Patient/volunteer name :-----------------------------
ID............................................

Date [Ref. doctor]..........................................................
........................................

Sex 1-male................ .........................
......................... female
Date of birth……………………

Long standing disease: present……………………abs cent…………………………………….

(1)…………………………………………………………………………………..

(2)…………………………………………………………………………………..

(3)…………………………………………………………………………………..

Family history for hyperthyroidism:

(1)abs cent ..................(2) present.........................

Family history for autoimmune disease/s

(1)abs cent .................

(2) present:

1)case
..........................,duration...........................
(......

2)case
..........................,duration...........................
(...

3)case
..........................,duration...........................
(......

Drugs on use:
1) Drug ..............

............................................, duration..........................................

2) Drug .... ....

............................................, duration..........................................

3) Drug ..............

............................................, duration..........................................

Appendix (1)
TSH
Thyrotropin

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results (thyroid volume and density) as well as criteria according to the guidelines of the National Academy of Clinical Biochemistry - NACB). Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Data was obtained in individual laboratories; data may differ.

**Precision**

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EPH-A) of the CLSI (Clinical and Laboratory Standards Institute) 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. Elecsys PreciControl TSH was determined once daily for 10 days (n = 10). The following results were obtained:

**Repeatability**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean SD</th>
<th>Linear regression</th>
<th>y = ax + b</th>
<th>r values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum 1</td>
<td>0.024</td>
<td>0.003</td>
<td>8.6</td>
<td>8.7</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>0.822</td>
<td>0.002</td>
<td>2.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>0.37</td>
<td>0.010</td>
<td>1.1</td>
<td>3.5</td>
</tr>
<tr>
<td>PreciControl Universal 1</td>
<td>0.955</td>
<td>0.014</td>
<td>1.5</td>
<td>0.316</td>
</tr>
<tr>
<td>PreciControl Universal 2</td>
<td>8.13</td>
<td>0.098</td>
<td>1.2</td>
<td>4.2</td>
</tr>
</tbody>
</table>

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

**References**


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*Elicys and cobas e analyzers*
Quality control

For quality control, use Elecsys PreC) control Universal 1 and 2. Follow the manufacturer's instructions.

Other suitable control material can be used in addition. Controls for the various concentration ranges should be run at single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Follow the applicable governmental regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analytic concentration of each sample either in \( \mu \text{g/mL} \) or \( \text{ng/mL} \) (selectable).

Limitations - interference

The assay is unaffected by drugs (up to 7 g/mL), calcium (up to 4 g/mL), and magnesium (up to 20 g/mL). No interference was observed from cryptocurrencies including: copper, iron, zinc, lead, tin, and tungsten. The presence of analyte(s) may influence high molecular weight complexes such as proteins, which may cause unexpected high values of TSH. In rare cases, interference due to extremely high levels of antibodies to analyte-specific antibodies, asparaginase or rhamnulose can occur.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination, and other findings.

Limits and ranges

**Measuring range:** 0.005-100.0 \( \mu \text{g/mL} \) (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.004 \( \mu \text{g/mL} \). Values below the detection limit are reported as < 0.005 \( \mu \text{g/mL} \). Values above the measuring range are reported as > 100.0 \( \mu \text{g/mL} \) (or up to 1000 \( \mu \text{g/mL} \) for 10-fold diluted samples).

**Lower limits of measurement:**

- **Lower detection limit:** 0.005 \( \mu \text{g/mL} \)
- **Lower detection limit:** 0.005 \( \mu \text{g/mL} \)

**Expected values:**

- **Expected values:** 0.07-0.90 \( \mu \text{g/mL} \)
- **Expected values:** 0.07-0.90 \( \mu \text{g/mL} \)

The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, reproductivity, \( n = 21 \)).

**Dilution:**

Samples with TSH concentrations above the measuring range can be diluted with Elecsys Diluent MUSE. The recommended dilution is 1:10 (either automatically by the MODULAR ANALYTICS E170, Elecsys 1010/2010 and cobas e analyzers or manually). The concentration of the diluted sample must be \( < 10 \mu \text{g/mL} \). After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 1010/2010 and cobas e software automatically takes the dilution into account when calculating the sample concentration.
**TSH**
Thyrotropin

<table>
<thead>
<tr>
<th>MODULAR ANALYTICS E170</th>
<th>cobas e 411</th>
<th>cobas e 501</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elecys 1010</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Elecys 2010</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

English

**Intended use**

Immunosay for the in vitro quantitative determination of thyrotropin in human serum and plasma.

**Summary**

Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein having a molecular weight of approx. 30,000 daltons and consisting of two subunits. The α-subunit carries the 53 amino-acid-sized polypeptide sequence, whereas the β-chain carries species-specific information and has an identical amino-acid sequence to the α-chains of LH, FSH and hCG.

TSH is formed in specific basophil cells of the anterior pituitary and is subject to a circadian secretion sequence. The hypophysial release of TSH (thyrotropic hormone) is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretion. It also has a proliferative effect.

The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypophysis/pituitary and thyroid gland.

The Elecsys TSH assay employs monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium complex consist of a chimeric construct from human and mouse-specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated.

**Test principle**

Sandwich principle. Total duration of assay: 18 minutes.

1. Incubation: 50 μL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2. Addition of streptavidin-coated microparticles, which becomes bound to the solid phase via interaction of both streptavidin.

3. Reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with PBS.

4. Application of a voltage to the electrode then induces chemiluminescence emission which is measured by a photomultiplier.

5. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via run correction.

**Reagents - working solutions**

| M | Streptavidin-coated microparticles (Opaemic cap) | 1 bottle, 12 mL | Streptavidin-coated microparticles 0.72 mg/mL, preservative.
| R1 | Anti-TSH-αb-biotin (gray cap) | 1 bottle, 14 mL | Biotinylated monoclonal anti-TSH antibody (mouse) 1.4 mg/mL, phosphate buffer 100 mM, pH 7.2, preservative.
| R2 | Anti-TSH-αb-Ruby2 (black cap) | 1 bottle, 12 mL | Monoclonal anti-TSH antibody (mouse) labeled with ruthenium complex 1.2 mg/mL, phosphate buffer 100 mM, pH 7.2, preservative.

**Precautions and warnings**

For in vitro diagnostic use.

- Exercise the normal precautions for handling all laboratory reagents.
- Handle waste material in accordance with local guidelines.
- Safety data sheet available for professionals on request.
- Avoid the formation of foam with all reagents and sample types (specimen, calibrators, and controls).

**Reagent handling**

- The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
- All information required for correct operation is read in via the respective reagent barcodes.

