
بسم الله الرحمن الرحیم

TSH receptor antibodies (TRAb)

in diagnosis & management of thyroid disorders

Maryam Tohidi
Associate professor of anatomical & clinical pathology
Research Institute for Endocrine Sciences
Shahid Beheshti University of Medical Sciences

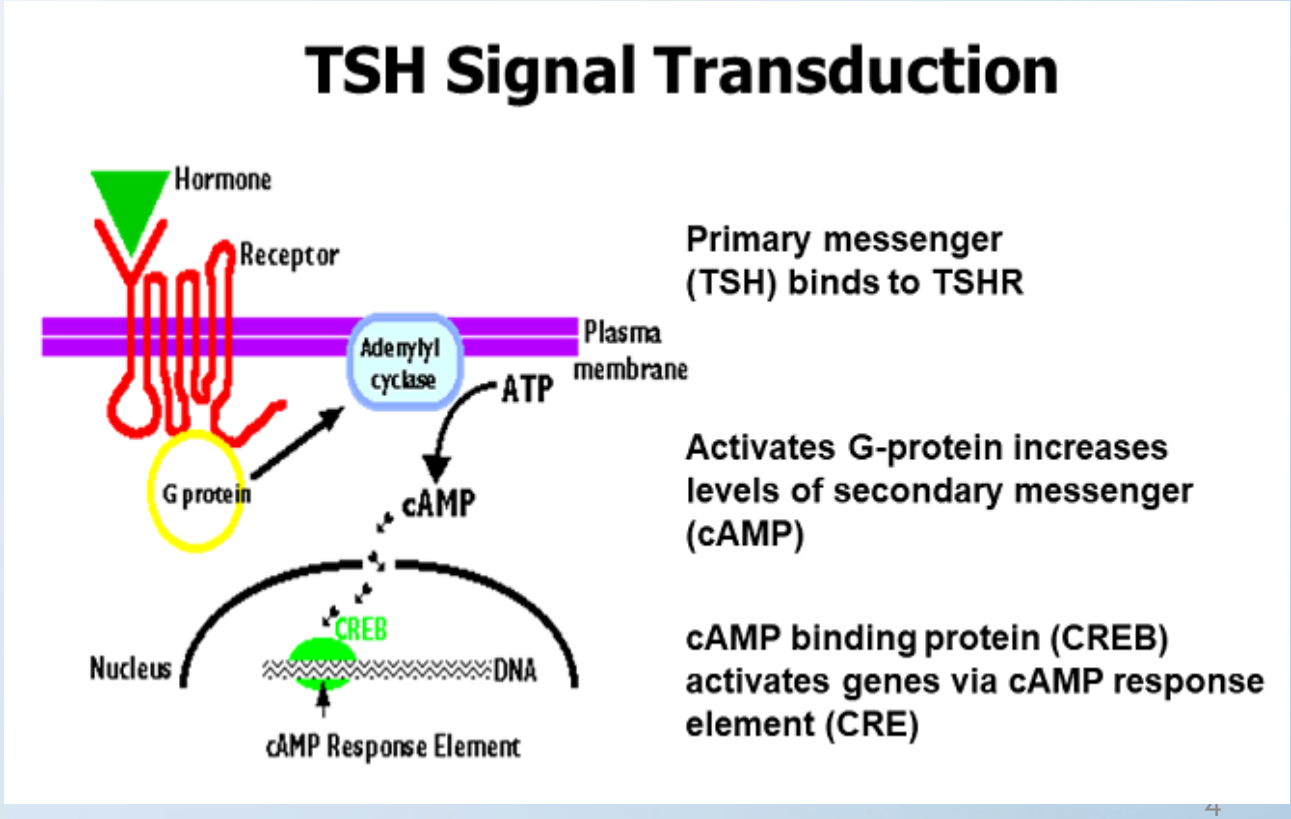
January 2022
Tehran, Iran

Agenda

- ✓ Introduction on autoantibodies to the TSH receptor (TSH-R-Abs, TRAb)
- ✓ Functional effects of TSH-R-Abs
- ✓ Nomenclature of TSH-R-Abs
- ✓ Assays for TSH-R-Abs & Cut-off
- ✓ Clinical usage of TSH-R-Abs

Introduction

- ✓ TSH-R-Ab, often referred to as TRAb, refers to any type of Ab interacting specifically with the TSH receptor.
  - ✓ TSH receptors are members of the large family of G-protein coupled receptors.
  - ✓ The major second messenger is cAMP.



Molecular characteristics of TSH-R & frequency of TRAb	
Antigen	TSH-R
Molecular weight (kDa)	85
Number Of aminoacids	764
Distribution in tissues	Thyroid, variety of extra-thyroidal tissues: lymphocytes, fibroblasts, adipocytes (retro-orbital)
Function	Transduction of TSH signal
TRAb frequency in general population (%)	1-2
TRAb frequency in Graves disease (%)	Close to 100
TRAb frequency in chronic autoimmune thyroiditis (%)	6-10

Klaus Zöphel , Dirk Roggenbuck, Matthias Schott Clinical review about TRAb assay's history. Autoimmunity Reviews 9 (2010) 695–700

