Anti-TSH Receptor ELISA (IgG)



Indication: Test system for the in vitro determination of antibodies against TSH receptor in human serum or plasma for the diagnosis of the following disease: Graves' disease.

Clinical significance: Graves' disease is an autoimmune disorder which manifests itself as hyperthyroidism. Other main symptoms are struma, exophthalmos, and tachycardia (Merseburg triad). Around 2% of the female and 0.2% of the male population are affected by this disease. Autoantibodies against the thyroid stimulating hormone (TSH) receptor (TRAb) are detected in about 90% of untreated Graves' disease patients.

Anti-TSH receptor autoantibodies (TRAb) are differentiated according to their biological functions, which include 1.) inhibiting the binding of TSH to its receptor (TBII, TSH binding inhibiting immunoglobulin), 2.) stimulating or blocking the function of the TSH receptor, and 3.) stimulating thyroid gland growth.

The determination of TRAb is of particular significance in suspected cases of Graves' disease and for differentiation of this condition from disseminated autonomy of the thyroid gland. Monitoring of TRAb concentrations during the course of Graves' disease allows a prognosis statement and provides an important aid for therapy management. High TRAb titers in Graves' disease patients following a long thyrostatic therapy indicate an increased risk of a relapse. Furthermore, the presence of TRAb in the third trimester of pregnancy in women with Graves' disease indicates hyperthyroidism in the foetus. TRAb are often determined in ophthalmology cases, since many patients with Graves' disease first attend an ophthalmologist because of exophthalmos.

Application of the Anti-TSH Receptor ELISA: Traditionally TRAb are detected by radio receptor assays (RRA) using ¹²⁵I-labelled TSH (first-generation assays) or by bioassays using cultured cells. Second-generation TRAb (TBII) assays, such as the EUROIMMUN ELISA, show higher diagnostic sensitivity and specificity. The development of monoclonal antibodies against a fragment of the porcine TSH receptor has made it possible to immobilize the TSH receptor to microplate wells. In the EUROIMMUN ELISA, TRAb (TBII) compete with biotinylated bovine TSH for binding to the immobilized TSH receptor. Bound TSH-biotin is then detected using an avidin-peroxidase conjugate.

Both porcine and recombinant human TSH receptors have been described in the literature as antigen substrates for anti-TSH receptor immunoassays. Equivalent first- or second-generation assays showed only marginal differences in diagnostic sensitivity and specificity, which were not attributable to the use of different receptors.

The EUROIMMUN ELISA has a similar precision to the ¹²⁵I-RRA (1st generation) and offers further advantages: 1.) a lower detection limit (0.5 IU/I compared to 3 IU/I); 2.) a nonradioactive assay format; 3.) compatibility with all commonly used microplate equipment, such as commercial washer, reader systems and fully automated ELISA processors and 4.) no false negative results due to the simultaneous presence of antibodies against TSH.

Correlation of the .EUROIMMUN Anti-TSH Receptor ELISA with two ¹²⁵I-RRA and a bioassay: Panels of sera from 56 or 60 patients with suspected Graves' disease were analysed for TRAb using the EUROIMMUN ELISA, two radio receptor assays (1st and 2nd gener-

ation) based on labelled ¹²⁵I-TSH, and a bioassay (based on an increase in cAMP in isolated porcine thyroid cells). There was a good agreement between different methods. the

		¹²⁵ I-RRA (1 st gen.) (n=56)		¹²⁵ I-RRA (2 nd gen.) (n=60)		Bioassay (n=56)				
		pos.	borderl.	neg.	pos.	borderl.	neg.	pos.	borderl.	neg.
EUROIMMUN Anti-TSH Receptor ELISA	pos.	38	0	0	37	5	2	34	0	4
	borderl.	0	2	0	3	6	2	1	2	0
	neg.	0	1	15	0	0	5	0	0	15

