



INSTRUCTION MANUAL

IVD

(February 01st, 2008)

SELco® TRAb

- 100 determinations -

European Patent
EP 1021721

REF 1040



Radioreceptor Assay for the determination
of TSH receptor autoantibodies (TRAb)
in human serum



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INTENDED USE

SELco® TRAb is used for the quantitative determination of Thyrotropin (TSH) receptor autoantibodies in human serum.

The majority of TSH receptor autoantibodies mimic the action of TSH on thyroid cells and thus increase blood levels of T4 and T3. Because they are not controlled by the negative feedback system, the stimulation of the thyroid often leads to the clinical thyrotoxic state of Graves' disease.

Consequently, the measurement of TRAb is valuable for the differential diagnosis of hyperthyroidism as well as for the follow-up of Graves' disease, both during and after its treatment by antithyroid drugs or 131-radioiodine or surgery.

Pregnant women developing Graves' disease can transfer their autoantibodies to the foeto placental unit. The higher the TRAb concentrations of the mother, the greater the risk for the infant to develop Graves' disease in utero and thus to present with congenital hyperthyroidism.

LITERATURE

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- Kakinuma A, I Morimoto, T Kuroda, T Fujihira, S Eto, SM McLachlan & B Rapoport: Comparison of recombinant human thyrotropin receptors versus porcine thyrotropin receptors in the thyrotropin binding inhibition assay for thyrotropin receptor autoantibodies; Thyroid 1999, 9:849-855
- Zimmermann-Belsing T, Nygaard B, Rasmussen AK & U Feldt-Rasmussen: Use of the 2nd generation TRAK human assay did not improve prediction of relapse after antithyroid medical therapy of Graves' disease; Eur. J. Endocrinol 2002, 146: 173-177
- Kamijo K: TSH-receptor antibody measurement in Patients with various Thyrotoxicosis and Hashimoto's Thyroiditis: a comparison of two two-step assays, coated plate ELISA using porcine TSH receptor and coated tube radioassay using human recombinant TSH-receptor: Endocrine J 2003, 50: 113-116

PRINCIPLE of the TEST

The SELco® TRAb is used for the determination of TSH receptor autoantibodies (TRAb) in human serum.

SELco® TRAb is a competitive radio receptor assay (RRA). During the first incubation step the TSH receptor autoantibodies (TRAb) of the patient samples or calibrators bind to the immobilized TSH receptors on the solid phase of coated tubes. After two washing steps bound TRAb are detected by their ability to inhibit the binding of 125-I-labelled TSH to the receptor coated tubes (second incubation).

The absence of autoantibodies against TSH receptor results in a complete saturation of the provided receptor by the 125-I-TSH. Thus, the more TRAb are present in the sample, the less 125-I-TSH is bound by the immobilized TSH receptors. This excessive 125-I-TSH is separated by decantation of the tubes.

Following two washing steps the radioactivity is measured by means of a γ -counter. The level of the measuring signal is inversely proportional to the TRAb concentration in the tested samples and can be read off from a calibrator curve with known TRAb concentrations .

IFU symbols radioactive assays MEDIPAN GMBH

IVD	In vitro diagnostic device	CE	EC Declaration of Conformity
REF	Catalogue number	LOT	Batch code
	Expiry date		Manufactured by
	Consult accompanying documents		Consult operating instruction
	Store at	D	Biological risk
	Radioactive component		
CT	Coated tubes	TRAC	Tracer
START	Start buffer	WASHB	Wash buffer
CAL	Calibrators	CONTROL	Control serum

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation.

Plasma must not be used in SELco® TRAb. The same applies for lipaemic and grossly hemolytic sera.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.


Repeated freezing and thawing should be avoided. For multiple use, initially aliquot samples and keep at - 20 °C.

Preparation and use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Before assayed the sera have to be free of any particulate matter (centrifuge, if necessary, and **use the clear supernatants** only).