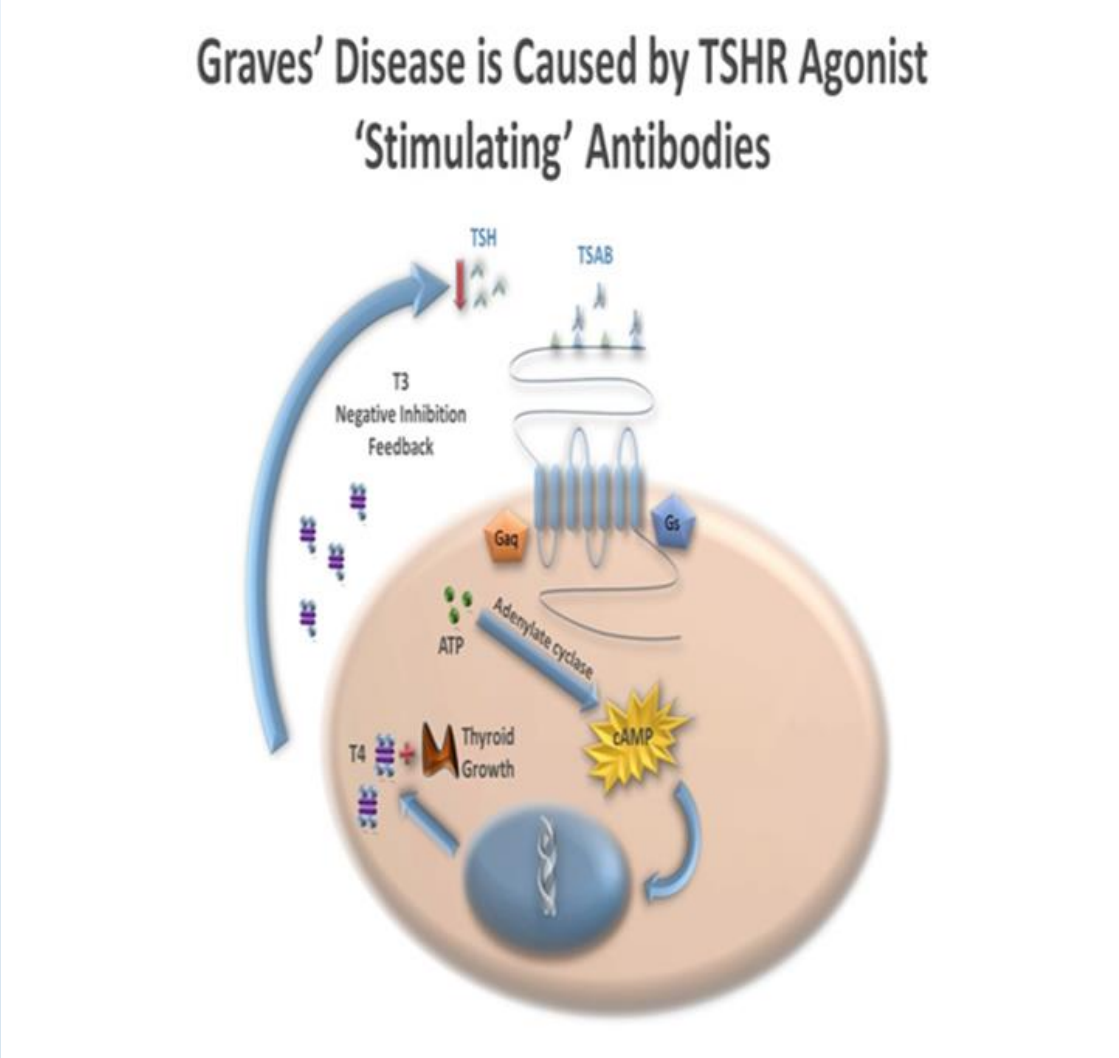
Types of TSH-R-Abs

✓TSH-R-Abs are subdivided according to their functional effects:

- ✓Thyroid-stimulating antibodies (TSAb)
- ✓ Thyroid-blocking antibodies (TBAb)
- ✓ Neutral antibodies
- ✓ Unknown antibodies

Thyroid-R-stimulating antibody (TSAb)

- TSAb, like TSH, bind primarily to the large amino terminal ectodomain of the TSH-R
- activate the cAMP signal transduction pathway
- leading to stimulation of:
  - ✓ thyroid hormone production
  - ✓ proliferation of thyrocytes



Graves' disease , an autoimmune disorder

- ✓ persistent stimulation of thyroid follicular epithelial cells
- ✓ uncontrolled by the hypothalamic–pituitary axis
- ✓ TSH-R-stimulating antibodies (TSAb) predominantly of IgG 1 subclass that activate the TSH-R
- ✓ oligoclonal generation, primarily by intra-thyroidal B cells, reflects the disease's primary autoimmune reaction.
- ✓ TSAb, specific for and central to Graves' disease

TSH-R-blocking Abs (TBAb)

- TSH-R-blocking antibodies can antagonize or block the action of TSH and in doing so cause hypothyroidism in certain patients with various types of autoimmune thyroiditis, particularly Hashimoto's thyroiditis.

Neutral TSH receptor antibodies

- TSH-R antibodies that neither induce the cAMP signal pathway nor block the binding of TSH are referred to as neutral or recently "cleavage" Abs and previously it was thought that they have no known functional influence.
- Recently, it has been found a more significant role such as regulating selective signaling cascades and inducing apoptosis in thyroid epithelial cells by signaling pathways distinct from the cAMP pathway.
- Another type of antibodies named as "Unknown antibodies" may also be included.

frontiers in Endocrinology

GENERAL COMMENTARY
published: 15 February 2017
doi: 10.3389/fendo.2017.00028

TSH Receptor Antibody Functionality and Nomenclature

George J. Kahaly and Tanja Diana*

Molecular Thyroid Research Laboratory, Department of Medicine I, Johannes Gutenberg University Medical Center, Mainz, Germany

Keywords: Graves' disease, functional TSH receptor antibodies, stimulating antibodies, blocking antibodies, nomenclature

A commentary on

Graves' disease
by Smith TJ, Hegedus LN. Engl J Med (2016) 375(16):1552–65. doi:10.1056/NEJMra1510030

TSH –R- Abs Nomenclature

- ✓ Different terms have been used to describe the different types of TSH-R-Abs.
- ✓ It is important for the clinician to be aware of the different nomenclature
- ✓ The used terminologies frequently reflect which assay is performed to determine TRAb.