**Storage and stability**

Store at 2-8 °C.

Store the Elecsys TSH reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

**Stability**

- Opened at 2-8 °C:
  - up to the stated expiration date after opening at 2-8 °C: 12 weeks
  - on MODULAR ANALYTICS E170 and cobas e 501: 5 weeks
  - on Elecsys 1010 and cobas e 411: 8 weeks
  - on Elecsys 1010: 8 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total)

**Specimen collection and preparation**

The specimens listed below were tested and found acceptable.

- Serum collected using standard sampling tubes or tubes containing separating gel.

**Cryopreservation**

LH: Na-EDTA, K-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.

**Cryopreservation**

- Cryopreservation within 90-110 °C of serum value or slope 0.9-1.1, intercept within 0.2 x analytical sensitivity (SD) x coefficient of correlation > 0.95.
- Stable for 2 days at 2-8 °C; 1 month at +6 °C. Freeze only once.
- The samples listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with acid.
- Use the patient's samples, calibrators, and controls at ambient temperature (20-25 °C) before measurement.

**Materials provided**

- See "Reagents - working solutions" section for reagents.

**Materials required (but not provided)**

- For 0,0640 mmol/L, TSH Calibr; 1 x 1.0 mL
- For 0,1776421123, PreciControl TSH; 4 x 2 mL
- For 0,0139841802, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- For 0,0127416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- For 0,01360687190, Dlient MulAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- Elecsys 10/2010, MODULAR ANALYTICS E170 or cobas e analyzer
Appendix (1I)
Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheet available for professional user on request.

Avoid the formation of foam with all reagents and sample types (vials, pipettes, and glassware).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Store the Elecsys FT4 reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

- unopened at 2-8 °C: up to the stated expiration date
- after opening at 2-8 °C: 12 weeks on MODULAR Analytics E170 and cobas e 601
- 6 weeks on Elecsys 1010 and cobas e 411
- 6 weeks on Elecsys 1010 (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C, up to 20 hours opened in total)

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

- Un diluted serum collected using standard sampling tubes or tubes containing separating gel.
- Li+, Na+, NH4+, heparin, K+, EDDA, sodium citrate, and sodium fluoride/potassium oxalate plasma (undiluted).

Criteria: Recovery within 80-110 % of serum value or slope 0.9-1.1 + intercept within ±2 x analytical sensitivity (0.1 DlU) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8 °C, 30 days at 20-25 °C. Freeze only once.

Stability of serum obtained with tubes containing separating gel: 48 hours at 2-8 °C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with arsenic.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- Cat. No. 1173986122, FT4 CatSet, 4 x 1 mL
- Cat. No. 1173981522, PreciControl Universal, 4 x 2 mL each of PreciControl Universal 1 and 2
- Cat. No. 1173981520, PreciControl Universal, 10 x 2 mL each of PreciControl Universal 1 and 2
- Cat. No. 1173981530, PreciControl Universal, 10 x 2 mL each of PreciControl Universal 1, 2, 3, 4
- General laboratory equipment
- Elecsys 1010/410, MODULAR Analytics E170 or cobas e analyzer
**FT4**

Free thyroxine

Accessories for Elecsys 1010/2010 and cobas e 411 analyzers:
- Cat. No. 116629630122, ProCell, 6 x 380 mL, system buffer
- Cat. No. 11953870122, CellClear, 3 x 380 mL, measuring cell cleaning solution
- Cat. No. 11953840122, ElyteWash, 13 x 250 mL, wash additive
- Cat. No. 119631590101, Adapter for CleanCell
- Cat. No. 11706628001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels
- Cat. No. 11706628011, Cobas 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706719001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MULLER ANALYTICS E170 and cobas e 601 analyzers:
- Cat. No. 04883040010, ProCell M, 2 x 2 L, system buffer
- Cat. No. 04809200010, CleanCell M, 2 x 2 L, measuring cell cleaning solution
- Cat. No. 12193527010, CleanCell M, 1 x 2 L, measuring cell cleaning solution (for USA)
- Cat. No. 03005712010, PC/CC/Cups, 12 cups to premix ProCell M and CleanCell M before use
- Cat. No. 03005712030, ProbeWash M, 12 x 70 mL, cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 12101856201, AssayTip/AssayCup Combination Pack, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- Cat. No. 03025915001, Waste Cup, waste bags
- Cat. No. 03025965001, SysClean Adapter M

Accessories for all analyzers:
- Cat. No. 11208500916, Elecsys TestCLean, 5 x 100 mL, system cleaning solution

Only available in the USA:
- Cat. No. 11778650010, Elecsys FT4 CalCheck, 3 concentration ranges

Assay

For optimum performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Reconstitution of the monoclonal takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MULLER ANALYTICS E170, Elecsys 2010 and cobas e analyzers:
- Bring the cooled reagents to approx. 20-25 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.
- Elecsys 1010 analyzer:
  - Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C).
  - Avoid the formation of foam. Open bottle caps manually before use and close manually after use. Store at 2-8 °C after use.

Calibration

Traceability: This method has been standardized against the Enzyme-Test FT4 method. This in turn was standardized using equilibrium dialysis. Every Elecsys FT4 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by use of the reagent v1+c Leiner.

Calibration frequency: Calibration must be performed once per reagent lot for fresh reagent (i.e., not more than 24 hours since the reagent lot was registered on the analyzer). Renewed calibration is recommended as follows:

MULLER ANALYTICS E170, Elecsys 2010 and cobas e analyzers:
- every 1 month (28 days) when using the same reagent lot
- every 7 days (when using the same reagent lot on the analyzer)

Elecsys 1010 analyzer:
- with every reagent kit
- after 7 days (ambient temperature 20-25 °C)
- after 3 days (ambient temperature 25-32 °C)

For all analyzers:
- as required; e.g., quality control findings outside the specified limits

Quality control

For quality control, use Elecsys ProControl Universal 1 and 2. Other suitable control material can be used in addition.

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analytic concentration of each sample either in pmol/L, ng/dL, or nG/L.

Conversion factors:
- pmol/L x 0.077688 = ng/dL
- pmol/L x 12.872 = nG/L
- pmol/L x 0.77588 = nG/L

Limitations - interference

The assay is unaffected by citrus (bicarbonate < 701 pmol/L or < 47 ng/dL), hemolysis (hemoglobin < 0.2 mol/L or < 2 g/dL), lipemia (triglycerides < 2000 pmol/L), and bilirubin < 409 pmol/L or < 100 nmol/L.

Circulation: Recovery within ± 10 % of initial value.

