RK-51CTACE250901

hTG [I-125] IRMA KIT

(REF: RK-51CT)

The hTG [I-125] IRMA system provides direct quantitative *in vitro* determination of human thyroglobulin (hTg) in human serum. hTg can be assayed in the range of 0.25-250 ng/mL using 100 µL serum samples.

Introduction

The Thyroglobulin is a iodoglycoprotein consisting of heterogeneous molecules, the composition of which is in part depending on the degree of iodination. The prevailing molecular form is 660 kDa (dimeric form, the two subunits, linked by noncovalent bounds), but both larger and smaller molecular forms exist in the thyroid gland. Thyroglobulin is the site of synthesis and storage of thyroid hormones produced by the thyroid gland. Thyroglobulin is synthesized and stored in thyroid follicles and some of the nonenzymatically digested protein is released into the circulation upon stimulation with thyrotropin (TSH).
Circulating Thyroglobulin can be elevated in different thyroid disorders, such as Grave's multinodular goitre, benign thyroid adenoma and acute phase thyroiditis.
The determination of Thyroglobulin by immunoassay methods is a useful and well accepted tool in monitoring differentiated thyroid cancer patients after total thyroidectomy with or without radioiodine ablation.
The sensitivity of the present hTG IRMA system makes it suitable for the measurement of subnormal Thyroglobulin levels, which is an early and reliable marker of tumour recurrence.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.
The ^{125}I labelled signal-antibody binds to an epitope of the hTg molecule spatially different from that recognised by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".
During an overnight incubation period the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.
The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hTg, the unknown concentration of hTg in patient samples can determined.

Contents of the kit

1. 1 bottle TRACER (21 mL), ready to use, containing about 980 kBq ^{125}I-anti-hTg and capture anti-hTg in buffer with red dye and 0.1 % NaN_3.

2. 6 vials STANDARD (6 x 1.0 mL), containing (S1-S6) 0.3, 1.0, 4.0, 20, 100, 250 ng/mL hTg (calibrated to BCR CRM457) in equine-swine serum with 0.1% NaN_3.

3. 2 vials CONTROL SERUM (2 x 1.0 mL), in equine-swine serum with 0.1% NaN_3. The concentration of the control sera is specified in the quality certificate enclosed.

4. SERUM DILUENT (5.0 mL), containing equine-swine serum and 0.1% NaN_3. Serum diluent is also zero calibrator.

5. RECOVERY SERUM (1.0 mL), containing human serum and 0.1% NaN_3. The concentration of the recovery serum is specified in the quality certificate enclosed.

6. 2 boxes COATED TUBE, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

7. 1 bottle WASH BUFFER CONCENTRATE (20 mL), containing 0.2 % NaN_3. See *Preparation of reagents.*

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (10, 100, 200 and 2000 µL), vortex mixer, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes (e.g., Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-mL pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C) up to 3 months. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, haemolyzed or turbid specimens. Samples with a hTg concentration higher than 250 ng/mL should be diluted with the serum diluent included in the kit and re-assayed.

Preparation of reagents, storage

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. Upon dilution store at 2-8°C until expiry date.
Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate for each standard (S1-S6), control serum (CI, CII), serum diluent (D) as zero calibrator and serum samples (Sx).
3. Homogenize all reagents and samples by gentle mixing. Avoid foaming.
4. Pipette 100 µL of standards into the standard tubes (S1-S6), 100 µL control into control tubes (CI, CII), 100µL sample into sample (Sx) tubes and 100µL serum diluent into the serum diluent tubes (D) as zero calibrator. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 200 µL of tracer into each tube.
6. Gently vortex all tubes. Seal all tubes with a plastic foil.
7. Incubate tubes for 15-24 hours at RT (room temperature).
8. Add 2.0 mL diluted wash buffer to each tube and decant the supernatant from all tubes by the inversion of the rack. In the upside-down position, place the rack on an absorbent paper for 2 minutes.
9. Return the tube-rack to an upright position, and repeat Step-9 two times more
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate the hTg concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres).
**Recovery measurement is optional.*