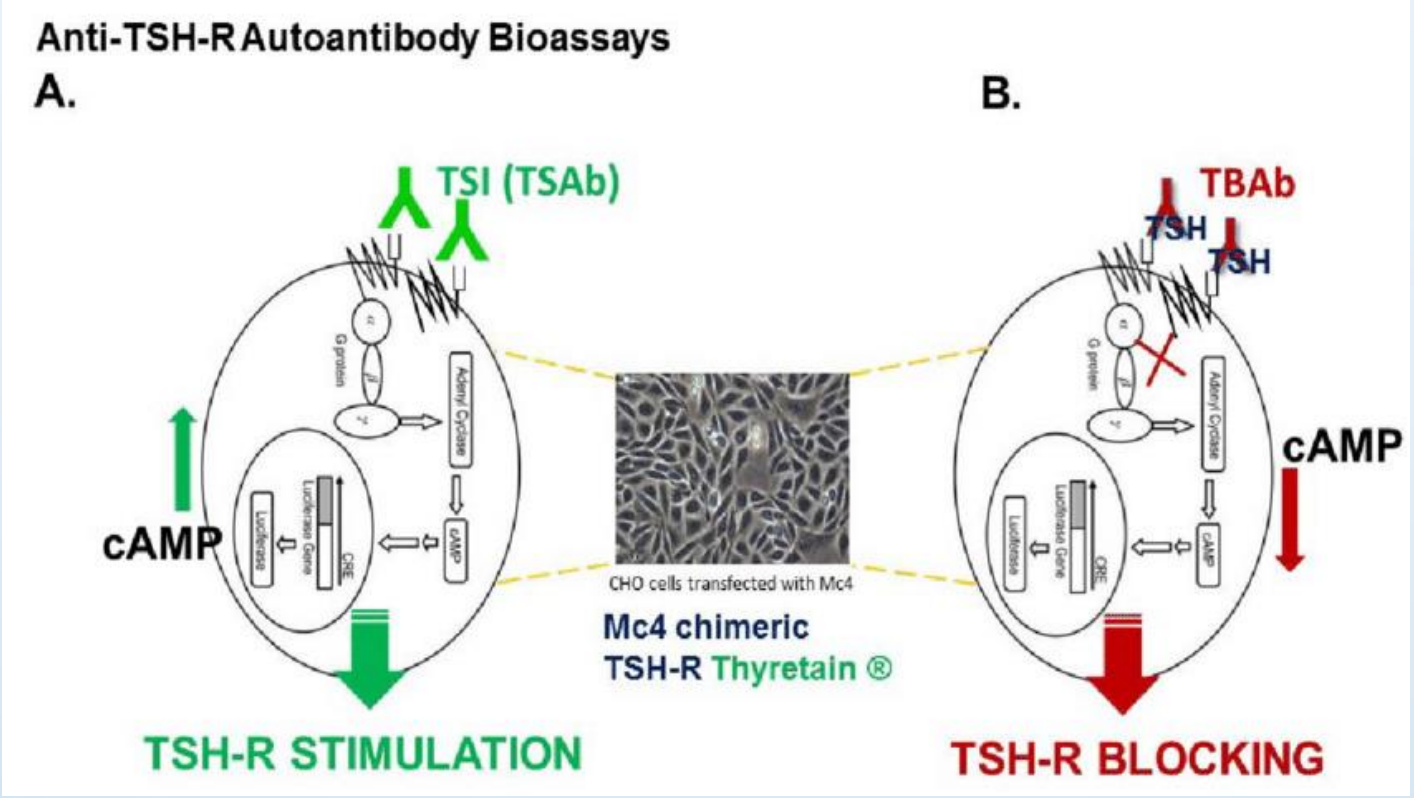
Assays performed for TRAb measurement

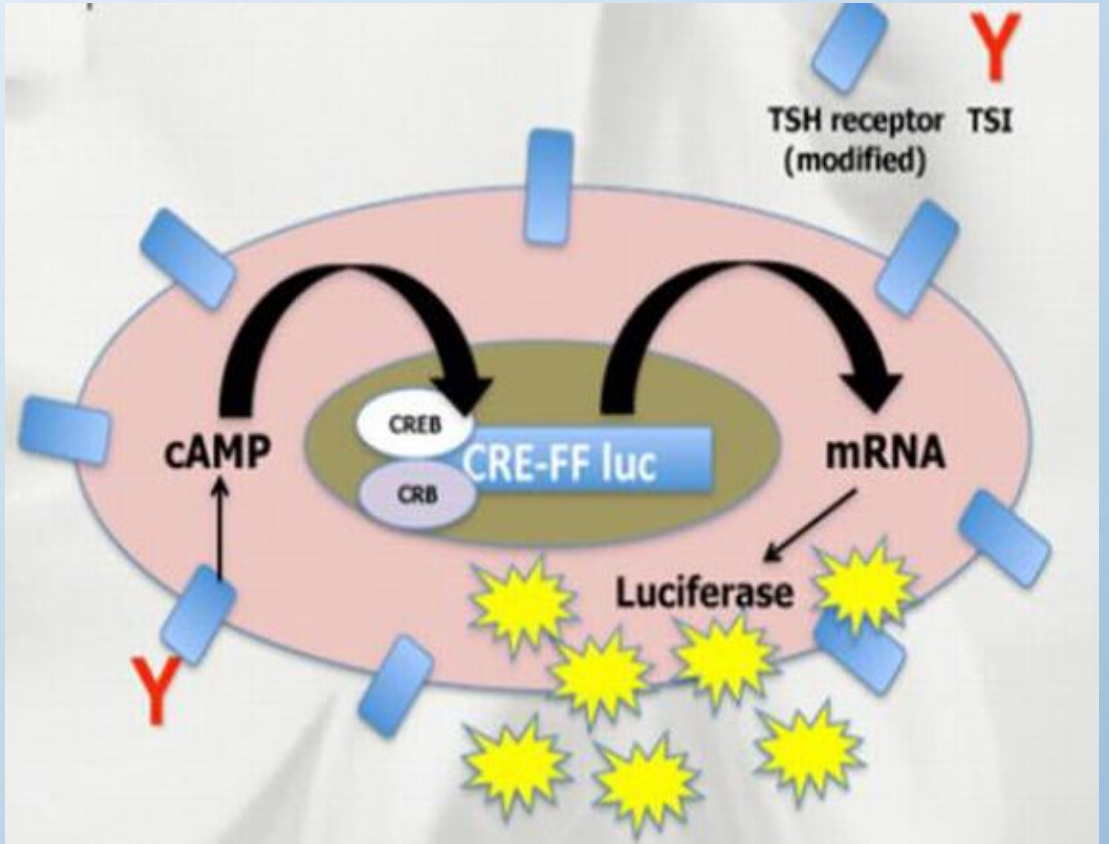
1) Cell-based bioassays
2) Competitive binding assay (competition-based assay or ligand assay)

✓ Bioassays for TRAb are more sensitive than the automated binding assays and exclusively differentiate between stimulatory and blocking Ab activity.

Cell-based bioassays for TRAb

- ✓ Bioassays for the TRAbs measure the ability of Abs to increase or decrease the intracellular level of cAMP directly or indirectly, e.g. from engineered Chinese Hamster Ovary (CHO) cells transfected with hTSH-R reported through luciferase production.
- ✓Such assays can differentiate between the TSH-R antibody types & specifically detect TSAb.


