



INSTRUCTION MANUAL

IVD

(July 21st, 2005)

SELco[®] Tg

- 100 determinations -

REF 1300



Immunoradiometric assay
for the determination of **Thyroglobulin (Tg)**
in human serum



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INTENDED USE

The SELco[®] Tg is used for the quantitative and very sensitive determination of human thyroglobulin (hTg) in serum. Biochemically, Tg is to be understood as a rather complex family of molecules. It is microheterogeneous with inter- and intraindividual variations (iodination degree, carbohydrate contents etc.). Dimers and several fragments also exist. Additional heterogeneity is due to malignant de-differentiation. Specifically and unspecifically interfering factors in individual sera cause further problems. Therefore, the Tg determination still represents a rather ambitious method.

On the other hand, Tg is the substratum of the thyroid hormone synthesis. Only thyroid tissue (even of malignant nature, if still differentiated) has the ability to produce, to store and to secrete Tg. Consequently, Tg is organ- and tissue specific.

This is the basis for the main indication of the Tg determination (**postoperative monitoring of differentiated thyroid carcinoma**). Its paramount clinical value consists in the early detection and exclusion of metastases or tumor relapses and in the reliable follow-up of radioiodine treatments. Tg-profiles are of particular meaningfulness. After total thyroidectomy (and ablation by radioiodine) **serum Tg is not detectable anymore** in patients who are free of metastases and tumor (**complete remission**). Even under endogenous TSH stimulation, Tg normally remains undetectable.

Detectable Tg values, however, are well accepted as important indication for still existing or newly developed **neoplasia**. Of particular significance are Tg values which are already detectable on TSH-suppressive thyroid hormone treatment or which show a **steady increase** during this drug regimen (**Tg profiles**). Another relevant criterion is a **significant Tg increase after thyroid hormone withdrawal**.

In the event, that any non-malignant thyroid remnants have been left, Tg is normally undetectable during TSH-suppressive thyroid hormone treatment. However, bigger remnants (> approx. 3 ml) or any co-existing non-malignant thyroid disease can lead in fact to detectable Tg. If the patient is on TSH stimulation, however, remnants as potential origin of measurable Tg have always to be taken into account.

In **benign thyroid diseases**, more or less elevated Tg values are regularly observed as compared to the reference range of healthy normal persons. Several factors (smoking, estrogens, pregnancy, goitrogen drugs, iodine deficiency, TRAb etc.) and, in particular, disturbances of the **morphological integrity** of the gland (goitre, nodules, cellular destructions or thyroid autonomy etc.) act often complex and frequently lead to Tg elevations. Serum Tg is stimulated by TSH and is normally decreased by thyroid hormone administration (and iodine under certain circumstances, as well).

PRINCIPLE of the TEST

The SELco[®] Tg is an immunoradiometric assay (IRMA). One of the two specially selected monoclonal anti-Tg antibodies is immobilized on the inner surface of the tubes (coated tube system). The other one is 125-iodine labelled and acts as tracer. Both monoclonals are used in excess and bind Tg at different epitopes, which are relatively free from interferences. As a result, solid-phase fixed sandwich-type complexes are formed. After removal of the non-bound radioactivity, two washing steps are performed in order to achieve highest possible sensitivity in the very low Tg concentration range, as well. Thereafter, the radioactivity remained in the tubes is measured.

The higher the Tg concentration of the sample the more tracer is specifically bound, that is, the signal (cpm) of the bound fraction is proportional to the Tg concentration. In case of Tg absence in the sample, no immunocomplex is formed. Using the measured cpm's or the binding B/T (%) and the corresponding Tg concentrations of the calibrators 1 - 6 the standard curve is constructed from which the Tg values of the unknown samples in the **measuring range 0.3 - 250 ngTg/ml** are read-off.

If Tg values are above approx. 200 ng/ml, the sample has to be **diluted 1 : 100** (and additionally 1 : 500 to be on the safe side) with the Tg-free serum diluent (**component 0**) provided. These samples are again analyzed. In that way, concentrations up to 25 000 and 125 000 ngTg/ml, respectively, become accessible.