Tube Reagents	Total (T)	Serum diluent (D)	Standard (S_1-S_6)	Sample (Sx)	Control serum (CI, CII)	*Recovery tubes* (Rx)*	*Recovery reference tubes* (D_R)*
Standard			100				
Sample				100		100	
Control serum					100		
Recovery serum						10	10
Serum diluent		100					100
Tracer	200	200	200	200	200	200	200
Incubate tubes for 15-24 hours at RT							
Wash buffer		2000	2000	2000	2000	2000	2000
Decant the fluid and blot on filter paper							

Repeat the washing step two times
Counting radioactivity (60 sec/tube)
Calculate the results

Interpretation of results:

When interpreting the test results, the possible presence of interfering Thyroglobulin autoantibodies in the sample should be ruled out. The validity of serum Thyroglobulin results should be verified preferably by the determination of anti-TG in the sample[15] (*for example using the RK-8CT Anti-hTG [I-125] RIA kit*) and in some cases recovery tests as described below may also prove to be useful.
The recovery test should be carried out as described in the assay procedure. The concentration of the recovery serum should be checked with serum diluent (recovery reference tubes (DR)).

1. Label coated tubes in duplicate for recovery reference (DR), and recovery serum (Rx).
2. Pipette 10µl recovery serum into the recovery reference tubes (DR) and into the sample recovery tubes (Rx).
3. Pipette 100µl sample into recovery tubes (Rx) and 100µl serum diluent into the recovery reference tubes (DR).
4. Same as assay procedure 5-11.

Calculate Recovery (in%) in the serum sample:

$$\frac{\text{ng hTg/mL } R_x - \text{ng hTg/mL } S_x}{\text{ng hTg/mL DR}} \text{ x100} = \% \text{ recovery}$$

Recoveries between 70% and 130% are considered valid. Levels of <70% or >130% are due to interference and the hTg level of the relevant original sample is considered invalid.

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.
Calculate the average count per minute (CPM) for each pair of assay tubes
Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}6} / C / Sx / Rx \text{ (cpm)} - D}{T(\text{cpm})} \text{ x } 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of hTg.
Determine the hTg concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	hTg ng/mL	Mean cpm (n = 20)	B/T%	hTg ng/mL
T		293137		
D (NSB)	0	110	0.04	
$S_{0.3}$	0.3	398	0.13	
$S_{1,0}$	1.0	916	0.31	
$S_{4,0}$	4.0	2648	0.89	
S_{20}	20	10154	3.43	
S_{100}	100	48363	16.33	
S_{250}	250	107279	36.23	
CI		1457	0.49	1.9
CII		36295	12.26	77.9

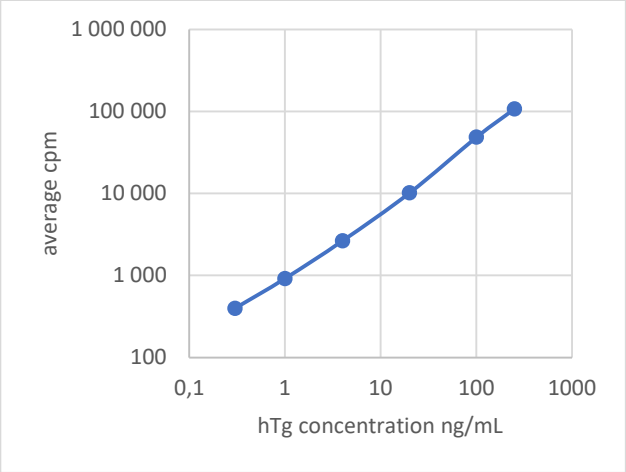

Figure 1: A typical standard curve
(*Do not use to calculate unknown samples)*

Characterization of assay

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17-A2.

Limit of Blank (LoB): 0.025 ng/mL
Limit of Detection (LoD): 0.10 ng/mL
Limit of Quantitation (LoQ): 0.20 ng/mL

Hook effect

There is no high dose hook effect up to an hTg concentration of 20000 ng/mL.

Linearity

The linearity was evaluated according to CLSI EP06-A guideline, using the polynomial method. The method was found linear from 0.1 ng/mL to 274 ng/mL, within 10% error in this interval.