Anti-TSH-R Autoantibody Bioassays
A.
B.
TSI (TSAb)
TBAb
TSH
TSH
cAMP
cAMP
CHO cells transfected with Mc4
Mc4 chimeric
TSH-R Thyretain ®
TSH-R STIMULATION
TSH-R BLOCKING


TSH receptor
(modified)
TSI
cAMP
CREB
CRB
CRE-FF luc
mRNA
Luciferase

Cell-based bioassays

Cell-based bioassays measure :

❖ ***Stimulatory Abs:***

- TSH-R stimulatory antibodies (TSAb)
- TSH-R-stimulating immunoglobulins (TSI)

❖ ***Blocking Abs:***

- TSH-R-blocking antibodies (TBAb)
- TSH-R-blocking immunoglobulins (TBI)

❖ *Terminologies for TSH receptor antibodies used in bioassays*

Cell-based bioassay	
• TSH-R-stimulating antibodies	TSAb
• TSH-R-stimulating immunoglobulins	TSI
▪TSH-R-blocking antibodies	TBAb, TSB-Ab, or TRBAb
▪TSH-R-blocking immunoglobulins	TBI

Competitive binding assays for TRAbs

- ✓ Competition assays measure inhibition of binding of either a labeled monoclonal anti-human TSH-R antibody or labeled TSH to a recombinant TSH-R.
- ✓ These TRAb assays are unable to distinguish the antibody types.
- ✓ Because these TSH receptor Abs are commonly assessed in a competitive binding assay, they are referred to as TSH-R-binding inhibitory immunoglobulins (TBII).

❖ *Terminology for TSH receptor antibodies binding assays*

Competitive-binding assay	
• TSH-R-binding inhibitory immunoglobulins	TBII

The word "inhibitory" in this terminology dose not indicate blocking antibodies.

It is believed that TRAbs assessed by the TBII assay are mostly TSAb and TBAb. (Ajjan RA, Weetman AP (2008) Techniques to quantify TSH receptor antibodies. Nat Rev Endocrinol 4(8):461)

History of competition-based TRAb assays

- ✓ 1974, Rees Smith and Hall, original receptor assay for TRAb
- ✓ employing particulate thyroid tissue from patients with GD and I-125-labelled bovine TSH
- ✓ Needs IgG preparations and physiological serum IgG led to a significant non-specific effect
- ✓ Low functional and diagnostic sensitivity

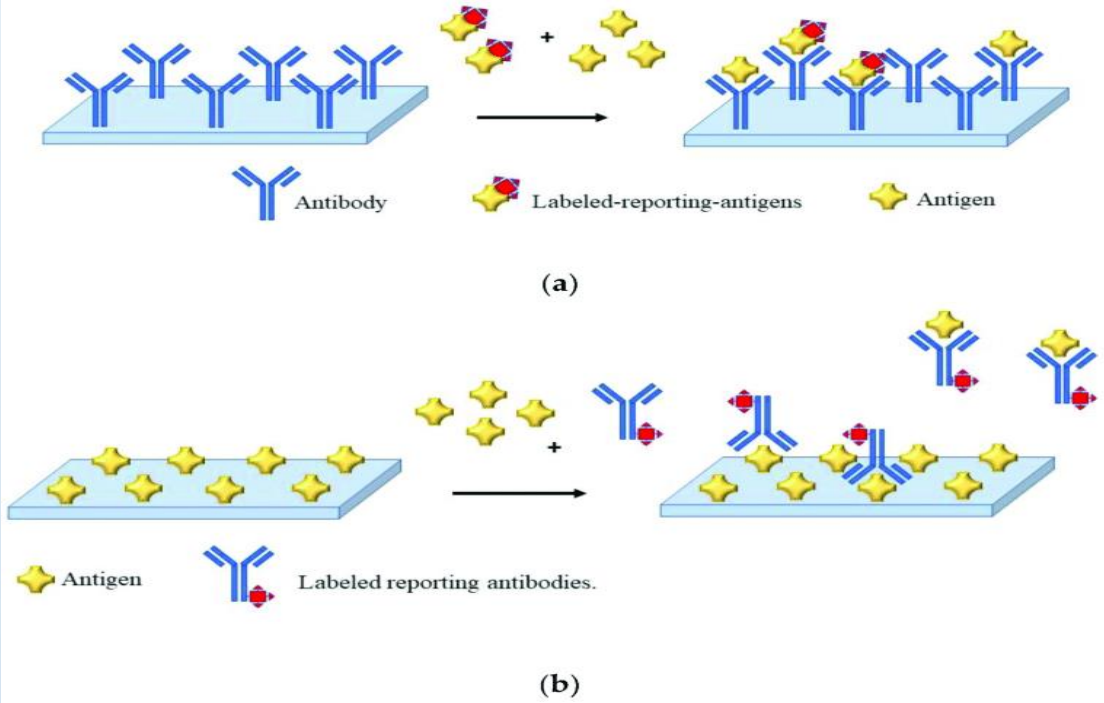
I. First generation competition-based TRAb assays

- ✓ Liquid phase TRAb assays with detergent solubilized TSHR preparations & radiolabeled I-125-labelled bovine TSH

  "porcine TRAb assay" or "human TRAb assay"

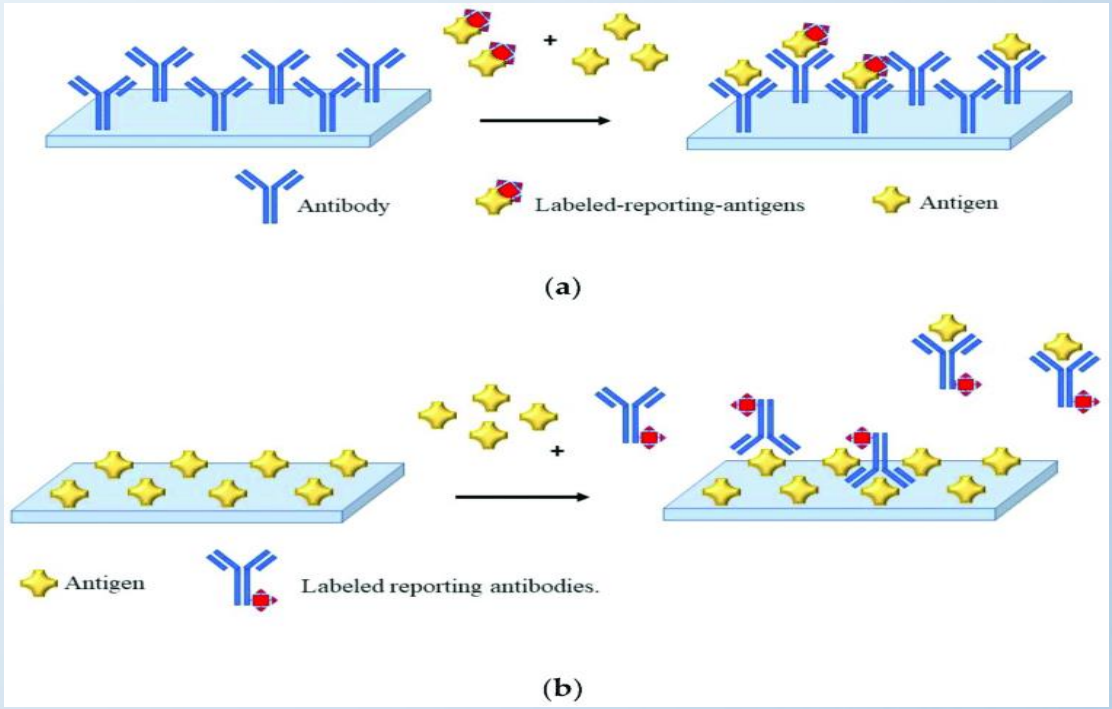
- ✓ Autoantibodies in the patient's serum compete with either porcine thyroid membrane or recombinant TSH receptor.