In patients receiving therapy with high bilirubin doses (i.e., > 5 mg/dL), no sample should be taken until at least 8 hours after the last bilirubin administration.

No interference was observed from neumatic factors up to a concentration of 339 fmol/L and samples from dialysed patients. Of 26 commonly used pharmaceuticals tested in vitro, only furosemide caused elevated FT4 findings at the daily therapeutic dosage level. The test cannot be used in patients receiving treatment with lipid-lowering agents containing D-74. If the thyroid function is to be checked in such patients, the laboratory should be informed.

Samples from neonates have not been tested with the Elecsys FT4 assay.

Automatisation as thyroid hormones can interfere with the assay.

Binding protein anomalies seen with FHDI (familial dysalbuminemic hyperthyroxinemia), for example, may cause values which, while characteristic of the condition, deviate from the expected results.

In rare cases, interference due to extremely high titer of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

0.900-100.0 pmol/L or 0.023-7.77 ng/dL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.900 pmol/L or < 0.023 ng/dL. Values above the measuring range are reported as > 100.0 pmol/L or > 7.77 ng/dL.

Dilution

Samples for FT4 determinations cannot be diluted, as T4 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Expected values

Euthyroid: 12-22 pmol/L (0.99-1.7 ng/dL)

These values correspond to the 2.5th and 97.5th percentile range of results from a total of 801 healthy test subjects studied.

Status: MCE Reference Range Thyroid, Status 1st quarter 1998.

For detailed information about reference intervals in children, adolescents and pregnant women, refer to the brochure "Reference Intervals for Children and Adults". Cat. No. English: 04840292; German: 04825899.

This booklet also contains results of a detailed study about influencing factors on thyroid parameters in a well characterized reference group of adults. Different inclusion and exclusion criteria were applied (e.g. sonographic results...
## References


For further information, please refer to the appropriate operator’s manual for the analyzer concerned. The respective application sheets, the product information, and the package inserts of all necessary components.

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---

### Table: FT4 (free thyroxine)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run precision</th>
<th>Total precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean pmoL/L</td>
<td>SD pmoL/L</td>
</tr>
<tr>
<td>HEP 1</td>
<td>8.7</td>
<td>0.68</td>
</tr>
<tr>
<td>HS 2</td>
<td>21.1</td>
<td>1.64</td>
</tr>
<tr>
<td>HS 3</td>
<td>50.8</td>
<td>3.05</td>
</tr>
<tr>
<td>PC 1/1</td>
<td>17.5</td>
<td>1.36</td>
</tr>
<tr>
<td>PC 1/2</td>
<td>26.1</td>
<td>2.03</td>
</tr>
</tbody>
</table>

**MOLAR ANALYTICS E170 and cobas e 601 analyzers**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run precision</th>
<th>Total precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean pmoL/L</td>
<td>SD pmoL/L</td>
</tr>
<tr>
<td>HEP 1</td>
<td>9.15</td>
<td>0.71</td>
</tr>
<tr>
<td>HS 2</td>
<td>16.9</td>
<td>3.30</td>
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<tr>
<td>HS 3</td>
<td>34.2</td>
<td>2.66</td>
</tr>
<tr>
<td>PC 1/1</td>
<td>11.4</td>
<td>0.89</td>
</tr>
<tr>
<td>PC 1/2</td>
<td>41.6</td>
<td>3.23</td>
</tr>
</tbody>
</table>

### Analytical sensitivity (lower detection limit)

0.30 pmoL/L (0.023 ng/dL)

The detection limit represents the lowest analyte level that can be distinguished from zero.

### Method comparison

A comparison of the Elecsys FT4 assay (x) using the Enzymun-test FT4 method (y) using clinical samples gave the following correlations (pmoL/L).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-run precision</th>
<th>Total precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean pmoL/L</td>
<td>SD pmoL/L</td>
</tr>
<tr>
<td>HEP 1</td>
<td>9.15</td>
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<tr>
<td>HS 2</td>
<td>16.9</td>
<td>3.30</td>
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<td>34.2</td>
<td>2.66</td>
</tr>
<tr>
<td>PC 1/1</td>
<td>11.4</td>
<td>0.89</td>
</tr>
<tr>
<td>PC 1/2</td>
<td>41.6</td>
<td>3.23</td>
</tr>
</tbody>
</table>

### Analytical specificity

For the antibody derivatives used, the following cross-reactivities were found:

- L-T4 and D-T4 100 %
- L-T3 1.53 %
- D-T3 1.38 %
- T4-T3 0.002 %

### Notes

- Cobas, Elecsys, Enzygnost, MCL, and MOLAR are trademarks of Roche Diagnostics.
- Genetic alterations are indicated by a change bar in the margin. Changes in target barcode test application which have already been listed in should be added manually.

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FT3 - free triiodothyronine

**Summary**

Triiodothyronine (T3) is one of the thyroid hormones present in serum which regulates metabolism. Determination of this hormone concentration is important for the diagnostic differentiation of euthyroid, hyperthyroid, and hypothyroid states. The major fraction of total triiodothyronine is bound to the transport proteins (TBG, prealbumin, albumin). Free Triiodothyronine (FT3) is the physiologically active form of the thyroid hormone triiodothyronine (T3). The determination of free T3 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-upper, TBG) is therefore unnecessary.

**Intended use**

Immunoassay for the in vitro quantitative determination of free triiodothyronine in human serum and plasma.

The electrochemiluminescence immunoassay "ECLA" is intended for use on Elecsys and cobas e immunoassay analyzers.

**Test principle**

Competition principle. Total duration of assay: 11 minutes.

1. **1st incubation**: 15 μL of sample and an anti-T3-specific antibody labeled with ruthenium complex.
2. **2nd incubation**: After addition of biotinylated anti-T3 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex is bound to the solid phase via interaction of biotin and streptavidin.
3. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
4. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcodes.

**Reagents - working solutions**

M  Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL
R  Anti-T3 Ab-Ruby (gray cap), 1 bottle, 18 mL
S  Monoclonal anti-T3 antibody (sheep) labeled with ruthenium complex
T3-biotin (black cap), 1 bottle, 18 mL

**Precautions and warnings**

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines.

**Safety data sheet**

Available for professional user on request. Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

**Reagent handling**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in via the respective reagent barcodes.

**Storage and stability**

Store at 2-8 °C.

Store the Elecsys FT3 reagent kit upright in order to ensure correct availability of the microparticles during automatic mixing prior to use.

**Specimen collection and preparation**

Only the specimens listed below were tested and found acceptable.

Undiluted serum collected using standard sampling tubes or tubes containing separating gel.

Coulter L-Heptan, K2-EDTA, and K3-EDTA plasma.

