Thyroid gland (Monkey) / Thyroglobulin
Instructions for the indirect immunfluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FA 1010 FA 1010-1 FA 1010-3 (see p. 12)	thyroid gland (MAb + TAb) mitochondria (AMA) thyroglobulin (TAb)	thyroid gland kidney thyroglobulin EUROPLUS	monkey rat ---	10 x 05 (050) 10 x 10 (100)

Indication: This test kit provides qualitative or semiquantitative in vitro determination of human antibodies of immunoglobulin class IgG against thyroid gland and thyroglobulin in patient samples to support the diagnosis of Hashimoto thyroiditis and Graves' disease.

Application: Autoantibodies against thyroid gland antigens are indicative in the diagnosis of Basedow's disease and Hashimoto's thyroiditis. The combination of the IIFT substrates thyroid gland tissue and thyroglobulin (TG) EUROPLUS enables the determination of the most important thyroid gland-specific antibodies and the monospecific confirmation of anti-TG antibodies in one step.

Test principle: Combinations of different substrates are incubated with diluted patient sample. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with FITC-labelled anti-human antibodies and made visible with a fluorescence microscope.

Contents of a test kit for 50 determinations (e.g. FA 1010-1005-1):

Description	Format	Symbol
1. Slides, each containing 5 x 2 BIOCHIPs coated with frozen sections of thyroid gland (monkey) and kidney (rat)	10 slides	SLIDE
2. FITC-labeled anti-human IgG (goat), ready for use	1 x 1.5 ml	CONJUGATE
3. Positive control: Autoantibodies against thyroid microsomes (MAb), human, ready for use	1 x 0.1 ml	POS CONTROL
4. Positive control: autoantibodies against mitochondria (AMA-M2), human, ready for use	1 x 0.25 ml	POS CONTROL
5. Negative control: Autoantibody negative, human, ready for use	1 x 0.1 ml	NEG CONTROL
6. Salt for PBS pH 7.2	2 packs	PBS
7. Tween 20	2 x 2.0 ml	TWEEN 20
8. Mounting medium, ready for use	1 x 3.0 ml	GLYCEROL
9. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
10. Instruction booklet	1 booklet	---

LOT Lot description	CE		Storage temperature
IVD In vitro diagnostic medical device			Unopened usable until

Single slides (e.g. EUROIMMUN order no. FB 1010-1005-1) are provided together with cover glasses. Additional positive control (e.g. order no. EUROIMMUN CA 1011-0101, CA 1622-0102) and negative control (e.g. order no. EUROIMMUN CA 1000-0101) can be ordered.
Performance of the test requires reagent trays TRAY, which are not provided in the test kits. They are available from EUROIMMUN under the following order no.:
- ZZ 9999-0110 Reagent tray for slides containing up to 10 fields.

Storage and stability: The slides and the reagents should be stored at a temperature between +2°C and +8°C. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, controls and slides are to be handled as potentially infectious materials. All reagents are to be disposed of in accordance with official disposal regulations.

Modifications to the former version are marked in grey.

Performing the test (reaction fields 5 x 5 mm)

The **TITERPLANE Technique** was developed by EUROIMMUN in order to standardise immunological analyses: Samples or labelled antibodies are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional “humidity chamber”. It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: The preparation of the reagents and of the serum and plasma samples is described on **page 4** of this test instruction.

Pipette: Apply **30 µl of diluted sample** to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate: Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