- ✓ This assay is in solution, making it impossible to wash.



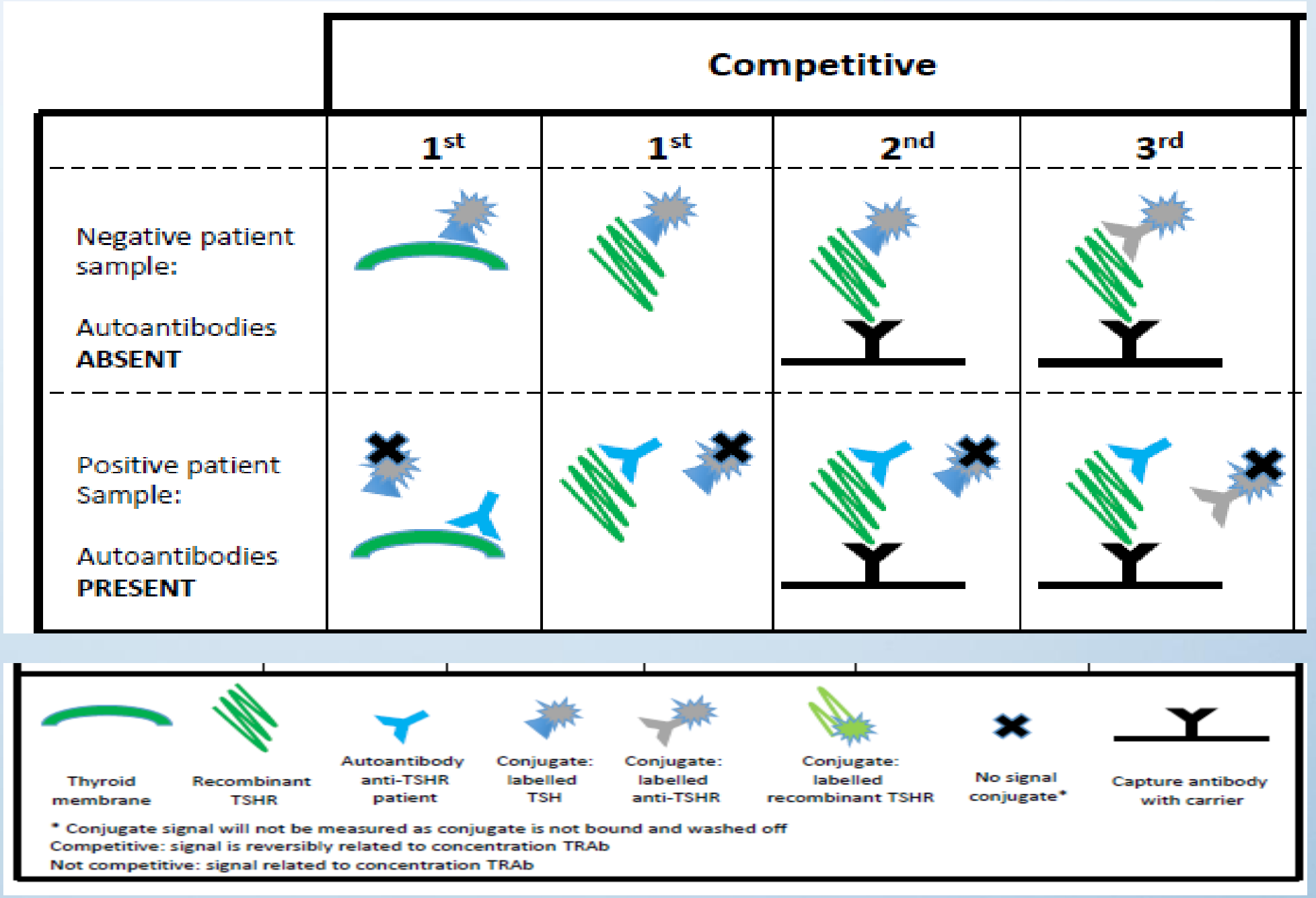
II. Second generation competition-based TRAb assays

- ✓ 1990s
- ✓ Solid phase TRAb assays, i.e. autoantibodies in patient's serum can bind the recombinant TSH receptor bound to a capture membrane.
- ✓ Fluorescent readout instead of radioactive one
- ✓ Easier & more accurate
- ✓ "porcine TRAb assay" or "human TRAb assay"



III. Third generation TRAb assays (2003)

- ✓ TRAb assays based on a thyroid stimulating monoclonal antibody of human origin (TSMAb) named M22
- ✓ The monoclonal antibody M22 was produced from lymphocytes of a GD patient
- ✓ Principle of assay: TRAb of patient sera inhibit the binding of M22 to immobilized porcine TSHR
- ✓ As with all autoantibody tests it is important to use an internationally accepted standard to allow comparison of results from different laboratories.
- ✓ New commercial assay are strictly calibrated to NIBSC 90/672 *(National Institute for Biological Standards and Control)*
- ✓ Approximately 5% of patients with newly diagnosed Graves' hyperthyroidism are TRAb negative in older assays , and 3% are negative in third-generation assays , especially those with milder disease.