EUROIMMUN Microplate ELISA

Autoantibody determination: AMA M2-3E (IgG) ANCA Profile (IgG) ANA Screen (IgG) ANA Screen 9 or 11 (IgG) BP180-NCT6A-4X (IgG) BP180-0CF (IgG) C1a (IgG) BP230-CF (IgG) C1q (IgG) cardiolipin (IgA, IgG, IgM, IgAGM) circulating immune complexes (CIC) cyclic circulinated peptide (CCP; IgG) centromere protein B (IgG) desmoglein 3 (IgG) double-stranded DNA (dsDNA, nDNA; IgG) double-stranded DNA (dsDN dsDNA-NcX (lgG) ENA Pool/lus (lgG) ENA Profile/lus 1 or 2 (lgG) ENA SLE Profile 1 or 2 (lgG) GAD GAD/IA-2 Pool glomerular basement membrane (GBM; IgG) 82-glycoprotein 1 (IgA, IgG, IgM, IgAGM) histones (IgG) IA-2 b2-grycoproteim r (gs/, igs/, Latex agglutination tests: spermatozou ovary zona pellucida Further autoimmune diagnostics: gliadin (GAF-3X; IgA, IgG) Saccharomyces cerevisiae (IgA, IgG) ous serology: vvirus (IgA, IgG, IgM) tella pertussis (IgA, IgG, IgM) tella FHA (IgA, IgG) ia (IgG, IgM) ia VIsE (IgG) Ia abottus (IgA, IgG, IgM) Chlamydia pnedriconiae (IgA, IgG, IgM) Chlamydia tradomatis (IgA, IgG, IgM) Chlamydia tradomatis (IgA, IgG, IgM) Dengue virus (IgG, IgM) Dengue virus (IgG, IgM) Diphtheria toxoid (IgG) Echinococcus granulosus (IgG) Epstein-Barr virus capsid ag (IgA, IgG, IgM) Epstein-Barr virus early ag (IgA, IgG, IgM) Epstein-Barr virus early ag (IgA, IgG, IgM) Epstein-Barr virus early ag (IgA, IgG, IgM) Helicobacter pylori (IgA, IgG, IgM) Helicobacter pylori (IgA, IgG, IgM) HSV-1 (g)coprotein C2; IgA, IgG, IgM) HSV-2 (g)coprotein C2; IgA, IgG, IgM) Influenza virus type 8 (IgA, IgG, IgM) Mesales virus (IgG, IgM) Mesales virus (IgG, IgM) Parainfluenza virus Ford (IgA, IgG, IgM) Parainfluenza virus Ford (IgA, IgG, IgM) RVV (IgA, IgG, IgM) Rvbella virus (IgG, IgM) Rvbella virus (IgG, IgM) Rvbella virus (IgG, IgM) Tetarus toxoid (IgG) Toxoplasma gondii (IgG, IgM) Vericella zoster virus (IgG, IgM) Verschild, IgG, IgM) Verschild, IgG, IgM) Versinia enterocol. virulence fact. (IgA, IgG, IgM) Versinia enterocol. virulence fact. (IgA, IgG, IgM) id ag (IgA, IgG, IgM) ag (IgA, IgG, IgM) ear ag, EBNA-1 (IgG) ⊦ "America" (IgG, IgM)

Allergology: total IgE Allercoat™ 6-ELISA (650 different allergens and allergen mixtures) Software EUROIMMUN Allercoat

Serum proteins and tumour markers: anti-p53

Bone metabolism marker: 25-OH-Vitamin-D Saliva diagnostics:

alpha-amvlase cortisol slgA

Software/Automation EUROLabOffice EUROIMMUN Analyzer I + I-2P

* Currently not available as IVD in the EU. Made in Germany



EUROIMMUN

Medizinische Labordiagnostika AG



EUROIMMUN Immunoblots

Autoantibody d

EUROASSAY:

flexible profiles of up to 7 antigens from: ENA and related antigens: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, dsDNA, histones, nucleosomes, CENP B, PM-Scl, ribosomal P-proteins, AMA M2 liver antigens: LKM-1, LC-1, SLA/LP, AMA M2, M4, M9 ANCA antigens: MPO, PR3 thyroid antigens:TG,TPO

EUROLINE:

ANA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, ScI-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins

nucleosomes, histones, ribosomal Pproteins ANA Profile 3: nRNP/Sm, Sm, SSA, Ro-52, SS-B, Sci-10, PM-Sci, Jo-1, CENP B, PCNA, dbDNA, nucleosomes, histones, ribosomal Pproteins, AMA M2 ANA Profile 5: nRNP/Sm, Sm, RNP70, RNPA, RNPC, SS-A, Ro-52, SS-B, Sci-10, PM-Sci, Jo-1, CENP B, PCNA, dsDNS, nucleosomes, histones, ribosomal Pproteins, AMA M2

Anti-ENA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, ScI-70, Jo-1

Systemic Sclerosis Profile: Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, Ro-52

Myositis Profile 3: Mi-2, Ku, PM-Scl100, PM-Scl75, SRP, Jo-1, PL-7, PL-12, OJ, EJ, Ro-52 Liver Profiles: AMA M2, 3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52

Neuronal Antigens Profile 2: amphiphysin, CV2.1** PNMA2 (Ma-2/Ta), Ri, Yo, Hu Anti-Ganglioside Profile 1: GM1, GD1b, GQ1b

Anti-Ganglioside Profile 2: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b ANCA Profiles: MPO, PR3, GBM EUROLINE-WB:

neuronal antigens (+ recomb. Hu, Yo, Ri) HEp-2 cell antigens (+ SS-A and Ro-52, CENP B)

Infectious serology:

EUROLINE: Bordetella pertussis (IgA, IgG) Bordetella pertussis (IgA, IgG) Bordetella VI, 1918, 2020, p21, p58, OspC, p39, p83, EBA, IbB, AlvEE BQ, VISE BD, VISE Ba) EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D) Hanta virus (IgG, IgM) TORCH Profile* (T. gond, rubella, CMV, HSV-1, -2)

Westernblot: Borrelia burgdorferi (IgG, IgM) Borrelia afzelii (IgG, IgM) Borrelia afzelii (IgG, IgM) Epstein-Barr virus (IgG, IgM) Rubella virus (IgG) Treponema pallidum (IgG, IgM) Yersinia enterocol. virulence fact. (IgA, IgG)