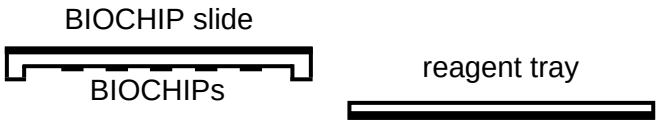
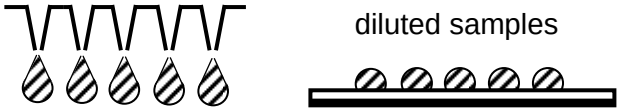
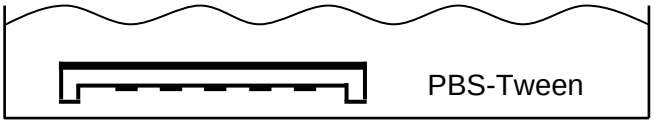
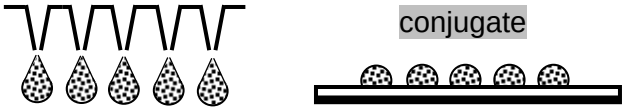
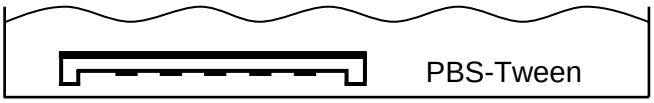
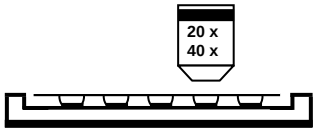
Pipette: Apply **25 µl of conjugate** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The conjugate should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

Incubate: Remove one BIOCHIP slide from the cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP slide. From now on, protect the slides from direct sunlight. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least **5 minutes**. Shake with a rotary shaker if available. Wash max. 16 slides, then replace PBS-Tween with new buffer.

Mount: Place mounting medium onto a cover glass – drops of **max. 10 µl per reaction field**. Use a polystyrene mounting tray. Remove one BIOCHIP slide from PBS-Tween and dry the back and all four sides with a paper towel. Put the BIOCHIP slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.

Evaluate: Read the fluorescence with the microscope.
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
Excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm.
Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight.

TITERPLANE Technique		
Pipette:	30 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Pipette:	25 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Mount:	max. 10 µl per field	
Evaluate:	fluorescence microscopy	

Automated Incubation: The test kit can be incubated by using automated devices, e.g. IF Sprinter, Sprinter XL, EUROLabLiquidHandler or others. The incubation and washing conditions programmed should be the same as described in the manual procedure. The test settings for EUROIMMUN devices are validated in combination with the kit. Any other combination has to be validated by the user. For details please refer to the device manual.

Preparation and stability of reagents

Note: After initial opening, the reagents are stable until the expiry date when stored between +2°C and +8°C and protected from contamination, unless stated otherwise below.

- **Slides:** Ready for use. Remove the protective cover only when the slides have reached room temperature (+18°C up to +25°C; condensed water can damage the substrate). Do not touch the BIOCHIPs. If the protective cover is damaged, the slide must not be used for diagnostics.

- **FITC-labeled secondary antibody:** Ready for use. Before using for the first time, mix thoroughly. The conjugate is sensitive to light. Protect from sunlight ✳.

- **Positive and negative controls:** Ready for use. Before using for the first time, mix thoroughly.

- **PBS-Tween:** 1 pack of “Salt for PBS” should be dissolved in 1 liter of distilled water (optimal: aqua pro infusione, aqua ad injectabilia) and mixed with 2 ml of Tween 20 (stir for 20 min until homogeneous). The prepared PBS-Tween can be stored at +2°C to +8°C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears.

- **Mounting medium:** Ready for use.

- **Reagent trays:** Reaction fields of the reagent tray must be hydrophilic and surrounding area hydrophobic. If necessary, leave in 2% Deconex 11 universal (EUROIMMUN order number: ZZ 9912-0101) for 12 hours. Afterwards rinse generously with water and dry. Cleaning: Rub reagent trays with 5% Extran MA 01 (EUROIMMUN order number: ZZ 9911-0130) and rinse generously with water. To disinfect: Spray reagent trays generously with Mikrozid AF (EUROIMMUN order number: ZZ 9921-0125), turn over and leave for 5 minutes. Afterwards, rinse generously with water and dry.

Warning: The BIOCHIPs coated with antigen substrates have been treated with a disinfecting fixing agent. Neither HBsAg nor antibodies against HIV-1, HIV-2, and HCV could be detected in the control sera using appropriate ELISA or indirect immunofluorescence tests. **Nevertheless, all test system components should be handled as potentially infectious materials.** Some of the reagents also contain sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of serum and plasma samples

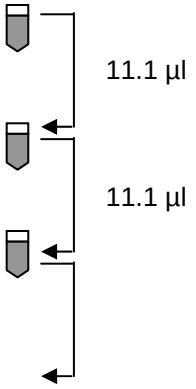
Samples: Human sera or EDTA, heparin or citrate plasma.