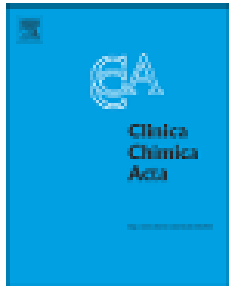

Competitive
1st
1st
2nd
3rd
Negative patient sample:
Autoantibodies ABSENT
Positive patient Sample:
Autoantibodies PRESENT
Thyroid membrane
Recombinant TSHR
Autoantibody anti-TSHR patient
Conjugate: labelled TSH
Conjugate: labelled anti-TSHR
Conjugate: labelled recombinant TSHR
No signal conjugate*
Capture antibody with carrier
* Conjugate signal will not be measured as conjugate is not bound and washed off
Competitive: signal is reversibly related to concentration TRAb
Not competitive: signal related to concentration TRAb

Clinica Chimica Acta 401 (2009) 84–89

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

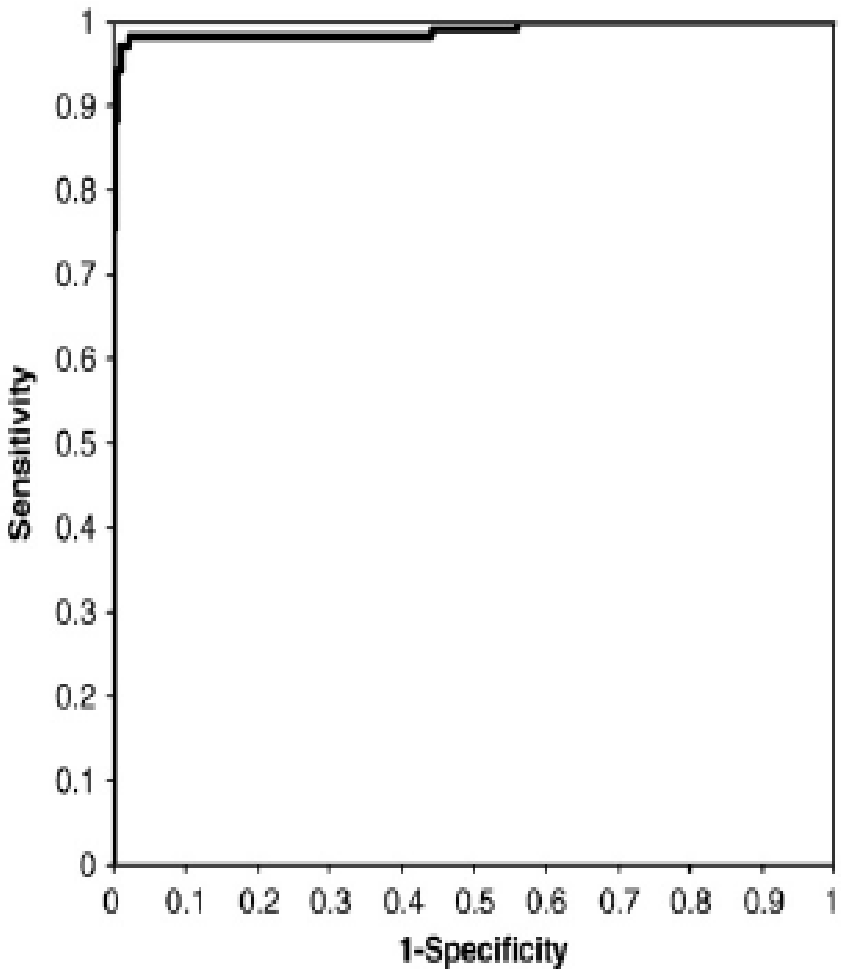


Technical evaluation of the first fully automated assay for the detection of TSH receptor autoantibodies

Derik Hermsen [a,*], Martina Broecker-Preuss [b], Marco Casati [c], Jordi Camara Mas [d], Anja Eckstein [e],

- ✓ A multi center study (nine clinical evaluation sites in Europe and Japan)
- ✓ A fully automated M22 based TRAb assay technique
- ✓ Strictly calibrated to NIBSC 90/672

Determination of the cut-off limit for positivity of the Elecsys Anti-TSHR assay



The ROC analysis was performed in:

- 102 patients with untreated Graves' disease (sensitivity cohort),
- 210 patients with other thyroid diseases
- 436 apparently healthy individuals (specificity cohort).

- ✓ Functional assay sensitivity of about 0.73 IU/L

- ✓ Based on ROC plot analysis, the cut-off for positivity was defined at:
  - ✓ 1.75 IU/L for GD
  - ✓ corresponding to sensitivity 97% & specificity 99%
  - ✓ AUC 0.99 (95% CI: 0.98–1.0)

  - ✓ The upper limit of TRAb in healthy individuals: 1.22 IU/L

  - ✓ The upper limit of TRAb in thyroid disease without Dx of GD: 1.58 IU/L

- ✓ The median CV at the cut-off (1.75 IU/L) was found to be 11%.

- ✓ Intra-assay and total imprecision CV were determined between 1.4%–14.9%, and 2.4%–28.8%, respectively.

Struja et al. BMC Endocrine Disorders (2019) 19:38
https://doi.org/10.1186/s12902-019-0363-6

BMC Endocrine Disorders

RESEARCH ARTICLE Open Access

Comparison of Five TSH-Receptor Antibody Assays in Graves' disease: *results from an observational pilot study*

Tristan Struja[1*†], Rebecca Jutzi[1†], Noemi Imahorn[1], Marina Kaeslin[1], Fabienne Boesiger[1], Alexander Kutz[1], Esther Mundwiler[2], Andreas Huber[2], Marius Kraenzlin[4], Beat Mueller[1,3], Christian Meier[3,4], Luca Bernasconi[2†] and Philipp Schuetz[1,3†]

- Observational study on 332 patients presenting with Graves' disease
- Aim: Assessment of abilities of four TSH-receptor antibody tests [TRAb] and one cyclic adenosine monophosphate bioassay for prediction of relapse of Graves' disease.
- Cut-offs suggested by the manufacturers were used to evaluate diagnostic performance.

Assays	Cut off
IMMULITE TSI	0.55 U/L
BRAHMS TRAK	1.8 U/L
EliA anti-TSH-R	2.9 U/L
RSR TRAb Fast	1.0 U/L
RSR-bioassay STIMULATION	150 %

Table 3 AUC for GD diagnosis and relapse compared to GREAT score and refitted with new TRAb's[b]

	DIAGNOSIS				GD RELAPSE PREDICTION			
Discriminator	Sensitivity [%]	Specificity [%]	AUC	95% CI	AUC	95% CI	Improved AUC	95% CI
GREAT score without routine TRAb					0.57	(0.43–0.71)		
GREAT score with routine TRAb[a]					0.69	(0.56–0.81)		
							GREAT score with new assay	
BRAHMS TRAK	86.7	93.7	0.96	(0.91–0.99)	0.71	(0.57–0.86)	0.67	(0.53–0.81)
IMMULITE TSI	94.0	91.7	0.97	(0.92–0.99)	0.69	(0.54–0.84)	0.66	(0.53–0.79)
EliA anti-TSH-R								
≥ 2.9 U/L	79.5	93.7	0.95	(0.90–0.98)	0.68	(0.52–0.83)	0.68	(0.54–0.82)
> 3.3 U/L	71.1	97.9	0.95	(0.90–0.98)				
RSR TRAb Fast	94.0	89.6	0.96	(0.91–0.99)	0.67	(0.50–0.83)	0.64	(0.50–0.78)
RSR-bioassay STIMULATION	81.9	87.5	0.90	(0.84–0.95)	0.62	(0.45–0.78)	0.62	(0.48–0.76)

Conclusion: The assays tested had good diagnostic power and relapse risk prediction with few differences among the new assays.