**Material provided**

See "Reagents - working solutions" section for reagents.

**Materials required (but not provided)**

- 00031994190, FT3 CalSet, for 4 x 1 mL.
- 1173441622, Precontrol Universal, for 2 x 3 mL each of Precontrol Universal 1 and 2.
- 11734416190, Precontrol Universal, for 2 x 3 mL each of Precontrol Universal 1 and 2.
- 1179449190, Precontrol Universal, for 2 x 3 mL each of Precontrol Universal 1 and 2 (for USA).
- General laboratory equipment.
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or cobas e analyzer.
- Accessories for Elecsys 1010/2010 and cobas e analyzers.
- 11662988120, PreCell, 6 x 360 mL system buffer.
- 11662970122, ClearCell, 6 x 380 mL measuring cell cleaning solution.
- 11993346122, Elecsys SysWash, 1 x 500 mL washer additive.
- 11993599001, Adapter for SysOpen.
- 11709682001, Elecsys 1010 Assay Cup, 12 x 32 reaction vessels.
- 11709882001, Elecsys 1010 Assay Cup, 60 x 60 reaction vessels.
- 11709799001, Elecsys 1010 Assay Tip, 30 x 120 pipette tips.
FT3
free triiodothyronine

Accessories for MODULAR ANALYTICS E170 and cobas e 601 analyzers:
- REF 04800340190, ProCell M, 2 x 2 L system buffer
- REF 04800501050, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 12150027190, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- REF 03023140100, PCC Cups, 12 cups to premix ProCell M and CleanCell M before use
- REF 03005712050, Prep Wash M, 12 x 70 ml cleaning solution for run initialization and rinsing during reagent change
- REF 12102137070, Assay Tip/Assay Cup Combinadagazine M, 48 magazines 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLine, waste bags
- REF 11350700010, System Clean Adapter M

Accessories for all analyzers:
- REF 11296500030, Eleeys SysClean, 5 x 100 ml, system cleaning solution
- REF 11296500060, Eleeys SysClean, 5 x 100 ml, system cleaning solution (for USA)

Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use. Read in the test-specific parameters via the reagent barcodes. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers:
MODULAR ANALYTICS E170, Eleeys 2010 and cobas e 601 analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.
Eleeys 1010 analyzer: Bring the cooled reagents to approx. 20-30 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. Open bottle caps manually before use. Store at 2-8 °C after use.

Calibration
Traceability: The Eleeys FT3 assay (REF 03051986) has been standardized against the Eleeys FT3 assay (REF 11731186). This line was standardized using equilibrium dialysis. Every Eleeys FT3 reagent set has a barcoded label containing the specific information for calibration of the particular reagent kit. The predefined master curve is adapted to the analyzer by the use of Eleeys FT3 CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR ANALYTICS E170, Eleeys 2010 and cobas e 601 analyzers:
- after 1 month (28 days) when using the same reagent kit
- after 7 days (when using the same reagent kit on the analyzer)
- Eleeys 1010 analyzer:
  - with every reagent kit
  - after 7 days (ambient temperature 20-25 °C)
  - after 3 days (ambient temperature 25-32 °C)
For all analyzers:
- as required; e.g. quality control findings outside the specified limits

Quality control
For quality control, use Eleeys PreControl Universal 1 and 2. Other suitable control material can be used in addition.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Follow the applicable government regulations and local guidelines for quality control.

Calculation
The analyzer automatically calculates the analytic concentration of each sample (either in pmol/L, pg/mL, or ng/dL).
Conversion factors:
- pmol/L x 0.691 = pg/mL
- pg/mL x 1.536 = nmol/L
- nmol/L x 0.1 = ng/dL

Limits - interference
The assay is unaffected by ketosis (blood ket < 644 pmol/L, or < 33 mg/dL), hemolysis (HB < 2.7 mmol/L, or < 4.3 g/dL), lipemia (triglyceride < 2000 mg/dL), and bilirubin < 100 mg/dL.
Criterion Recovery: Within ± 10 % of initial value.
In patients receiving therapy with high estrogen doses (i.e. > 5 mg/day), no sample should be taken until at least 6 hours after the last estrogen administration.
CT, in commonly used pharmacological tests in vitro, only furnished specificity increased FT3 findings at the daily therapeutic dosage level.
Any influence that might affect the binding behavior of the binding proteins can alter the result of the FT3 tests (e.g. drugs, NTIs, and other factors). It is known that some factors in the patient's medical history, clinical examination, and other

Limits and ranges
- Measuring range: 0.400-50.00 pmol/L, or 0.260-32.55 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.400 pmol/L, or 0.260 pg/mL. Values above the measuring range are reported as > 50.0 pmol/L, or > 32.55 pg/mL.

Lower limits of measurement
Lower detection limit: 0.400 pmol/L, or 0.260 pg/mL.

Dilution
Samples for FT3 measurements cannot be diluted, as T3 in the blood is present in free and protein-bound forms which are in equilibrium. A change in the concentration of the binding proteins alters this equilibrium.

Expected values
Establishing values in reference range studies is based primarily on samples obtained from outpatient clinics, hospitals, and commercial laboratories in which TSH and FT4 levels are found to be in the euthyroid range. These patients often have non-thyroid diseases which might influence the thyroid function in general, and especially the FT3 level. This may explain the differences observed when comparing the reference range values used in different population groups using the same FT3 method. Besides local differences in some cases the overall health status of the individuals involved is decisive for the outcome of the reference intervals. The Eleeys FT3 assay was used to determine reference ranges in the following groups of individuals from different locations in Germany:

Adults:
- 1.6-4.4 pmol/L (0.9-2.4 pg/mL)

From a consistently situated commercial laboratory in Germany, S566 routine samples with TSH between 1 and 3 mU/L, were evaluated by non-parametric calculation of the central 95 % limits and corresponding 95 % confidence intervals (CI) for FT3 concentration.
Appendix (1II)
### FT3

**FT3**
- **free triiodothyronine**

#### Median 2.5th percentile 90th percentile
- 3.9-6.7 pmol/L (2.5-4.3 mg/mL)
- 870 samples derived from apparently healthy blood donors aged 20 to 69 years from a central German site were evaluated by non-parametric calculation of the central 95% limits and corresponding 95% confidence intervals (CI) for FT3 concentration.