Stability: The patient samples to be investigated can generally be stored up to 14 days at a temperature between +2°C and +8°C. Diluted samples must be incubated within one working day.

Recommended sample dilution for qualitative evaluation: The sample to be investigated is diluted 1:10 in PBS-Tween. For example, dilute 11.1 µl sample in 100 µl PBS-Tween and mix thoroughly, e.g. vortex for 4 seconds.

Recommended sample dilution for semiquantitative evaluation: The dilution of samples to be investigated is performed using PBS-Tween. Add 100 µl of PBS-Tween to each tube and mix with 11.1 µl of the next highest concentration, e.g. vortex for 2 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:10.

Dilution	Dilution scheme
1:10	100 µl PBS-Tween + 11.1 µl u undiluted sample
1:100	100 µl PBS-Tween + 11.1 µl 1:10 diluted sample
1:1000	100 µl PBS-Tween + 11.1 µl 1:100 diluted sample
⋮	⋮



After every two dilution steps, a new pipette tip should be used to prevent carryover.

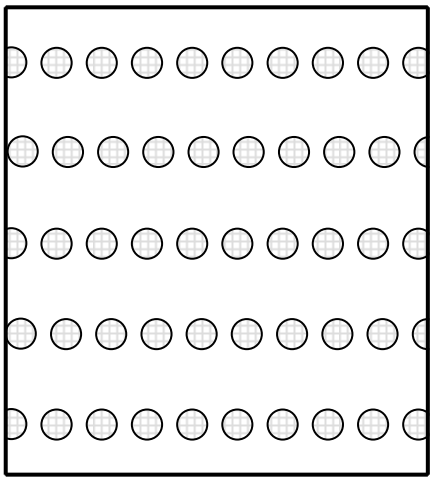
Evaluation

Fluorescence pattern (positive reaction): Antibodies against **thyroid microsomes (MAb)** react with the standard substrate primate thyroid and give a granular staining in the cytoplasm of the follicle epithelium. The target antigen has been identified as thyroid peroxidase (TPO). The pattern shown is essentially the same as that obtained for the positive control serum.

The determination of autoantibodies against thyroid microsomes requires a positive differentiation from **mitochondria antibodies (AMA)** because they show a similar fluorescence pattern. For the targeted identification of these antibodies, frozen sections of rat kidney are used as standard substrate. The cytoplasm of the proximal and distal tubule cells shows a granular, basally emphasized fluorescence. The glomeruli are only weakly stained by AMA. The pattern shown is essentially the same as that obtained for the positive control serum. Any fluorescence of the luminal tubule sections (brush border) is not taken into account.

Autoantibodies against **thyroglobulin (TAb)** react with all follicles of the thyroid tissue and cause a reticular fluorescence pattern. Any colloid staining found only in sporadic follicles is not evaluated.

On the BIOCHIP coated with thyroglobulin green circular areas appear in front of a dark background.
In case of a negative reaction the entire thyroglobulin-coated BIOCHIP remains dark. For a secure differentiation between positive and negative results, the controls, several normal sera if necessary, must be tested simultaneously and compared with the patient samples.



Thyroglobulin-coated BIOCHIP

If the cell nuclei or the cytoplasm of <u>all</u> cells are stained, antinuclear antibodies or antibodies against mitochondria and other cell antigens are present.

If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence, the results are not to be used and the test is to be repeated.

A large range of fluorescence images can be found on the EUROIMMUN website (www.euroimmun.com).

Recommended qualitative evaluation:

Anti-microsomes (MAb) reactivity (IgG)	**Evaluation**
No reaction at 1:10	Negative. No antibodies against thyroid microsomes detected in the patient sample.
Positive reaction at 1:10	Positive. Indication of an autoimmune thyroid disease.