The Netherlands Journal of Medicine

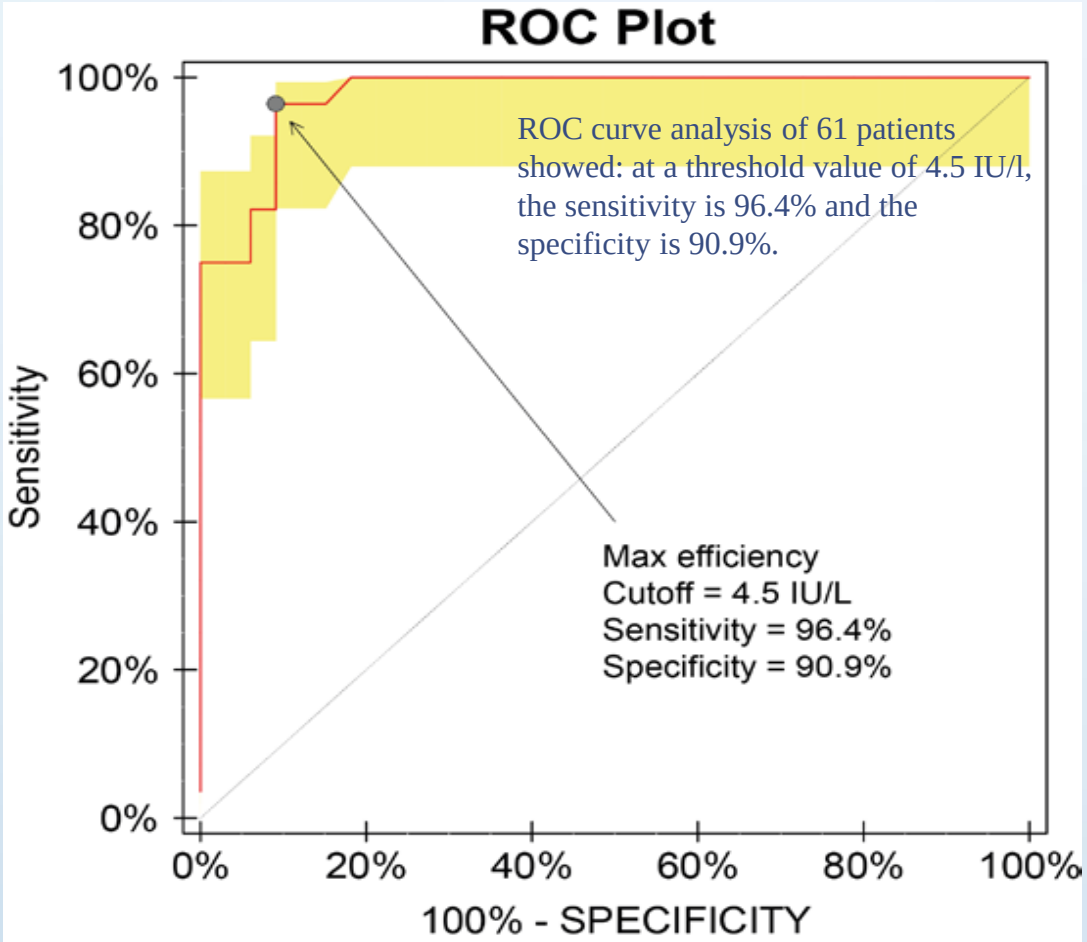
ORIGINAL ARTICLE

Measurement of anti-TSH receptor antibodies: what is the correct cut-off value?

M.A. Smit[1], C.M.J. van Kinschot[2], J. van der Linden[2], C. van Noord[2], S. Kos[1]*

Departments of [1]Clinical Chemistry, [2]Internal Medicine, Maasstad Hospital, Rotterdam, the Netherlands. *Corresponding author: koss@maasstadziekenhuis.nl

- 61 patients consisted (28 Graves' disease, 15 MNG or toxic adenoma, and 18 thyroiditis)
- newly diagnosed for the most frequent causes of hyperthyroidism
- a third-generation competitive immunoassay: EliA-anti-TSH-R (Thermofisher Scientific, Phadia, Sweden)
- according to the manufacturer, the interval of 2.9-3.3 IU/l is referred to as grey zone area and a concentration above 3.3 IU/l is considered positive.
- ROC curve analysis of 61 patients showed: at a threshold value of 4.5 IU/l, the sensitivity is 96.4% and the specificity is 90.9%.



N=61	Negative cases	Positive cases
Samples	15 MNG/toxic adenoma 18 Thyreoiditis	28 Graves' disease
Range	1.5 to 6.1 IU/l	3.4 to 52 IU/l

Conclusion: Extending the grey zone to 4.5 IU/l is probably more appropriate and will more accurately discriminate between Graves' disease and non-Graves' disease causes of hyperthyroidism.

Table 3. *Sensitivity and specificity of TRAb measurement at different cut-off values*

		Negative TRAb (# patients)	Positive TRAb (# patients)	Sensitivity	Specificity
TRAb cut-off value 2.9 IU/l	lower limit of the grey zone proposed by the manufacturer			100	58
Non-Graves' disease*		19	14		
Graves' disease**		0	28		
TRAb cut-off value 3.3 IU/l	cut-off for positivity of the manufacturer			100	79
Non-Graves' disease*		26	7		
Graves' disease**		0	28		
TRAb cut-off value 4.5 IU/l	cut-off for positivity based on the study population			96	91
Non-Graves' disease*		30	3		
Graves' disease**		1	27		

M.Tohidi

- ✓ The proposed cut off of TRAb in management of hyperthyroidism in pregnancy is a value > 5 IU/L (approximately 3 times of upper limit of normal range). *

Abeillon-du Payrat J, et al. Predictive value of maternal second-generation thyroid-binding inhibitory immunoglobulin assay for neonatal autoimmune hyperthyroidism. European Journal of Endocrinology .2014, 171, 451–460.

Besancon A, et al . Management of neonates born to women with Graves' disease: a cohort study. Eur J Endocrinol. 2014 170:855–862.

Clinical use of TSH receptor antibodies

- ✓ As diagnostic test
- ✓ For management of hyperthyroidism
- ✓ Clinical use in pregnancy
- ✓ For clinical assessment of Graves' ophthalmopathy

Diagnostic use of TRAb test

The etiology of thyrotoxicosis should be determined.