#### Table: FT3 Median and 2.5th percentile

<table>
<thead>
<tr>
<th>Median</th>
<th>2.5th percentile</th>
<th>90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>3.9</td>
<td>6.7</td>
</tr>
<tr>
<td>3.3</td>
<td>2.9-2.9</td>
<td>4.3</td>
</tr>
</tbody>
</table>

The following parameters were recorded in these individuals: the concentration of TSH, FT4, and auto-antibodies to Tg and TPO; the volume and density of the thyroid gland measured by ultrasonograph; their case history, family, and personal thyroid history; their gender, age, and iodine intake; and whether or not they smoked or were taking oral contraceptives. For results based on a variety of different inclusion and exclusion criteria, please refer to the separate information given in the brochure "Reference Intervals for Elecsys Thyroid Assays".

### Children and Adolescence
- Samples from newborns, infants, and adolescents up to 18 years of age, characterized as apparently healthy by experts from a medical center in central Germany.

### Table: FT3 Median and 2.5th percentile

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-30 days</td>
<td>5.2</td>
<td>3.0</td>
<td>81</td>
<td>7.3</td>
<td>8.4</td>
<td>56.4</td>
</tr>
<tr>
<td>3-12 months</td>
<td>5.9</td>
<td>3.5</td>
<td>84</td>
<td>6.7</td>
<td>8.4</td>
<td>45.6</td>
</tr>
<tr>
<td>2-6 years</td>
<td>6.1</td>
<td>3.0</td>
<td>91</td>
<td>8.2</td>
<td>9.6</td>
<td>58.6</td>
</tr>
<tr>
<td>7-11 years</td>
<td>6.1</td>
<td>3.0</td>
<td>91</td>
<td>8.2</td>
<td>9.6</td>
<td>58.6</td>
</tr>
<tr>
<td>12-19 years</td>
<td>5.9</td>
<td>3.5</td>
<td>77</td>
<td>7.3</td>
<td>8.4</td>
<td>56.4</td>
</tr>
</tbody>
</table>

The following exclusion criteria were stipulated in these individuals (both outpatient and hospitalised): no preneuro or acute thyroid disease, no family history of thyroid disease, no coronary disease, no intensive care, and no postoperative medical care.

### Specific performance data

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Precision

#### Table: Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean pmol/L</th>
<th>SD pmol/L</th>
<th>CV</th>
<th>Mean pmol/L</th>
<th>SD pmol/L</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS 1</td>
<td>2.86</td>
<td>0.06</td>
<td>5.8</td>
<td>2.09</td>
<td>0.06</td>
<td>2.3</td>
</tr>
<tr>
<td>HS 2</td>
<td>3.85</td>
<td>0.08</td>
<td>5.2</td>
<td>2.05</td>
<td>0.08</td>
<td>2.1</td>
</tr>
<tr>
<td>HS 3</td>
<td>19.5</td>
<td>0.53</td>
<td>2.7</td>
<td>17.5</td>
<td>0.53</td>
<td>2.9</td>
</tr>
<tr>
<td>PC 1U</td>
<td>4.98</td>
<td>0.24</td>
<td>5.0</td>
<td>4.74</td>
<td>0.24</td>
<td>4.9</td>
</tr>
<tr>
<td>PC 2U</td>
<td>22.0</td>
<td>0.33</td>
<td>1.5</td>
<td>21.2</td>
<td>0.33</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Repeatability: within-run precision
- a) HS = human serum
- b) PC = PreControl Universal

### Methods

#### Table: Method comparison

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean pmol/L</th>
<th>SD pmol/L</th>
<th>CV</th>
<th>Mean pmol/L</th>
<th>SD pmol/L</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS 1</td>
<td>2.83</td>
<td>0.04</td>
<td>1.9</td>
<td>2.09</td>
<td>0.04</td>
<td>2.4</td>
</tr>
<tr>
<td>HS 2</td>
<td>3.87</td>
<td>0.05</td>
<td>1.4</td>
<td>3.85</td>
<td>0.05</td>
<td>1.2</td>
</tr>
<tr>
<td>HS 3</td>
<td>19.5</td>
<td>0.49</td>
<td>2.5</td>
<td>19.5</td>
<td>0.49</td>
<td>2.5</td>
</tr>
<tr>
<td>PC 1U</td>
<td>4.98</td>
<td>0.18</td>
<td>3.8</td>
<td>4.98</td>
<td>0.18</td>
<td>3.8</td>
</tr>
<tr>
<td>PC 2U</td>
<td>22.0</td>
<td>0.32</td>
<td>1.5</td>
<td>22.0</td>
<td>0.32</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Repeatability: within-run precision
- a) HS = human serum
- b) PC = PreControl Universal

### Notes

- n = 21
- The following results were obtained:
FT3
Free thyroxine (FT3)

References
9. Marsh J. Nichols Institute, CA, USA.

For further information, please refer to the appropriate operator’s manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

For our customers only: Limited Warranty
Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling. Roche Diagnostics will repair or replace, at no charge to the customer, any Roche Diagnostics product which is defective in material or workmanship. If the product is returned to Roche Diagnostics at the customer’s expense within the warranty period, Roche Diagnostics’ obligations under this warranty will be limited to pro-rata repair or replacement of the product. This warranty is in lieu of any other warranty, express or implied, including any implied warranty of merchantability or fitness for particular purpose. In no event shall Roche Diagnostics be liable for incidental, indirect, special or consequential damages.

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Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-459-2338

Elecsys and cobas e analyzers
4/4
2006-11-V 12 English
Appendix (1V)
**Anti-TSH Receptor (TRAb) Fast ELISA (IgG)**

**Test instruction**

<table>
<thead>
<tr>
<th>ORDER NO.</th>
<th>ANTIBODIES AGAINST</th>
<th>Ig CLASS</th>
<th>SUBSTRATE</th>
<th>FORMAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>EA 1015-9601-1 G</td>
<td>TSH Receptor (thyroid-stimulating hormone receptor)</td>
<td>IgG</td>
<td>Ag-coated microplate wells</td>
<td>96 x 01 (96)</td>
</tr>
</tbody>
</table>

**Indication:** Detection or exclusion and management of the treatment of Graves' disease.

**Principle of the test:** The Anti-TSH Receptor (TRAb) Fast ELISA (IgG) test kit provides a quantitative in vitro assay for human autoantibodies against thyroid-stimulating hormone (TSH) receptor (TRAb). The test kit contains microplate strips, each with 8 break-off reagent wells coated with TSH receptor. In the first reaction step, patient sera are incubated in the wells. If samples are positive, specific antibodies bind to the TSH receptors. Bound antibodies are able to inhibit the binding of a thyroid-stimulating human monoclonal antibody (M2G2, in the form of M22-peroxidase), which is added in a second incubation step. To detect the bound M22-peroxidase, a third incubation is carried out using peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) catalysing a colour reaction. The intensity of the colour formed is inversely proportional to the concentration of antibodies against TSH receptor.