The use of any (artificially defined) zero calibrator is not required. It is recommended to analyze the serum diluent 0 as unknown sample 1, repre-

IFU symbols radioactive assays MEDIPAN GMBH

	In vitro diagnostic device		EC Declaration of Conformity
	Catalogue number		Batch code
	Expiry date		Manufactured by
	Consult accompanying documents		Consult operating instruction
	Store at		Biological risk
	Radioactive component		
	Coated tube		Dilution
	Tracer		Wash buffer
	Control serum		Calibrators
	Recovery sample		

sending the Tg-negative control. Its corresponding recovery sample 0R serves as control for the 100 % value of the recovery experiment.

PATIENTS SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Lipaemic and hemolytic samples should not be employed.

The samples may be kept at 2 - 8 °C for up to three days. For long-term storage, - 20 °C are necessary. Repeated freezing and thawing should be avoided. If required, the samples have to be initially frozen in aliquots.

Preparation before use

Prior to assay, allow the samples to reach room temperature. Take care to agitate serum samples gently in order to ensure homogeneity.

TESTCOMPONENTS for 100 DETERMINATIONS

A <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">CT</div>	Coated tubes , Coated with anti-Tg, monoclonal (mouse)	2 x 50 tubes, ready for use
D <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">TRAC</div>	Tracer , 125-I-anti-Tg, monoclonal (mouse) < 700 kBq	 1 bottle 21 ml ready for use
B <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">WASHB</div>	Wash buffer (Concentrate for 500 ml)	1 bottle 20 ml
1 - 6 <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">CAL</div>	Tg calibrators 1 - 6 Conc.: see leaflet enclosed Any Tg zero standard is not necessary.	 6 vials 0.7 ml serum, each ready for use
C I - II <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">CONTROL</div>	Tg control sera I and II Conc.: see leaflet enclosed	 2 vials 0.7 ml serum, each ready for use
0 <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">DIL</div>	Serum diluent (Tg-free), (Tg-negative control, as well)	1 vial 5 ml ready for use
R <div style="border: 1px solid black; padding: 2px; width: fit-content; margin: 2px auto;">REC</div>	Recovery sample (for recovery test) Conc.: 500 ngTg/ml	1 vial 1 ml ready for use

Size and storage

The SELco® Tg has been designed to allow 100 determinations. This is sufficient for the analysis of 20 unknown samples with their respective recovery tests as well as calibrators and control sera, all assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit (max. 8 - 9 weeks) on the box label.

Upon receipt, all components of the SELco® Tg have to be kept at 2 - 8 °C in the original kit box. The coated tubes remain in their foil-covered boxes.

Preparation before use

Before using the components in the assay, allow all of them to reach room temperature. During this time, the coated tubes remain in their foil-covered boxes. All components of the kit are ready for use except

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reagent B (**wash buffer**) provided as concentrate. It has to be filled up to **500 ml final volume** by distilled water before use.

ASSAY PROCEDURE

- Use two non-coated tubes for the determination of the total radioactivity T.

- Number the test tubes (A) appropriately.
- Pipette into the corresponding tubes according to assay scheme
 - 100 µl calibrators,
 - 100 µl control sera,
 - 100 µl patient's sample 1
 - 100 µl patient's sample 1 **plus 10 µl recovery sample R** etc.
- Add into **all tubes** 200 µl tracer (D), each, including the tubes for total radioactivity T.

(The two tubes T are now kept separately until radioactivity is measured - working step 8).
- After short-term agitation of the tubes (in order to ensure homogeneous reaction conditions), **incubate over night** (minimum 15 hours) at room temperature.
- Add 1 ml washing solution (made from B) to each tube.
- Aspirate or decant.
- Repeat washing procedure (**working steps 5 and 6**) two times more. These steps have to be carried-out with great care.

For removal of any remaining liquid turn tubes upside down (5 - 10 minutes) and absorb any droplets by tapping on blotting paper.
- Measure radioactivity of **all** tubes including T in the Gamma-Counter, recommended time: 1 minute

DATA PROCESSING

The mean cpm-values of calibrators 1 - 6 are plotted on the ordinate (log. scale) versus their respective Tg-concentrations (abscissa, log. scale, as well) with the subsequent construction of the standard curve.

By means of the respective cpm mean values, the Tg concentrations of the controls and of the unknown samples as well as the recovery results can be directly read off in ngTg/ml.

Instead of the cpm mean values, the respective percent binding rates related to the total radioactivity (B/T %) can be used, as well. Here, a log. scale is also recommended.

This data processing can also be made by a computer assisted analysis using a software program suitable for sandwich-type assays (IRMA).