EUROLINE-WB:

Anti-Borrelia (B. afzelii + rec. VIsE) Anti-HSV (HSV-1 + HSV-2 gG2) Helicobacter pylori (VacA, Cag A; IgA, IgG) Treponema pallidum + cardiolipin

Allergology: EUROASSAY

Food Profile (IgE) Inhalation Profile (IgE) Pediatric/Atopy Profile (IgE) Insect Venom Profile (IgE) EUROLINE:

EuroLuive. Atopy Profile (IgE; also region-specific profiles) Food Profile (IgE; also region-specific profiles) Paediatric Profile (IgE) Pollen-Food Cross Reaction Profile (IgE) Insect Venom Profile (IgE)

Software/Automation: EUROLineScan camera system EUROBlotCamera scanner system EUROBlotScanner incubation processor EUROBlotMaster

EUROIMMUN Radioimmunoassavs

Autoantibody determination

Autoantioody determination: thyroid peroxidase (TPO; IgG) thyroglobulin (TG; IgG) TSH receptor (IgG) acetylcholine receptor (ACHR; IgG) glutamic acid decarboxylase (GAD; IgG) insulin (IAA; IgG) P/Q calcium channel* (VGCC; IgG) tyrosine phosphatase (IA2; IgG) dsDNA (IgA/IgG/IgM)

Antigen determination thyroglobulin (TG)

Hormone determination: free triiodothyronine (FT3) free thyroxine (FT4) thyrotropin (TSH) calcitonin

Currently not available as IVD in the EU.
** CV2 partial protein, which only contains N-terminally localised epitopes of the an

Made in Germany

Version: 06/04 EA_1015_D_UK_A03

Test Characteristics Anti-TSH Receptor ELISA (IgG)

Reproducibility: To assess the reproducibility, the coefficients of variation (CV) were determined using 4 sera with values at different points on the standard curve. The intra-assay CVs are based on 12 determinations for each serum and the interassay CVs on 6 determinations.

	Intra-assa variation, n	ay = 12	Inter-assay variation, n= 6		
Serum	Mean value (IU/I)	CV (%)	Mean value (IU/I)	CV (%)	
1	1.0	15.5	1.0	18.0	
2	3.0	3.9	2.8	13.0	
3	8.0	2.5	8.5	6.9	
4	22.0	5.5	15.8	6.5	

Reference range: The levels of TRAb were analyzed in sera from 150 healthy blood donors. In 98% of the sera concentrations of less than 1 IU/I were found, which corresponds to an inhibition of TSH binding of under 10%. The upper limit of the normal range (cut-off value) recommended by EUROIMMUN is 2IU/I.

Comparison of porcine and human TSH receptors: Sera from 136 patients from an endocrinology clinic were analyzed using an assay based on porcine TSH receptor and a comparable assay based on recombinant human TSH receptor in parallel. The porcine TSH receptor-based assay was slightly more sensitive for detecting anti-TSH receptor autoantibodies than the human TSH receptor-based test.

No interference from antibodies against TSH: Anti-TSH-

positive sera from Graves' disease patients were analyzed using the EUROIMMUN ELISA and a ¹²⁵I-RRA with PEG precipitation in parallel. In the ¹²⁵I-RRA, anti-TSH antibodies interfer with measurement during the test procedure. In contrast, this

interference is avoided in the EUROIMMUN ELISA by removal of the sera following incubation in the receptor-coated wells.

n=1	36	Anti-human TSH receptor assay				
		pos.	borderl.	neg.		
Anti- porcine TSH receptor assay	pos.	37	0	3		
	border.	0	0	5		
	neg.	0	2	89		

	n=48	TSH-antibody-positive Graves`disease patients			
		125I-RRA	ELISA		
TRAb	pos.	0	30		
TRAb	border.	0	8		
TRAb	neg.	48	10		

Technical Data:

Antigen	Porcine TSH receptor immobilized in microplate wells by means of mouse monoclonal antibodies against the TSH receptor.			
Calibration	Semiquantitative, in inhibition of TSH binding (%). Quantitative, in international units per liter (IU/I).			
	Calibrator 1:40 IU/ICalibrator 2:8 IU/ICalibrator 3:2 IU/I; cut-off valueCalibrator 4:1 IU/INegative control:0 IU/I			
Sample dilution	Serum; 75 µl undiluted.			
Reagents	Ready for use, with the exception of the wash buffer (10x), TSH (lyophi- lized), and enzyme conjugate (20x). Colour-coded solutions.			
Test procedure	120 min / 25 min / 20 min -/ 30 min. Room temperature. Fully automatable. (Sample / TSH / conjugate / substrate incubation, 1 st incubation on a microplate shaker at 500-700 rpm.)			
Measurement	450 nm. Reference wavelength 620-650 nm.			
Kit format	96 individual break-off wells. Kit includes all necessary reagents.			
Order no.	EA 1015-9601 G			