**Contents of the test kit:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Colour</th>
<th>Format</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Microplate wells coated with antigens: 12 microplate strips each containing 5 individual break-off wells in a frame, ready for use</td>
<td>—</td>
<td>12 x 8</td>
<td>STRIPS</td>
</tr>
<tr>
<td>2. Calibrator 1</td>
<td>colourless</td>
<td>1 x 1.0 ml</td>
<td>CAL 1</td>
</tr>
<tr>
<td>3. Calibrator 2</td>
<td>colourless</td>
<td>1 x 1.0 ml</td>
<td>CAL 2</td>
</tr>
<tr>
<td>4. Calibrator 3</td>
<td>colourless</td>
<td>1 x 1.0 ml</td>
<td>CAL 3</td>
</tr>
<tr>
<td>5. Calibrator 4</td>
<td>colourless</td>
<td>1 x 1.0 ml</td>
<td>CAL 4</td>
</tr>
<tr>
<td>6. Negative control</td>
<td>colourless</td>
<td>1 x 1.0 ml</td>
<td>NEG CONTROL</td>
</tr>
<tr>
<td>7. Positive control</td>
<td>yellow</td>
<td>1 x 10 ml</td>
<td>POSITIVE CONTROL</td>
</tr>
<tr>
<td>8. M22-Peroxidase, lyophilized</td>
<td>colourless</td>
<td>2 x 6 ml</td>
<td>M22</td>
</tr>
<tr>
<td>9. M22-Peroxidase buffer, ready for use</td>
<td>colourless</td>
<td>1 x 15 ml</td>
<td>M22 BUFFER</td>
</tr>
<tr>
<td>11. Wash buffer, 10x concentrated</td>
<td>colourless</td>
<td>1 x 100 ml</td>
<td>WASH BUFFER ON</td>
</tr>
<tr>
<td>12. Peroxidase substrate (TMB), ready for use</td>
<td>colourless</td>
<td>1 x 15 ml</td>
<td>SUBSTRATE</td>
</tr>
<tr>
<td>13. Stop solution, ready for use</td>
<td>colourless</td>
<td>1 x 10 ml</td>
<td>STOP SOLUTION</td>
</tr>
<tr>
<td>14. Protective foil</td>
<td>—</td>
<td>1 piece</td>
<td>—</td>
</tr>
<tr>
<td>15. Test instruction</td>
<td>—</td>
<td>1 protocol</td>
<td>—</td>
</tr>
<tr>
<td>16. Protocol with target values</td>
<td>—</td>
<td>1 protocol</td>
<td>—</td>
</tr>
</tbody>
</table>

**Lot:** Lot

**In vitro determination:** CE

**Storage temperature:** 4°C

**Unopened usable until:**

**Storage and stability:** The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

**Waste disposal:** Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents are to be disposed of according to official regulations.
Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+20°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- Coated wells: Ready for use. Tear open the protective wrapping of the microplate. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C up to 12 weeks.

- Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use.

- M22-Peroxidase: Lyophilized. Reconstitute the contents of one vial with 6.0 ml M22-Peroxidase buffer. The reconstituted M22-Peroxidase is stable until the indicated expiry date at +2°C to +6°C.

- M22-Peroxidase buffer: Ready for use.

- Sample buffer: Ready for use.

- Wash buffer: The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water). For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water. The working strength wash buffer is stable until the indicated expiry date when stored at +2°C to +8°C and handled properly.

- Peroxidase substrate (TMB): Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The peroxidase substrate (TMB) solution must be clear on use.

- Stop solution: Ready for use.

Warning: Calibrators and controls used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: Human serum.

Stability: Serum samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Do not use grossly haemolysed or lipaemic serum samples. Do not use plasma samples.
Incubation

For quantitative analysis incubate the calibrators 1-4, negative control, positive control and patient samples.

(Parity) manual test performance

Sample incubation:
(1st step)
Add 75 µl of sample buffer into each of the microplate wells used. Transfer 75 µl calibrators, negative and positive controls or patient samples into the individual microplate wells according to the pipetting protocol. Cover the reagent wells and incubate for 1 hour at room temperature (+20°C to +25°C) on a microplate shaker set at 500 rpm.

Washing:
Manual: Empty the wells and add once 350 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells once with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Leave the wash buffer in each well for 30 to 60 seconds, then empty the wells. After manual washing, thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

M22-Peroxidase incubation:
(2nd step)
Pipette 100 µl of reconstituted M22-Peroxidase into each of the microplate wells and incubate for 25 minutes at room temperature (+20°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash twice using 350 µl of working strength wash buffer for each wash followed one wash with 350 µl of deionised or distilled water.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Modus”).

Substrate incubation:
(3rd step)
Pipette 100 µl of peroxidase substrate TMB into each of the microplate wells. Incubate for 25 minutes at room temperature (+20°C to +25°C) (protect from direct sunlight).

Stopping the reaction:
Pipette 50 µl of stop solution into each of the microplate wells in the same order and at the same speed as the peroxidase substrate TMB solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 520 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using the analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I or the DXS from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.

Automated test performance using other fully automated, open system analysis devices is possible, however, the combination should be validated by the user.
Pipetting protocol

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>C1</td>
<td>P3</td>
<td>P11</td>
<td>P19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>C2</td>
<td>P4</td>
<td>P12</td>
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<td>C</td>
<td>C3</td>
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<td>P21</td>
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<tr>
<td>D</td>
<td>C4</td>
<td>P6</td>
<td>P14</td>
<td>P22</td>
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<tr>
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<td>P15</td>
<td>P23</td>
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<tr>
<td>F</td>
<td>pos.</td>
<td>P8</td>
<td>P16</td>
<td>P24</td>
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<td>G</td>
<td>P1</td>
<td>P9</td>
<td>P17</td>
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<tr>
<td>H</td>
<td>P2</td>
<td>P10</td>
<td>P18</td>
<td>P26</td>
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</tbody>
</table>

The pipetting protocol for microtiter strips 1-4 is an example for the quantitative analysis of 26 patient samples (P 1 to P 26).

The calibrators (C 1 to C 4), the negative (neg.) and positive (pos.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample. Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

**Quantitative:** The calibration curve from which the concentration of TDI receptor antibodies in the serum samples can be obtained is obtained by plotting the extinction values measured for the 4 calibrators and the negative control as zero calibrator (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis). The results for unknowns can be calculated using spline fits. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of TSH receptor antibody concentrations in patient samples.

