



Immunoradiometric Assay for the measurement of Thyroglobulin in Human Serum - Instructions for use

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Human thyroglobulin (Tg) is measured in an IRMA using two antibodies, which recognize different epitopes on the antigen (Tg). One antibody is coated onto the assay tube and when the other 125 I-labelled antibody and sample are incubated together in the tubes, a sandwich-type complex is formed. This complex is bound to the tube so separation of bound and free antigen is simply accomplished by aspiration and washing the tubes. Radioactivity measured in the tube is directly proportion to the Tg concentration in the sample. The method is highly sensitive, being able to detect 1 ng/mL of Tg and has a measuring range up to 250 ng/mL. Interference from Tg autoantibodies in the assay is minimal.

Intended use of the test: The RSR Tg IRMA is used for reliable and quantitative determination of human thyroglobulin in serum. Tg is the precursor of thyroid hormones and therefore specific to the thyroid and thyroid derived tissue. Because of this, Tg determinations are important in the post-operative monitoring of differentiated thyroid carcinoma patients. Following total thyroidectomy and successful radioiodine treatment, Tg levels should fall to zero in patients who are free from metastases and tumour. A small quantity of Tg is continuously released into the serum of healthy individuals. However, in various non-malignant thyroid disorders, elevated levels are often observed, due to destruction or hyperactivity of thyroid follicles. These disorders include Hashimoto's thyroiditis, hyperthyroid Graves' disease and toxic nodular goitre.

Specificity: Quantitative determination of serum Tg is complicated by the frequent presence of specific (Tg autoantibodies) and non-specific interfering factors. For this reason, the validity of the test must be assessed by performing a recovery test on every serum sample, the procedure for which is given later.

Reagents provided in kit for 100 tubes: Store all reagents at 2-8°C before use. Use within the designated shelf life stated on the label

- 1. Coated tubes** (2 x 50 tubes) Coated with anti-hTg antibody. Allow tubes to reach room temperature before removing from bag.
- 2. Tracer** (21 mL) 125I-anti hTg antibody. Ready for use.
- 3. Calibrators** (6 x 0.75 mL) 1 set of human thyroglobulin calibrators (0.3, 1, 4, 20, 100, 250, ng/mL). Ready for use.
- 4. Wash buffer** (20 mL) 1 bottle of concentrated buffer. Dilute contents to 500 mL with distilled water.
- 5. Control sera I and II;** 0.75 mL of each. Concentrations – see label enclosed.
- 6. Recovery test sample** (1.0 mL) Approximately 550 ng/mL Tg.
- 7. Diluent for serum** (5.0 mL) To be used for dilution of sera with Tg concentrations greater than 500 ng/mL.

Allow all reagents to reach room temperature before use. Mix reagents gently before use but avoid vigorous mixing.

Equipment required but not provided in the kit: -

1. Suitable rack for assay tubes
2. Pipettes for 100 μ L, 200 μ L and 1 mL
3. Vortex mixer
4. Gamma Counter
5. Uncoated tubes for total counts

Handling of serum samples: Sera to be analysed should be stored, preferably in aliquots, at below – 20°C. 0.5 mL is sufficient for one assay. Subsequent freezing and thawing, or increases in storage temperature should be avoided. Do not use grossly haemolysed or lipaemic samples. Do not use plasma in the assay. After thawing at room temperature, agitate serum samples gently to ensure homogeneity and centrifuge at about 1500 g for 10 minutes (at room temperature or below) or preferably for 5 minutes in a microfuge.

Assay Protocol: **1.** Label coated tubes serially in duplicate. Use two uncoated tubes for measuring total counts. **2.** Pipette 100 μ L calibrator, assay control, or unknown, into appropriate tubes. **3.** Pipette 200 μ L 125I-anti Tg into all tubes including totals. **4.** Vortex-mix tubes. Cover and incubate overnight at room temperature. **5.** Keeping total tubes separate until radioactivity is measured, dispense 1 mL wash buffer into each tube then decant or aspirate liquid. **6.** Dispense 1 mL wash buffer into each tube; decant or aspirate and blot dry. **7.** Repeat step 6 and drain tubes upside down

for more than 5 mins. **8.** Count activity in tubes for 60 seconds and plot log₁₀ of cpm bound versus Log₁₀ of concentration of Tg to construct a standard curve. Read unknown values off the standard curve. For samples with concentrations of 10 ng/mL Tg or less, assay precision will be improved by counting standard and sample tubes for longer times, eg 5 mins.

TYPICAL RESULTS (Example only, not for use in calculation of actual results)

Calibrator ng/mL	% B	Conc. ng/mL
Total counts	413,098	
0.3	0.08	
1	0.26	
4	0.95	
20	4.2	
100	21.3	
250	49.0	
Positive Control I	0.54	2.2
Positive Control II	12.7	59.4

Extrapolation of the standard curve to Tg values above 250 ng/mL is not recommended and sera with such high Tg levels should be diluted with the diluent provided (component 7).

Recovery Test procedure: Inaccuracies in serum thyroglobulin measurements can be caused by interference from Tg autoantibodies or unspecific effects in a patient's serum. Consequently all sera need to be assessed for such interferences by carrying out a recovery test as follows: -

In the case of the recovery test, the recovery sample (containing 550 ng/mL of Tg) is diluted 1 in 10 (i.e. 10 µL recovery sample in 90 µL sample) and the final 100 µL is assayed as usual.

The calculation is as follows: -

If x = concentration of Tg in test sample alone

y = concentration of Tg in test sample + recovery sample, and

z = concentration of Tg in sample diluent plus recovery sample,

The % recovery

$$= \frac{y - x}{(0.9x + z) z} \times 100 = \frac{y - x}{z - 0.1x} \times 100$$

Recovery range = 80 – 120%

Safety Considerations: This kit is for in vitro use by professional persons only. Follow the instructions carefully and observe the expiry dates stated on the labels. Store all reagents at 2–8°C in their original containers. Refer to the Material Safety Data Sheet for more detailed safety information. When handling the kit and all of its reagents, the following precautions should be observed: -1. Do not pipette by mouth. 2. Do not smoke, eat or drink whilst handling the reagents. 3. Always wear protective gloves whilst handling reagents. In addition, the following precautions should be observed when handling radioactive materials: - 1. Always use procedures set down in the local rules and regulations for handling radioactive materials. 2. Any spillage should be wiped up quickly and thoroughly and any contaminated materials should be treated as radioactive waste. Materials of human origin used in the preparation of the kit have been tested and found non-reactive for HIV-1 and 2 and HCV antibodies and HBsAg but should be handled as potentially infectious. Avoid ingestion, inhalation, injection and contact with skin, eyes and clothing. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens before disposal. Materials of animal origin, which have been used in the preparation of the reagents, have been obtained from healthy animal as certified by a veterinary surgeon but these should be handled as potentially infectious. Some reagents contain small quantities of sodium azide (0.1% w/v) as preservative. Do not swallow any reagents and avoid reagent contact with skin or mucous membranes. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.