Recovery – addition test

The recovery test was performed as specified before in this instruction for use. 92 individual human serum samples, previously tested negative for anti-hTg, were spiked with the recovery serum included in the kit. The base hTg concentration of the samples (Sx) and the hTg concentration of the spiked samples (Rx) were measured. The expected increase (DR) is that measured when spiking the Serum Diluent (with zero hTg concentration) with the Recovery Serum. The Recovery % is calculated as percentage of measured increase per expected increase of hTg in each spiked sample:

$$Recovery\ \% = \frac{Rx\ -\ Sx}{DR}\ x\ 100$$

The average recovery was 109.9%. The highest result is 119% and the lowest result is 93.1%.

Precision

Single-site precision

Single-site precision was calculated using 5 serum pools at different hTg concentrations, according to CLSI document EP05-A3. Samples were measured in twenty testing days, two runs per day and using two replicates per run.

Sample	Mean (ng/mL)	*Repeatability*		*Within-laboratory Precision*	
		SD	CV%	SD	CV%
Pool 1	0.27	0.03	10.83	0.04	15.54
Pool 2	5.43	0.23	4.19	0.25	4.67
Pool 3	20.18	0.47	2.31	0.65	3.22
Pool 4	52.89	0.92	1.73	1.39	2.62
Pool 5	136.55	4.41	3.23	4.63	3.39

Multisite precision

Multisite precision was calculated using 5 serum pools at different hTg concentrations, according to CLSI document EP05-A3. Samples were measured in three different sites, at each site five runs were performed, one run per day and using five replicates per run.

Sample	Mean (ng/mL)	*Repeatability*		*Within-laboratory Precision*		*Reproducibility*	
		SD	CV%	SD	CV%	SD	CV%
Pool 1	0.258	0.032	12.46	0.036	13.92	0.039	14.95
Pool 2	5.075	0.334	6.59	0.344	6.78	0.366	7.21
Pool 3	18.86	0.977	5.18	1.003	5.32	1.017	5.39
Pool 4	49.58	1.892	3.82	2.108	4.25	2.128	4.29
Pool 5	129.84	6.554	5.05	6.886	5.30	6.941	5.35

Reference Interval

Reference Interval was established following the EP28-A3c CLSI Guideline, using the non-parametric method. 484 presumably healthy blood donors were evaluated.
The central 95% reference limits obtained (with 90% confidence intervals) is from **0.3 ng/mL** (0.22 – 0.51 ng/mL) **to 50 ng/mL** (40.6 – 69.7 ng/mL).
It is recommended that each laboratory determine a reference range for healthy persons for its own patient population, since this may vary in different laboratories or regions.

Interference:

Interference testing was performed according to CLSI document EP7-A2. Predefined acceptable interference threshold: <15%. Interference could not be detected for endogenous substances and drugs up to the following concentration:

Bilirubin	684 µmol/L
Triglycerides	16.94 mmol/L
Haemoglobin	10 g/L
Rheumatoid Factor	400 IU/mL
Biotin	100 ng/mL
Acetaminophen	15.6 mg/dL
Acetylsalicylic acid	3.0 mg/dL
Ascorbic acid	5.25 mg/dL
Diclofenac	2.4 mg/dL
Ibuprofen	21.9 mg/dL
Levothyroxine (LT4)	10 µg/mL
hTSH	1.5 µg/mL
Sorafenib	500 µg/mL
Lenvatinib	15 µg/mL

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Addition of wash buffer.** For the addition of wash buffer, the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 75 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 67 days from availability.

Literature

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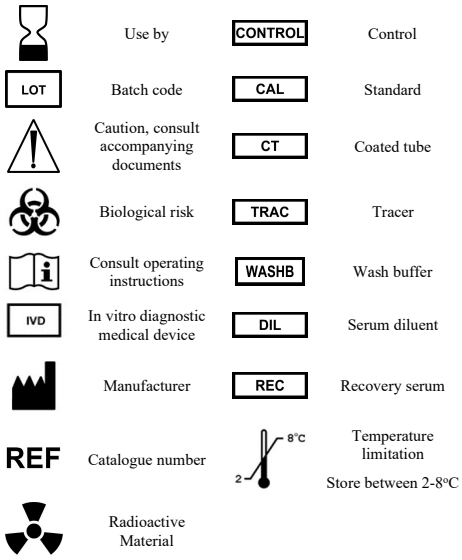

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