Anti-thyroglobulin reactivity (IgG)	**Evaluation**
No reaction at 1:10	Negative. No antibodies against thyroglobulin found in the patient sample.
Positive reaction at 1:10	Positive. Indication of an autoimmune thyroid disease.

AMA reactivity* (IgG)	**Evaluation**
No reaction at 1:100	Negative. No antibodies against mitochondria detected in the patient sample.
Positive reaction at 1:100	Positive. Indication of various diseases, e.g. primary biliary cirrhosis (PBC), autoimmune hepatitis, chronic hepatitis C and other diseases.

* The substrate rat kidney in the substrate combination FA 1010-####-1 only serves to differentiate between MAb and AMA. A positive AMA result with a titer of 1: < 100 is not diagnostically relevant.

Recommended semiquantitative evaluation: The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared to the reaction obtained with an equivalently diluted negative serum.

Antibody titers can be determined according to the following table from the fluorescence of the different sample dilutions.

fluorescence at				Antibody titer
1:10	1:100	1:1000	1:10,000	
weak	negative	negative	negative	1:10
moderate	negative	negative	negative	1:32
strong	weak	negative	negative	1:100
strong	moderate	negative	negative	1:320
strong	strong	weak	negative	1:1000
strong	strong	moderate	negative	1:3200
strong	strong	strong	weak	1:10,000
⋮	⋮	⋮	⋮	⋮

Limitations of the procedure

1. A diagnosis should not be made based on a single test result. The clinical symptoms of the patient should always be taken into account along with the serological results by the physician.
2. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
3. Mishandling of slides during the staining procedure, especially allowing slides to dry between steps, may result in a “washed out” pattern appearance and/or a high level of background staining.
4. Coplin jars used for slide washing should be free from all residues. Use of coplin jars containing residues may cause staining artefacts.
5. The light source, filters and optical unit of the fluorescence microscope can influence the sensitivity of the assay. Using traditional mercury vapour lamp systems, the performance of the microscope depends on correct maintenance, especially alignment of the lamp and replacement of the lamp after the recommended period of time. The EUROIMMUN fluorescence microscopes with LED Bluelight as the light source offer many advantages. Contact EUROIMMUN for details.

Test characteristics

Antigen: For the detection of autoantibodies against thyroid microsomes (MAb, ATMA) using indirect immunofluorescence, unfixed **thyroid tissue** is used as the standard substrate. With a substrate combination of primate thyroid and rat kidney (EUROIMMUN order no.: FA 1010-1005-1), it is possible to achieve a reliable differentiation between thyroid microsomes and antibodies against mitochondria (AMA) in one test procedure.

For the detection of autoantibodies against thyroglobulin (TAb) using indirect immunofluorescence BIOCHIPs coated with a native, highly specific **thyroglobulin** are used (EUROPLUS Technique [10]). Ideally, antibodies against thyroid microsomes (MAb) and thyroglobulin (TAb) are determined using a substrate combination of thyroid tissue and thyroglobulin (EUROPLUS: thyroid monkey/thyroglobulin, EUROIMMUN order no: FA 1010-1005-3).

For the detection of autoantibodies against mitochondria (AMA) by indirect immunofluorescence **rat kidney** is used as a standard substrate. These antibodies mainly react with biochemically defined antigens of the mitochondrial membranes.

Measurement range: The dilution starting point for this measurement system is 1:10. Samples can be further diluted by a factor of 10 so that the dilution series is 1:100, 1:1000, 1:10,000 etc. There is no upper limit to the measurement range.

Reproducibility:

Reproducibility	Inter-lot	Intra-assay	Inter-assay
Minimum requirement	3 lots x 3 samples x 1 run x single determination: max. ± 1 titer step	1 lot x 3 samples x 1 run x tenfold determination: max. ± 1 titer step	1 lot x 3 samples x 2 runs x double determination: max. ± 1 titer step
Thyroid gland (monkey)	Maximum deviation ± 1 titer step	Is assured since inter-lot reproducibility was investigated with more than 10 lots.	Is assured since inter-lot reproducibility was investigated with more than 10 lots.
Thyroglobulin EUROPLUS	Maximum deviation ± 1 titer step	Maximum deviation ± 1 titer step	No deviation

Cross-reactivity:

Substrate	Cross-reactivity
Thyroid gland (monkey)	There is no scientific literature known to EUROIMMUN in which cross-reactivity was described.
Thyroglobulin EUROPLUS	

Interference: Haemolytic, lipaemic and icteric samples showed no influences on analysis results.