Any **extrapolation** of the standard curve to Tg values above 250 ng/ml (calibrator 6) is **not permitted**. Patients sera with such high Tg levels have to be diluted 1 : 100 (additionally 1 : 500 to be on the safe side) with the Tg-free serum diluent (**component 0**) provided. These samples have to be analyzed again (including recovery tests). In this way, values up to 25 000 and 125 000 ngTg/ml, respectively, become available.

Note: Tg values above 2 millions ng/ml have already been observed.

Any "lengthening" of the standard curve to values below 0.3 ngTg/ml (calibrator 1) is also misleading, even with the help of any artificially defined type of zero-standard or of the serum diluent (component 0). Therefore, the range of measurable Tg concentrations in the SELco® Tg starts at its first standard (0.3 ngTg/ml) in a technical sense, too.

CHARACTERISTICAL ASSAY DATA

TYPICAL EXAMPLE

(approx. 4 weeks before expiry)

Do not use for evaluation!

Test tubes	cpm (a)	cpm (b)	cpm (mean)	$\frac{B}{T}$ %	ngTg/ml
Total radioactivity T	287463	288145	287804	---	---
Calibrator 1	395	365	380	0.13	0.3
Calibrator 2	738	806	772	0.27	1.0
Calibrator 3	2175	2321	2248	0.78	4.0
Calibrator 4	10562	10902	10732	3.73	20.0
Calibrator 5	51694	51270	51482	17.9	100.0
Calibrator 6	103461	104161	103811	37.1	250.0
Control I	---	---	---	---	---
Control II	---	---	---	---	---
Sample 1	207	253	230	0.08	< 0.3
Sample 1 R	27364	27111	27238	9.46	50

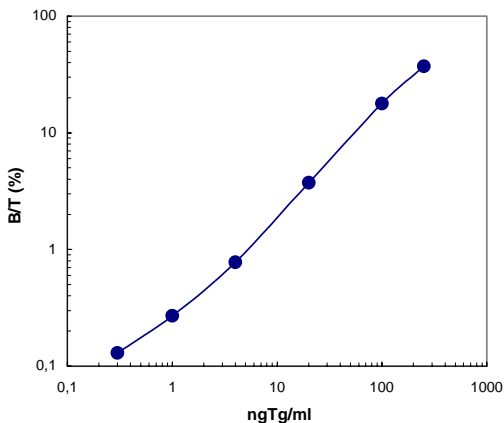
Calculation of the recovery

$$\frac{\text{Sample 1 R (ngTg/ml)} - \text{Sample 1 (ngTg/ml)}}{50 \text{ (ngTg/ml)}} \times 100 = \% \text{ Recovery Sample 1}$$

Instead of the 50 ngTg/ml as denominator, the result of the recovery sample 0R (ngTg/ml) of the serum diluent 0 should be used.

STANDARD CURVE

Typical example



REFERENCE VALUES

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

- Differentiated thyroid carcinoma in complete remission after total thyroid ablation:**
 Below 0.5 - 1 ngTg/ml on TSH-suppressive thyroid hormone treatment, no Tg-increase under TSH-stimulation.

 Tg-values above this "grey zone" (particularly, in case of TSH-suppressive treatment) strongly indicate the necessity of extensive diagnostic investigations.
Verification: Significant Tg-increase under TSH-stimulation.

 Any thyroid remnants are to be taken into account, particularly under endogenous TSH-stimulation (thyroid hormone withdrawal).
- Healthy normal persons:**
 approx. 2 - 70 ngTg/ml (median approx. 13 ng/ml), slightly lower in areas of sufficient iodine supply (approx. 1 - 35 ng/ml, median at approx. 10 ng/ml).

Binding capacity

The maximum binding capacity of the SELco® Tg is defined by means of the highest standard (B_0/T %) and is normally 30 to 40 %.

Calibration

The calibration of the SELco® Tg is orientated to the first International Tg Reference Material CRM 457 (Community Bureau of Reference, BCR, European Union, Brussels, Belgium), which, however, is still preliminary.

Parallelism of standards and serum samples

Defined dilutions of the reference material in human serum (Tg-free) measured in the SELco® Tg show results as expected. Human sera of high Tg contents also lead to the expected results within the usual margins of error after appropriate serial dilution with Tg-free human serum. When using the Tg-free serum diluent 0, equivalent data are observed.