If the extinction of a serum sample lies below the value of calibrator 1 (40 IU/l), the result should be given as ">40 IU/l". It is recommended that the sample be re-tested at a dilution of 1:10 in TRAb negative serum. The result in IU/l read from the calibration curve for this sample must then be multiplied by a factor of 10.
The upper limit of the normal range (cut-off value) recommended by EUROIMMUN is 1 international unit per litre (IU/L). EUROIMMUN recommends interpreting results as follows:

- <1.0 IU/L: negative
- ≥1.0 IU/L: positive

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be repeated.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Results can also be expressed as inhibition (%) of M22 binding calculated using the formula:

\[ \text{Inhibition} \% = 100 \times \left(1 - \frac{\text{Extinction of the serum sample}}{\text{Extinction of the negative control}}\right) \]

Test characteristics

Calibration: The calibration is performed in international units (IU) using the 1st International Standard for thyroid stimulating antibody (WHO, 1996, standard 90/672, National Institute for Biological Standards and Control, Hertfordshire, England). The NIBSC 90/672 standard contains 0.1 IU per ampoule by definition.

For every group of tests performed, the international units determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these target values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Antigen: The microplate wells were coated with porcine TSH receptor. Mouse monoclonal antibodies specific for TSH receptor were used to immobilise the receptor onto microplate wells. Human autoantibodies against thyroid-stimulating hormone (TSH) receptor show similar reactivity to porcine and to human TSH receptor.

Detection limit: The kit negative control was assayed 54 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 0.16 IU/L.

Cross reactivity: The Anti-TSH Receptor (TRab) FAST ELISA specifically detects human autoantibodies against thyroid-stimulating hormone (TSH) receptor with the ability to inhibit the binding of M22-Peroxidase to the TSH receptor. Analysis of sera from patients with different autoimmune diseases other than Graves’ disease indicated no interference in the Anti-TSH Receptor (TRab) FAST ELISA from autoantibodies to thyroglobulin, thyroid peroxidase, dsDNA or rheumatoid factors.

Interference: No effect is observed with intralipid up to 30 mg/ml, human LH up to 10 U/ml, hCG up to 160 U/ml, human FSH up to 70 U/ml and human TSH up to 3 mU/ml. Neither haemoglobin up to 5 mg/ml nor bilirubin up to 0.2 mg/ml had any influence in the ELISA.
Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 2 sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 20 determinations.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Mean value (IU/l)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.96</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
<td>7.14</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Clinical sensitivity and specificity: Clinical sensitivity and specificity: 82 samples from patients diagnosed with Graves’ disease (treated and untreated patients) were assayed using the Anti-TSH Receptor (TRab) FAST ELISA. 85% of the patients were identified as being positive for TSH receptor autoantibodies. 104 samples (44 females) from healthy blood donors were tested negative. The sensitivity determined for the ELISA was 85%, at a specificity of 100%.

Method comparison: The TRab concentration was determined in 82 sera of Graves’ disease patients using the 3rd generation Anti-TSH Receptor (TRAb) Fast-ELISA (IgG) and the 2nd generation Anti-TSH Receptor (TRAb)-ELISA (IgG). The sensitivity of the 3rd generation with respect to the clinical picture was 85%, the sensitivity of the 2nd generation was 77%. The specificity of the test systems determined with 100 control sera amounted to 100%, respectively.

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 slow down the emission of TSH (thyroid-stimulating hormone) that is produced in the pituitary gland inducing 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 growth of the total organism [1].

An increase of T3 and T4 levels are in general an indication of a hyperthyroid functional disorder (hyperthyroidism), whereas low levels of T3 and T4 hormones in serum are allocated to a hypothyroid functional disorder (hypothyroidism) [2]. Independent from the cause of the varying thyroid hormone levels the clinical symptoms of hyper- and hypothyroidism are largely the same.

The symptoms of a hyperthyroidism are nervousness, irritability, restlessness, trembling hands, insomnia, perspiration, warm damp hands, ravenous appetite, thirst, and weight loss despite a good appetite and in women menstruation cycle disorders (irregular or increased bleeding, or absence of the menstruation) [1].

The symptoms of a hypothyroidism are low body temperatures, increased sensitivity to coldness, edemas (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 edemas), 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 [1].

Aside from a disorder of the thyroid hormone regulation, thyroiditis (thyroid inflammation) can be the cause of the symptoms of either hyper- or hypothyroidism [3]. These include several diseases: A differentiation is made between an acute (bacterial infection), a subacute (non infectious), and a chronic thyroiditis (autoimmune disease) [4]. Autoimmune thyroidopathy are chronic inflammatory thyroid diseases that are caused by disregulation of specific immune defences (B-cells and T-cells) [5].
They occur most often after a virus infection and sometimes also after a subacute thyroiditis. Genetic factors play a role in their development. During an autoimmune process antibodies against one or several of the three autoantigens, thyroid peroxidase (TPO), thyroglobulin (TG) and TSH receptor (TR), of the thyroid are formed [4, 5, 7, 8, 9].

TSH receptor autoantibodies (TRAb) are heterogeneous regarding their biological effect. There are antibodies that have a stimulating or blocking effect on the TR, antibodies that stimulate thyroid growth and others that inhibit the binding of TSH and TR [6, 7, 10]. 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 [7, 10].

The determination of TRAb is mainly performed if there is the suspicion of Graves' disease, an autoimmune disease that shows not only the symptoms of a hyperthyroidism, but additional symptoms such as struma, exophthalmos and tachycardiae (Mersburg triad). Severe cases are characterised by weight loss, heart insufficiency and coma [2]. Approximately 2% of the female and 0.2% of the male population are affected by a manifest Graves' disease. Graves' disease often appears in women during hormonal changes (puberty, pregnancy, menopause). 60% of all cases of hyperthyroidism can be ascribed to Graves' disease.

