

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 sample¹⁵ (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:

$$\frac{\text{ng hTg/mL Rx} - \text{ng hTg/mL Sx}}{\text{ng hTg/mL DR}} \times 100 = \% \text{ recovery}$$

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:

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

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

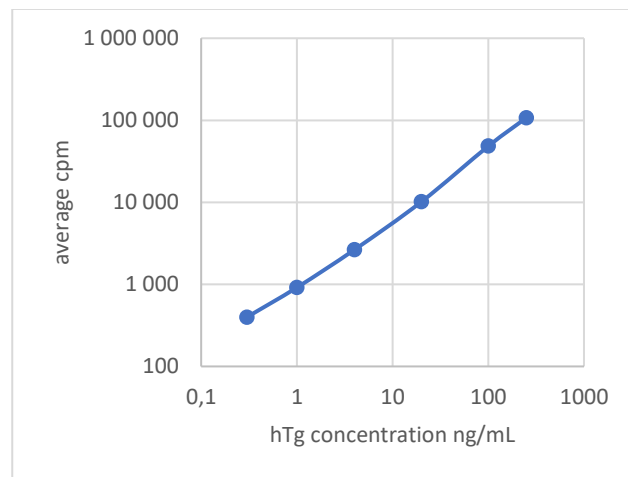


Figure 1: A typical standard curve
(Do not use to calculate unknown samples)

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:

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

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

1: Feldt-Rasmussen U et al. Ann Biol Clin (Paris). 1996;54(10-11):337-42. Human thyroglobulin reference material (CRM 457). 1st Part: Assessment of homogeneity, stability and immunoreactivity.

2: Feldt-Rasmussen U et al. Ann Biol Clin (Paris). 1996;54(10-11):343-8. Human thyroglobulin reference material (CRM 457). 2nd Part: Physicochemical characterization and certification.

3: Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016 Jan;26(1):1-133.

4: F. Pacini, M. G. Castagna, L. Brilli & G. Pentheroudakis. Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 23 (Supplement 7): vii110-vii119, 2012.

5: Yoo JY, Stang MT. Current Guidelines for Postoperative Treatment and Follow-Up of Well-Differentiated Thyroid Cancer. Surg Oncol Clin N Am. 2016 Jan;25(1):41-59.

6: Giovanella L, Castellana M, Trimboli P. Unstimulated high-sensitive thyroglobulin is a powerful prognostic predictor in patients with thyroid cancer. Clin Chem Lab Med. 2019 Dec 18;58(1):130-137.

7: Kelly A, Barres B, Kwiatkowski F, Batisse-Lignier M, Aubert B, Valla C, Somda F, Cachin F, Tauveron I, Maqdasy S. Age, thyroglobulin levels and ATA risk stratification predict 10-year survival rate of differentiated thyroid cancer patients. PLoS One. 2019 Aug 19;14(8):e0221298.

8: Farnaz Nesari Javan, Narjes Ayati, Kayvan Sadri, Esmat Ramezanzadeh, Fateme Farahmandfar, Somaye Beheshti, Seyed Rasoul Zakavi. Evaluation thyroglobulin level during suppressive therapy measured by ultrasensitive technique in the prediction of excellent response in patients with differentiated thyroid cancer. Iran J Nucl Med 2022;30(2):109-114.

9: van Kinschot CMJ, Peeters RP, van den Berg SAA, Verburg FA, van Noord C, van Ginhoven TM, Visser WE. Thyroglobulin and thyroglobulin antibodies: assay-dependent management consequences in patients with differentiated thyroid carcinoma. Clin Chem Lab Med. 2022 Jan 28;60(5):756-765.

10: CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

11 NCCLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A. Wayne, PA; 2003.

12: Clinical and Laboratory Standards Institute. Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition. CLSI document EP7-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2005.

13: CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI

document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

14: CLSI. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition*. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

15: Giovanella L, D'Aurizio F, Algeciras-Schimmich A, Görges R, Petranovic Ovcaricek P, Tuttle RM, Visser WE, Verburg FA; hsTg&TgAb Consensus Working Group. Thyroglobulin and thyroglobulin antibody: an updated clinical and laboratory expert consensus. *Eur J Endocrinol*. 2023 Aug 2;189(2):R11-R27.

	Use by	CONTROL	Control
LOT	Batch code	CAL	Standard
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	WASHB	Wash buffer
IVD	In vitro diagnostic medical device	DIL	Serum diluent
	Manufacturer	REC	Recovery serum
REF	Catalogue number	 8°C	Temperature limitation
	Radioactive Material		Store between 2-8°C



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