Specificity (recovery test, high dose hook effect)

The falsification of any Tg determination by specifically (anti-Tg, anti-TPO) and unspecifically acting serum factors can in principle not be excluded. Therefore, every Tg value has to be checked for accuracy by means of the **recovery test**: **Parallel determination** of the sample in the same assay after a known amount of Tg (10 µl recovery test serum R) has been added.

In case of uninterfered recovery (100 %) in the SELco® Tg, the Tg value of the respective recovery test sample is 50 ngTg/ml higher than that of the corresponding original specimen. Routinely, recovery results between 70 and 130 % are assessed as correct. In contrast, values below 70 % and above 130 % represent incorrect recovery and normally indicate a falsely-low Tg value in the corresponding original serum.

In order to check the basic reliability of the performance of that test, it is recommended to run the recovery test for the serum diluent 0, as well. The result of the sample 0R shows the experimental 100 % value. The sample 0 itself simultaneously represents the Tg-negative control.

In SELco® Tg, serum levels above 250 ng/ml always correspond to cpm values higher than those of the calibrator 6 provided that the serum Tg remains below approx. 20 000 ng/ml. However, Tg concentrations above approx. 20 000 ng/ml result in cpm values below standard 6 (**high dose hook effect**). Consequently, the resulting Tg values become more and more falsely-low, if these genuine sera are measured undiluted. In case of suspicion of such a phenomenon the respective serum should be re-tested at 1 : 100 predilution.

Any **cross reactivity** with thyroxin or tri-iodothyronin and HSA, respectively, could not be detected even in supra-physiological concentrations.

Sensitivity (lower detection limit)

The most appropriate and statistically reasonable definition of the lower detection limit of any assay is at present the so-called **functional assay sensitivity**.

The functional assay sensitivity generally represents that concentration, which corresponds to the 10 % (within-assay) and to the 20 % (between-assay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of the SELco® Tg, this value is at approx. 0.5 - 1 ngTg/ml.

Formally determined Tg values below this Tg level do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and can, therefore, not be distinguished from zero with the statistically necessary certainty.

Tg concentrations above approx. 0.5 - 1 ng/ml, however, fulfil these criteria and are consequently assessed as valid.

LIMITATIONS of the METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.




SELco[®] Tg

ASSAY SCHEME

1	Number	Test tubes A	S 1 - 6	I - II	Pat. 1	Pat. 1 R (recovery)	T
2	Pipette	Calibrators 1 - 6 Controls CI / CII Patient's sample 1 Patient's sample 1 + Recovery sample R etc.	100 µl	100 µl	100 µl	100 µl 10 µl	
3	Pipette	Tracer D	200 µl	200 µl	200 µl	200 µl	200 µl
4	Incubate*	Over night (15 hours minimum) at room temperature					
5	Pipette	Washing solution	1 ml	1 ml	1 ml	1 ml	
6	Decant or aspirate						
7	Wash. procedure (5 + 6)		repeat two more times				
8	Count radioactivity		counting time: 1 minute				

* Prior to incubation, agitate the tubes briefly in order to ensure homogeneous reaction conditions

SAFETY PRECAUTIONS

- This kit is for **in vitro use only**. Follow the working instruction carefully.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- All reagents should be kept at 2 - 8 °C in the original shipping container before use.
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 - Some of the reagents contain small amounts (< 0.1 % w/w) of sodium azide as a preservative. Nevertheless, they must not be swallowed or allowed to come into contact with skin or mucosae. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
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 - Source material derived from human body fluids or organs and used in the preparation of this kit were tested and found negative for both hepatitis and HIV antibody. However, no known test can guarantee the absence of such viral agents. Therefore, handle all components (and all patient samples) as if potentially hazardous.
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 - Since the kit contains radioactive material the following precautions should be observed:
 - Do not smoke, eat or drink while handling radioactive material,
 - Always use protective gloves,
 - Never pipette radioactive material by mouth,
 - Wipe up spills promptly, washing the affected surface with a decontaminant thoroughly,
 - Place contaminated tissues, tubes, bench covers, gloves etc. in a special marked container, discard liquid and solid radioactive waste only as permitted by State, Federal or local authorities and regulations.
- It is the responsibility of the user of this product to handle radioactive material in accordance to the national rules given by law or other statements of the local authorities.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.