Reference range: Titer 1: < 10

The following antibody prevalences were determined using a panel of samples from healthy blood donors (origin: Germany):

Substrate	Antibodies against	Conjugate	Prevalence	Cut-off	Number of samples
Thyroid gland (monkey)	thyroid gland (MAb)	IgG	3.0%	1:10	200
Thyroglobulin EUROPLUS	thyroglobulin (TAb)		2.5%		200

Specificity and Sensitivity:

Substrate	Ig class	Reference (number of samples, origin of samples)	Specificity	Sensitivity
Thyroid gland (monkey): MAb	IgG	Anti-TPO ELISA (n = 107, Germany)	95.5%	100%
Thyroglobulin EUROPLUS: TAb	IgG	Anti-Tyroglobulin ELISA (n = 199, Germany)	99.2%	98.4%

Clinical significance

The functions of the thyroid are controlled by the hypothalamus in the brain stem via the pituitary gland. The releasing and inhibition factors formed in the hypothalamus stimulate or inhibit the release of TSH (thyroid stimulating hormone = thyrotropin), which is produced in the pituitary gland and induces the thyroid gland to release the thyroid hormones T3 (triiodothyronine) and T4 (tetraiodothyronine = thyroxine).

The free thyroid hormones T3 and T4 belong to the vitally important hormones that regulate the metabolism of almost all organs. On a cellular level they are responsible for the oxygen consumption, warmth production and also for the mental development and growth of the total organism.

An increase of T3 and T4 levels are in general an indication of a hyperthyroid functional disorder (hyperthyrosis), whereas low levels of T3 and T4 hormones in serum are allocated to a hypothyroid functional disorder (hypothyrosis). The clinical signs of thyroid hyperfunction or thyroid hypofunction are largely standard, regardless of the cause of the varying thyroid hormone levels.

Symptoms of thyroid overfunction The symptoms of a hyperthyrosis are nervousness, irritablility, restlessness, trembling hands, insomnia, perspiration, warm damp hands, ravenous appetite, thirst, and weight loss despite a good appetite and in women men-struation cycle disorders (unregular or increased bleeding, or absence of the menstruation).

The symptoms of a hypothyreosis are low body temperatures, increased sensitivity to coldness, oedema (particularly on eye lids, face and extremities), feeling of pressure on or in the throat, feeling of strangulation (also only sporadic), frequent clearing of the throat and coughing, hoarse or throaty voice (vocal cord oedemas), depressive moods, listlessness, concentration and memory disorders, sleepiness, weak muscles, muscle hardening, dry, chapped skin and accompanying itchiness, dry mucosa, brittle hair and fingernails, high weight increase, decreased libido, menstrual cycle change in women, joint pain.

Aside from a disorder of the thyroid hormone regulation, thyreoiditis (thyroid inflammation) can be the cause of the symptoms of either hyper- or hypothyrosis. These include several diseases:
- Acute (bacterial) thyroiditis
- Subacute (non-infectious) thyroiditis
- Chronic thyroiditis (autoimmune thyroiditis)

Autoimmune thyreopathies are chronic inflammatory thyroid diseases that are caused by disregulation of specific immune defences (B cells and T cells). They occur most often after a virus infection and sometimes also after a subacute thyreoditis. Genetic factors play a role in their development. During the autoimmune process antibodies against one or more of the three autoantigens of the thyroid are formed:
- Thyroperoxidase (TPO)
- Thyroglobulin (TG)
- TSH receptor (TR)

TSH receptor autoantibodies (TRAb) are heterogeneous regarding their biological effect.
- Antibodies which stimulate or inhibit the functioning of the TR (TSH receptor).
- Antibodies which stimulate growth of the thyroid gland.
- Antibodies which inhibit the binding of TSH to the TR

The biological effect of TRAb for one individual patient can change during the course of the disease, e.g. from a blocking TR to a stimulating TR or the reverse, which is more rare.

