagents (suggestion: check the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) -incorrect temperature or incorrect incubation time (suggestion: more care in the incubator maintenance; note down the beginning of the incubation) -error in washing or in photometer reading (suggestion: check operating or settings of respective instruments) -contamination of Substrate or Conjugate (suggestion: use only disposable and clean plastic containers)
Low reproducible results	-not constant dispensing volume of samples or reagents (suggestion: check the pipettes precision and the correspondence between the volume dispensed by the pipette and the one required by the assay; re-calibrate again pipettes) -error in washing or in reading (suggestion: check operating or settings of respective instruments) -contamination of Substrate (suggestion: use only disposable and clean plastic containers) -pollution or degradation of reagents (suggestion: use appropriate tips, disposable and clean plastic containers for reagents and high quality distilled or equivalent water)
no colorimetric reaction after addition of substrate	-some reagent not pipetted - strong contamination of Conjugate or Substrate -errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

too low reaction (too low ODs)	-incubation time too short, incubation temperature too low -incorrect conjugate dilution
too high reaction (too high ODs)	-incorrect conjugate dilution -incubation time too long, incubation temperature too high -water quality for wash solution insufficient (low grade of deionization) -insufficient washing (conjugates not properly removed)
unexplainable outliers	-contamination of pipettes, tips or containers -inconstant and insufficient washing (conjugates not properly removed)
too high within-run CV%	-reagents and/or strips not pre-warmed to Room Temp. prior to use - plate washer is not washing correctly (suggestion: clean washer head)
too high between-run CV%	-incubation conditions not constant (time, temperature) -controls and samples not dispensed at the same time (with the same intervals) (check pipetting order) -person-related variation

BIBLIOGRAPHY

1. Ingbar, S.H. and Beaverman, L.E. , Active Form of the Thyroid Hormone, Ann. Rev. Med. (1975) 26: 443-449.
2. Chattoraj, S.C. and Watts, N.B., Endocrinology - Thyroid Function, in "Textbook of Clinical Chemistry", ed, N.W. Tietz, W.B. Saunders Co (1986) 1116-1136.
3. Chopra, I.J., An Assessment of Daily Production and Significance of Thyroidal Secretion of 3,3',5'-triiodothyronine (reverse T3) in Man, J. Clin. Invest. (1976) 58: 32-40.
4. Burr, W.A., Black, E.G., Griffiths, R.S. and Hoffenberg, R., Serum Triiodothyronine and Reverse Triiodothyronine Concentrations After Surgical Operation, Lancet (1975) ii 1277-1279.
5. Nadamanee, K., Singh, B.N., Hendrickson, J.A., Reed, A.W., Melmed, S. and Hershman, J., Pharmacokinetic Significance of Serum Reverse T3 Levels During Amiodarone Treatment, Circulation (1982) 66: 202-211.
6. Surks, M.I., Schadlow, A.R., Stock, J.M. and Oppenheimer, J.H., Determination of Iodothyronine Absorption and Conversion of L-Thyroxine (T4) to L-Triiodothyronine (T3) Using Turnover Rate Techniques, J. Clin. Invest. (1973) 52: 805-811.
7. Prince, H.P. and Ramsden, D.B., A New Theoretical Description of the Binding of Thyroid Hormones by Serum Proteins, Clin. Endocr. (1977) 7: 307-324.
8. Snyder, P.J. and Utiger, E.D., Inhibition of Thyrotrophin Response to Thyrotrophin-Releasing Hormones by Small Quantities of Thyroid Hormones, J. Clin. Invest. (1972) 51: 2077-2084.
9. Martino, E., Pacchiarotti, A., Aghini-Lombardi, F., Grasso, L., Bambini, G., Baschieri, L. and Pinchera, A., Serum Free-Thyroxine in Patients with T3-Toxicosis, Acta Endocrin. (1985) 110: 354-359.
10. Evered, D.C., "Disease of the Thyroid", Pitman Medical, Tunbridge Wells (UK) (1976).
11. Evered, D.C., Ormston, B.C., Smith, P.A., Hall, R. and Bird, T., Grades of Hypothyroidism, Brit. Med. J. (1973) 1: 657-662.
12. McLarty, D.G. et al, Thyroid Hormones in Serious Non-Thyroidal Illness, Lancet (1976) i 1070.
13. Evered, D.C., Tunbridge, W.M.G., Hall, R., Appleton, D., Brewis, M., Clark, F., Manuel, P. and Young, E., Thyroid Hormone Concentrations in a Large Scale Community Survey. Effect of Age, Sex, Illness and Medication, Clin. Chim. Acta (1978) 83: 223-229.
14. Larsen, P.R., Triodothyronine: Review of Recent Studies of its Physiology and Pathophysiology in Man, Metabolism (1972) 21: 1073-1092.
15. Hotelling, D.R. and Sherwood, L.M., The Effects of Pregnancy on Circulating Triiodothyronine, J. Clin. Endocr. Metab. (1971) 73: 783-786.
16. Stubb, P., Gatz, J., Heidemann, P., Muhlen, A. and Hesch, R., Thyroxine-Binding Globulin, Triiodothyronine, Thyroxine and and Thyrotrophin in Newborn Infants and Children, Horm. Metab. Res. (1978) 10: 58-61.
17. Boscato, L.M.., Stuart, M.C., Incidence and specificity of interference in two-site immunoassays. Clin. Chem. 32 (8): 1491, 1986.
18. Kinders, R.J., Hass, G.M., Interference in immunoassay by human anti-mouse antibodies. Eur. J. Cancer. 26 (5):647, 1990.
19. Boscato, L.M., Stuart, M.C., Incidence and specificity of interference in two-site immunoassays. Clin. Chem. 32 (8): 1491, 1986.