- ✓ If the etiology is not obvious based on the clinical presentation and initial paraclinical assessment, diagnostic testing is indicated and can include:

(1) Measurement of TRAb

(2) Determination of the radioactive iodine uptake (RAIU)

(3) Measurement of thyroidal blood flow on ultrasonography

R1- Strong recommendation, moderate-quality evidence.

- ✓ The choice of initial diagnostic testing depends on cost, availability, local expertise, resource and the choice of therapy [methimazole (MMI) versus RAI treatment].
- ✓ If third-generation TRAb assays are not readily available, RAIU is preferred for initial testing.
- ✓ In a thyrotoxic patient with a non-nodular thyroid and no definite orbitopathy, measurement of TRAb or RAIU can be used to distinguish GD from other etiologies.

American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid, 2016 (26):1343-1421.

Diagnostic use of TRAb test

In the setting of overt thyrotoxicosis, newer TRAb binding and bioassays have a sensitivity of 96–97% and a specificity of 99% for GD.

- ✓ If TRAb is positive it confirms the diagnosis of GD.
- ✓ If it is negative it does not distinguish among other etiologies.
- ✓ It can be negative in very mild GD.

American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid, 2016 (26):1343-1421.

Diagnostic use of TRAb test

- ✓ TRAb may also be of diagnostic value in the euthyroid patient with exophtalmus especially when it is unilateral.

- ✓ To differentiate between postpartum thyroiditis versus relapse of GD after delivery.

TRAb test in management of GD

- ✓ **An indicator of the degree of disease (GD) activity**
- ✓ **Assisting to decide about treatment modality**

ATDs is suggested for patients with high likelihood of remission including who with negative or low-titer TRAb)

- ✓ **To determine the duration of anti-thyroid drug (ATD) therapy for GD**

- ❑ Measurement of TRAb levels prior to stopping ATD therapy is suggested. Normal levels indicating greater chance for remission.

  R-21- Strong recommendation, moderate-quality evidence

- ❑ To decide to discontinue MMI chosen as the primary therapy for GD after 8-12 months, if the TSH and TRAb levels are normal at that time.

  R-22- Strong recommendation, high-quality evidence

TRAb test in management of GD

✓ **To assess the possibility of relapse of GD at the end of the course of ATD therapy**

❑ Persistently elevated TRAb:

unlikely to be in remission (80-100% relapse rate)

❑ Low or undetectable TRAb:

higher probability of permanent remission (20-30% relapse rate)

The role of TRAb test in pregnancy

- ✓Diagnosis of hyperthyroidism in pregnancy

- ✓ Management of hyperthyroidism in pregnancy

The Diagnostic role of TRAb test in pregnancy

- ✓ Once the diagnosis of hyperthyroidism is made in a pregnant woman, attention should focus on determining the etiology (gestational hyperthyroidism vs GD) and whether it warrants treatment.
- ✓ TRAb levels should be measured when the etiology of hyperthyroidism in pregnancy is uncertain.

[R 92-* Strong recommendation, low-quality evidence*]

The diagnostic sensitivity of good assays is around 95% and the specificity is 99%.

- ✓ The two best indicators of the activity of GD during pregnancy are thyroid function in the untreated patient and measurement of TRAb levels in the serum.

The role of TRAb Management of GD in pregnancy

GD diagnosed during or after first trimester

- ➢ Measuring TRAb at diagnosis and, if elevated, repeat at 18–22 weeks and again at 30–34 weeks of gestation.
- ✓ If a TRAb-positive woman becomes TRAb-negative during pregnancy, this may indicate a need to reduce or stop ATD therapy to avoid fetal hypothyroidism.
- ✓ TRAb (TBII or TSI) measurement may be useful to assist in the evaluation of disease activity in a woman being treated with ATDs for GD during pregnancy.
- ✓ If the ATD-treated mother has high TRAb values in late pregnancy, this indicates a risk of delayed neonatal hyperthyroidism

The role of TRAb Management of GD in pregnancy

GD diagnosed and treated prior to pregnancy

I. In remission after stopping ATD medication

- If euthyroid state is confirmed by thyroid function testing, TRAb measurement not necessary.

II. Currently taking methimazole

Switch to PTU or withdraw ATD therapy as soon as pregnancy is confirmed with early testing. Measure TRAb initially and, if elevated, again at 18–22 weeks and 30–34 weeks of gestation.

[R- 94-Strong recommendation, low-quality evidence]

The role of TRAb Management of GD in pregnancy

III. Previous treatment with RAI or surgery

✓ Measure TRAb initially during the first trimester and, if elevated, again at 18–22 weeks of gestation using a sensitive assay.

If the mother has undergone some type of thyroid ablation (RAI or surgery) for GD and TRAb is high, evaluate fetus carefully for hyperthyroidism in second half of pregnancy and adjust or begin ATD therapy accordingly.

[R- 93- Strong recommendation, low-quality evidence]

- Thyroidectomy is often followed by a decrease or disappearance of TRAb from circulation, whereas RAI is often followed by a transient increase in TRAb.
- If the mother still produces TRAb, the antibodies will cross the placenta and may affect fetal thyroid function in the last half of the pregnancy. Because of the slow clearance of maternal immunoglobulin G from the neonatal circulation, thyroid dysfunction in the child may last for several months after birth.

For clinical assessment of Graves' ophthalmopathy

- ✓ T- cells play a major role in initiating the disorder
- ✓ Locally produced TRAb effect many of characteristic orbital tissue change
- ✓ Patients with the most severe orbithopathy have highest titer of TRAb.
- ✓ The level of TRAb often correlates with the severity of the eye disease.
- ✓ Data presently available support the routine use of TRAb in confirming the diagnosis in patients with euthyroid Graves' ophthalmopathy.

THYROID
Volume 23, Number 1, 2013

DOI: 10.1089/thy.2012.0374

REVIEW

Thyrotropin-Blocking Autoantibodies and Thyroid-Stimulating Autoantibodies: Potential Mechanisms Involved in the Pendulum Swinging from Hypothyroidism to Hyperthyroidism or Vice Versa

Sandra M. McLachlan and Basil Rapoport

- ✓ A small proportion of TBAb-positive hypothyroid patients treated with LT4 switch to TSAb and hyperthyroidism.
- ✓ Some Graves' patients treated with ATD switch from TSAb to TBAb-induced hypothyroidism.
- ✓ These changes arise because of differences in individual patients of the relative concentrations, affinities, or potencies of TBAb and TSAb.
- ✓ The occurrence of functional TSHR antibody "switching" emphasizes the need for careful patient monitoring and the difficulties sometimes encountered in regulating thyroid function.