Graves’ disease is another autoimmune thyroid disease. Symptoms are hyperthyreosis and also disease signs such as struma, exophthalmus and tachycardia (Merseburger triad). A severe disease course is characterised by emaciation, heart insufficiency and coma. Around 2% of women and 0.2% of men are affected by manifest Graves’ disease. Graves' disease often appears in women during hormonal changes (puberty, pregnancy, menopause). 60% of all hyperthyreosis cases are attributed to Graves’ disease.

The serological determination of TRAb is used to confirm suspected clinical cases of Graves' disease. The prevalence is 90 to 100%. Thus TRAb are considered to be diagnostic markers and are utilised for differential diagnostics compared to a disseminated autonomy of the thyroid gland. Monitoring TRAb concentrations during the course of Graves’ disease allows a prognostic statement and provides an important decision-making aid for management of therapy. High TRAb titers in patients with Grave’s disease following a long thyreostatic therapy show an increased risk of reoccurrence of the disease. Moreover increased TRAb concentrations in the third trimester of pregnant women with Grave's disease indicate a hyperthreosis in the foetus. Where normal values are found, the diagnosis can be supported by the determination of antibodies against TPO with a prevalence of 60 to 70%. Additionally antibodies against TG are found in 20 to 50% of cases. Since there are associations with other autoimmune diseases, e.g. myasthenia gravis, pernicious anaemia, chronic-atrophic gastritis and autoimmune polyendocrinopathies, it is likely that further autoantibodies are found (e.g. ANA in approx. 30% of cases, AMA, ASMA, PCA). TRAb determinations are indications for ophthalmology as many patients first visit the optometrist.

Hashimoto's thyroiditis is one of the most common autoimmune diseases in humans and the most frequent cause of primary thyroid gland hypofunction. Hashimoto’s thyroiditis (autoimmune thyropathy type 1A and 2A with struma) is a chronic thyroiditis with progressive destruction of the thyroid tissue by T-lymphocytes. Ord’s thyroiditis (autoimmune thyropathy type 1B and 2B) is a special form of Hashimoto Thyreoiditis and is characterised by an atrophy of the thyroid gland. These two conditions (the

hypertrophic and the atrophic form) lead to a thyroid hypofunction, with possible phases of hyperfunction (so-called hyperthyrodism, in extreme cases hashitoxicosis) at the onset of the disease due to the destruction of thyroid tissue.

There is a genetic predisposition for Hashimoto's thyroiditis. Women are affected significantly more often than men (approx. ratio 8:1 to 10:1). The disease can be triggered by stress, severe virus infections (e.g. infectious mononucleosis, shingles), dysfunction of the adrenal cortex or, as in patients with Graves' disease, high levels of iodine (iodine excess). So far, Hashimoto's thyroiditis cannot be cured; however, the thyroid hypofunction must be treated. From a serological point of view, antibodies against TPO can be detected with a prevalence of 60 to 70%. Antibodies against thyreoglobulin are in 90 to 100% of the cases initially high. In Hashimoto's disease and myxoedema, blocking TRAb may cross the placenta in pregnant women and lead to transient neonatal hypothyroidism.

Approximately 5% of women have postpartum thyroiditis, which is a transient hypothyrote autoimmune thyroiditis with a very high risk of a simultaneously present insulin-dependent diabetes mellitus. Due to the therapeutic consequences, all women who have just given birth should be tested for antibodies against TPO.

The determination of antibodies against TG is particularly important in the diagnosis of differentiated thyroid carcinoma, since the presence of these antibodies can interfere with the measurement of TG concentrations in serum.

Autoimmune test methods which have proven successful are the indirect immunofluorescence test (IIFT) and the EUROASSAY for the detection of autoantibodies against TPO and TG, the Enzyme Linked Immunosorbent Assay (ELISA) and the radio-immunoassay for the detection of autoantibodies against TPO, TG and TR. Today, enhanced ELISA tests which are highly sensitive and specific for the determination of autoantibodies against TR are available.