TEST COMPONENTS for 100 DETERMINATIONS

A	Coated tubes coated with pTSH receptor		5 x 20 tubes, ready for use
CT			
B	Wash buffer Concentrate for 500 ml wash buffer solution. Dilute to a final volume of 500 ml with distilled water before use.		1 vial 50 ml
WASHB			
D	Tracer (125-Iodine-TSH) < 0.180 MBq, t _{1/2} = 59 days gamma radiation 35 keV, x radiation 27 keV, 31 keV		1 vial, 11 ml red colored ready for use
TRAC			
H	Start Buffer		1 vial, 10 ml yellow colored ready for use
START			
0 - 4	Calibrators conc.: 0; 1; 2; 8; 40 IU/l		5 vials; 0.7 ml, each, ready for use
CAL			
C	Positive control (human serum) conc.: cf. leaflet enclosed		1 vial, 0.7 ml, ready for use
CONTROL			

Materials required

- Precision pipettes 50 - 100 µl, 1000 µl
- Disposable pipette tips
- Graduated cylinders
- Distilled or de-ionized water
- Absorbent paper or paper towel
- Foil
- Orbital shaker (> 250 rpm)
- γ-counter

Size and Storage

SELco® TRAb has been designed for 100 determinations. This is sufficient for the analysis of 44 unknown samples as well as calibrators and control serum, assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit (max. 6 weeks) on the box label.

Upon receipt, all components of the have to be kept SELco® TRAb at 2 - 8 °C preferably in the original kit box.

Preparation before use

All components of the kit must be stored at 2 - 8 °C but allowed to reach room temperature for at least 30 min before use.

- A Coated tubes:**
Any unused coated tubes should be kept in the original foil bag(s) (reseal with adhesive tape), sealed in the self seal.
- B Wash Buffer:**
Dilute wash buffer concentrate (B) to a final volume of 500 ml with distilled water before use.
- D Tracer:** all of these reagents
- H Start buffer:** are ready for use
- 0 - 4 Calibrators:** and must be pipetted
- C Control serum:** at room temperature.

ASSAY PROCEDURE

- Duplicates are recommended.

1. Label test tubes appropriately.
2. Pipette into each tube 50 µl start buffer H and in the corresponding tubes according to assay scheme
 - 100 µl of calibrators 0 - 4
 - 100 µl of control serum
 - 100 µl of patient's sample 1, 2 etc.
3. **Incubate 2 hours** at room temperature on an orbital shaker (> 250 rpm).
4. Pipette 1 ml washing solution (diluted from B) into each tube. For removal of any remaining liquid turn tubes upside-down (5 minutes) and absorb any droplets by tapping on blotting paper.
Repeat washing step.
5. Pipette 100 µl tracer (D) into each tube.
6. **Incubate 1 hour** at room temperature on an orbital shaker (> 250 rpm).
7. Pipette 1 ml washing solution (diluted from B) into each tube. For removal of any remaining liquid turn tubes upside-down (5 minutes) and absorb any droplets by tapping on blotting paper.
Repeat washing step.
8. Measure radioactivity of all tubes.
Recommended counting time: **1 minute**

DATA PROCESSING

We recommend lin / log processing for best results!

The standard curve is established by plotting the mean calculated cpm or binding rates of the calibrators 0 - 4 on the ordinate, y-axis, (log. or linear scale) versus their respective TRAb-concentrations on the abscissa, x-axis, (log. scale).

The TRAb concentrations of the control and the unknown samples are **directly read off** in IU/ml against these cpm or respective binding rate values. SELco® TRAb may also be used with Computer Assisted Analysis with software able to plot lin/log curves with spline smoothing, such as for RIAs.

TYPICAL EXAMPLE

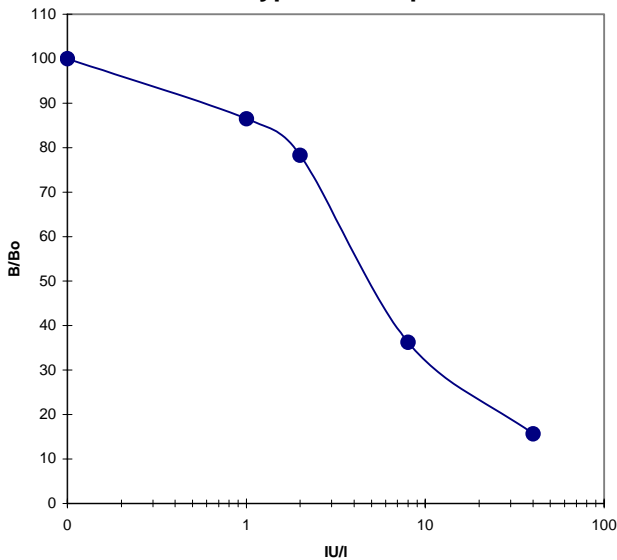
(approx. 4 weeks before expiry)

Do not use for evaluation!