Literature references

1. Akamizu T, Kohn LD, Hiratani H, Saijo M, Tahara K, Nakao K. **Hashimoto's thyroiditis with heterogeneous antithyrotropin receptor antibodies: unique epitopes may contribute to the regulation of thyroid function by the antibodies.** J Clin Endocrinol Metab 85 (2000) 2116-2121.
2. Bolton J, Sanders J, Oda Y, Chapman C, Konno R, Furmaniak J, Rees Smith B. **Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA.** Clinical Chemistry 45 (1999) 2285-2287.
3. Davies TF. **Ord-Hashimoto's Disease: Renaming a Common Disorder - Again.** Thyroid 13 (2003) 317.
4. EUROIMMUN AG. Stöcker W, Schlumberger W, Krüger C. **Alle Beiträge zum Thema Autoimmundiagnostik.** In: Gressner A, Arndt T (Hrsg.) Lexikon der Medizinischen Laboratoriumsdiagnostik. 2. Auflage. Springer Medizin Verlag, Heidelberg (2012).
5. Hamada N, Noh JY, Okamoto Y, Ueda M, Konishi T, Fujisawa T, Ito K, Ito K. **Measuring thyroglobulin autoantibodies by sensitive assay is important for assessing the presence of thyroid autoimmunity in areas with high iodine intake.** Endocr J 57 (2010) 645-649.
6. 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 Journal 50 (2003) 113-116.
7. Kohn LD, Harii N. **Thyrotropin receptor autoantibodies (TSHRAbs): epitopes, origins and clinical significance.** Autoimmunity 36 (2003) 331-337.
8. Latrofa F, Phillips M, Rapoport B, McLachlan SM. **Human monoclonal thyroglobulin autoantibodies: epitopes and immunoglobulin genes.** J Clin Endocrinol Metab 89 (2004) 5116-5123.
9. Meng W. **Diagnostik der Hyperthyreose.** Z ärztl Fortbild Qual sich (ZaeFQ) 95 (2001) 51-60.

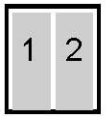
10. Orgiazzi J, Madec AM, Ducottet X. **The role of stimulating, function-blocking and growth-blocking anti-TSH receptor antibodies (TRAbs) in GD, Hashimoto's disease and in atrophic thyroiditis.** Ann Endocrinol (Paris) 64 (2003) 31-36.

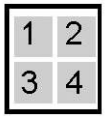
11. Rho MH, Kim DW, Hong HP, Park YM, Kwon MJ, Jung SJ, Kim YW, Kang T. **Diagnostic value of antithyroid peroxidase antibody for incidental autoimmune thyroiditis based on histopathologic results.** Endocrine 42 (2012) 647-652.

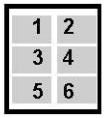
12. Saravanan P, Dayan CM. **Thyroid autoantibodies.** Endocrinology and Metabolism Clinics of North America 30 (2001) 315-337.

13. Willms A, Bieler D, Wieler H, Willms D, Kaiser KP, Schwab R. **Correlation between sonography and antibody activity in patients with hashimoto thyroiditis.** J Ultrasound Med 32 (2013) 1979-1986.

BIOCHIP position on the subfields:







This test instruction is valid for the following test kits (#### is a place holder for different test formats, e.g. 1005 = 10 slides with 5 fields):

Order no.	Description			Field size (mm)
		1	2	
FA 1010-####	IIFT: Thyroid Gland (Monkey)	Thyroid Gland (Monkey)		5 x 5
FA 1010-####-1	IIFT Mosaic: Thyroid Gland (Monkey)/Kidney (Rat)	Thyroid Gland (Monkey)	Kidney (Rat)	5 x 5
FA 1010-####-3	EUROPLUS: Thyroid gland (Monkey)/Thyroglobulin	Thyroid Gland (Monkey)	Thyroglobulin	5 x 5