Test tubes	cpm (a)	cpm (b)	cpm (mean)	B ₀ /T (%)	B/B ₀ (%)	IU/l
Total radioactivityT	59 075	59 308	59 192		---	---
calibrator 0	7 396	7 141	7 269	12.2	100	0
calibrator 1	6 321	6 263	6 292		86.5	1
calibrator 2	5 800	5 579	5 690		78.3	2
calibrator 3	2 689	2 574	2 632		36.2	8
calibrator 4	1 151	1 115	1 133		15.6	40
control		cf. leaflet enclosed				
patient 1	3 666	3 463	3 566		49.0	5.3

STANDARD CURVE

Typical example



REFERENCE VALUES

SELco [®] TRAB	IU/l
negative	< 1
grey zone	1 - 1.5
positive	> 1.5

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.

Healthy individuals should be tested negative by using the SELco[®] TRAB. In active Graves' disease positive values of TSH receptor autoantibodies are found in approx. 70 - 85 % of all cases, whereas hyperthyroidism due to thyroid autonomy normally results in negative TRAB value.

CHARACTERISTIC ASSAY DATA

Parallelism of standard

Dilutions of specimen in antibody-free human sera are determined according to their expected theoretical values with SELco[®] TRAB. On the basis of the heterogeneous nature of the autoantibody population in human serum and in view of epitope specificity and affinity of the autoantibodies in some cases are not determined the expected theoretical values.

50 % Intercept Point

Represents the concentration corresponding to 50 % specific binding and is regularly found about 5-6 IU/l.

Calibration

SELco[®] TRAB is calibrated according to the WHO reference standard 90/672. Results are expressed in International Units (IU/l).

Specificity

Human TSH levels up to 100 mU/l did not show any significant cross reactivity in SELco[®] TRAB.

Sensitivity

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

This 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 SELco[®] TRAB, this value is found at approx. 1.0 IU/l.

SELco[®] TRAB values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

SELco[®] TRAB concentrations above approx. 1.0 IU/l, however, fulfill these criteria and are consequently assessed as valid.

Intra - and inter-assay variation

Intra-assay			Inter-assay		
Sample no.	Mean Concentration (IU/l)	CV (%)	Sample no.	Mean Concentration (IU/l)	CV (%)
1	1	10	5	2	8
2	5	7	6	4	7
3	10	6	7	6	5
4	18	9	8	8	6

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[®] TRAb

ASSAY SCHEME

1	Label	Test tubes	Cal 0 - 4	C	Pat. 1 etc.	T
2	Pipette	Start buffer H Cal 0 - 4 Control serum Patients sera 1 etc.	50 µl 100 µl --- ---	50 µl --- 100 µl ---	50 µl --- --- 100 µl	
3	Incubate		2 hours at room temperature on an orbital shaker (> 250 rpm)			
4	Pipette	Washing solution (diluted from B) Decant the supernatant Repeat washing step	1 ml	1 ml	1 ml	
			leave tubes 5 minutes upside down			
5	Pipette	Tracer	100 µl	100 µl	100 µl	100 µl
6	Incubate		1 hour at room temperature on an orbital shaker (> 250 rpm)			
7	Pipette	Washing solution (diluted from B) Decant the supernatant Repeat washing step	1 ml	1 ml	1 ml	
			leave tubes 5 minutes upside down			
8	Measure radioactivity		Recommended measuring time: 1 minute			

* Use clear sera only (centrifuge, if necessary)

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- 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 before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 %) of sodium azide as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for both Hepatitis an HIV antibody. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains radioactive material the following precautions should be observed:
 - Do not smoke, eat or drink while handling radioactive material in any room designated for working with radioactive material,
 - Always use protective gloves,
 - Never pipette radioactive material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant,
 - Place contaminated tissues, tubes, bench covers, gloves etc. in a specially marked container, discard liquid and solid radioactive waste only as permitted by federal, state 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 with all general and individual regulation has to be applied to the use of this kit.

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