TRAb determinations are performed for the confirmation of Graves' disease with a prevalence of 90-100%. Thus TRAb are considered to be diagnostic markers and are utilization for differential diagnostics compared to a disseminated autonomy of the thyroid gland [17]. 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 Graves' disease following a long thyroidatic therapy show an increased risk for reoccurrence of the disease [11]. Moreover increased TRAb concentrations in the third trimester of pregnant women with Graves' disease indicate a hyperthyrosis in the foetus. Where normal values are found, the diagnosis can be supported by the determination of antibodies against TPO (thyroid peroxidase) with a prevalence of 60-70% [2, 8]. Additionally antibodies against TG in 20-50% of the cases are found [9]. Since there are also associations with other autoimmune diseases, e.g. myasthenia gravis, pernicious anemia, chronic-atorrophic 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 frequently found autoimmune diseases in humans and is the most frequent cause of primary thyroid hypofunction. Hashimoto's thyroiditis (autoimmune thyroiditis type 1A and 2A with struma) is a chronic thyroiditis with progressive destruction of the thyroid tissue by T-lymphocytes [13]. One's thyroiditis (autoimmune thyroiditis type 1B and 2B) is a special form of Hashimoto's thyroiditis and is characterised by an atrophy of the thyroid gland [14]. These two conditions (the hyperrophic and the atrophic form) lead to a thyroid hypofunction, with possible phases of hyperfunction (so-called hyperthyroidism, in extreme cases hashitotoxicosis) 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 [15]. From a serological point of view, antibodies against TPO can be detected with a prevalence of 60-70%. Antibodies against thyroglobulin are in 90-100% of the cases initially high [8, 16]. In Hashimoto's disease and myxoedema, blocking TRAb may cross the placenta in pregnant women and lead to transient neonatal hypothyroidism [13, 17].

Approximately 5% of women have postpartum thyroiditis, which is a transient hypothyrotic 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 radioimmunosay 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 [5, 7, 10, 16, 18, 19, 20, 21].

Literature references


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Thyrotropin receptor autoantibodies (TSHRAbs): epitopes, origins and 

Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA. 

19. Stöcker W, Schatz H. Sind die neueren Methoden zur Bestimmung von 
Autoantikörpern gegen Schilddrüsen-Antigene den Säuren überlegen? 


Sanders J, Furmaniak J 2004 A new assay for thyrotropin receptor autoantibodies. Thyroid 14: 
830-835.
Pipetting Scheme for ELISA

<table>
<thead>
<tr>
<th>Sample buffer</th>
<th>Calibrators</th>
<th>Controls</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
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<td></td>
</tr>
<tr>
<td>Calibrator 1 - 4</td>
<td>75 µl</td>
<td></td>
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</tr>
<tr>
<td>Negative control</td>
<td>75 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>75 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample serum</td>
<td>75 µl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incubate for 1 hour at room temperature on a shaker set at 500 rpm

wash 1x

M22-Peroxidase 100 µl

Incubate for 25 minutes at room temperature

wash 3x

Substrate (TMB) 100 µl

incubate for 25 minutes at room temperature in the dark

Stop solution 50 µl

Shake briefly and read absorbance at 450 nm.
Appendix V
Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) around 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of the partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for a minimum of 4 months.

  - Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use.
  - Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.
  - Sample buffer: Ready for use.
  - Wash buffer: The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).
    For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
    The ready-to-use diluted wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

  - Chromogen/substrate solution: Ready for use. Close the bottle immediately after use as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

  - Stop solution: Ready for use.

Warning: Calibrators and controls used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid contact with the skin.

Preparation and stability of the patient samples

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 28 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted 1:201 in sample buffer. For example, dilute 5 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.
Incubation

For semiquantitative analysis incubate calibrator 2 along with the positive and negative controls and patient samples. For quantitative analysis incubate calibrators 1, 2 and 3 along with the positive and negative controls and patient samples.

Sample incubation:
(1. step)
Transfer 100 µl calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for 30 minutes at room temperature (+15°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 400 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downward to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to falsely low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to falsely high extinction values.

Conjugate incubation:
(2. step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for 30 minutes at room temperature (+15°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation:
(3. step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to 25°C) protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the micro-plate to ensure a homogeneous distribution of the solution.
The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24). The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample. The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes reagent wastage. Both positive and negative controls serve as internal controls for the reliability of the test procedure.

### Calculation of results

**Semiquantitative:** Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

\[
\text{Ratio} = \frac{\text{Extinction of control or patient sample}}{\text{Extinction of calibrator}}
\]

**EUROIMMUN** recommends interpreting results as follows:

- Ratio < 1.0: negative
- Ratio ≥ 1.0: positive

**Quantitative:** The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.
**EUROIMMUN**

**Linearity:** The linearity of the test was investigated by assaying serial series dilutions of patient samples with high antibody concentrations. The chart below shows the typical linearity of samples on the basis of 4 patient samples. The Anti-TPO ELISA is linear in the measurement range 10 - 500 IU/ml.

![Graph showing linearity](image)

**Detection limit:** The detection limit is defined as a value three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The detection limit of the Anti-TPO ELISA is approximately 5 IU/ml.

**Cross reactivity:** This ELISA showed no cross reactivity.

**Interference:** Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

**Reproducibility:** The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

**Intra-Assay Variation, n = 20**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Mean value (IU/ml)</th>
<th>CV (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>151</td>
<td>3.7</td>
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<tr>
<td>2</td>
<td>245</td>
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<td>3</td>
<td>370</td>
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</table>

**Inter-Assay Variation, n = 4 \times 6**

<table>
<thead>
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<th>Serum</th>
<th>Mean value (IU/ml)</th>
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<tbody>
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<td>1</td>
<td>146</td>
<td>2.8</td>
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<tr>
<td>2</td>
<td>254</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>383</td>
<td>3.5</td>
</tr>
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</table>

**Prevalence and specificity:** Sera from 9 patients with Hashimoto's thyroiditis, 23 patients with Grave's disease, 11 patients with other hyperthyroidism and 200 healthy blood donors were examined with the EUROMMUN Anti-TPO ELISA. The test showed a specificity of 95%.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Anti-TPO positive</th>
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<tbody>
<tr>
<td>Hashimoto's thyroiditis</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Grave's disease</td>
<td>17 (74%)</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>16 (63%)</td>
</tr>
<tr>
<td>Blood donors</td>
<td>10 (5%)</td>
</tr>
</tbody>
</table>

**Reference range:** The levels of the anti-TPO antibodies (IgG) were analyzed with this EUROMMUN ELISA in a collective of 200 healthy blood donors. With a cut-off of 50 IU/ml, 5.0% of the blood donors were anti-TPO positive (IgG).
Correlation of the ELISA with the indirect immunofluorescence test (IFT): 153 uncharacterized sera were investigated with the EUROIMMUN Anti-TPO ELISA (IgG) and the EUROIMMUN Anti-Thyroid Microsomes IFT (IgG) as reference. The ELISA had a specificity of 100% and a sensitivity of 98% with reference to the EUROIMMUN IFT.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>IFT (thyroid microsomes)</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>153</td>
<td>106</td>
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<tr>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Clinical significance

Autoimmune thyroid diseases can manifest themselves as both hyper- and hypothyroid-functional disorders. They occur considerably more often in women than in men. The symptoms in young persons are characteristic, in later years they are increasingly masked.

Approximately 60% of all cases of hyperthyroidism can be ascribed to Grave's disease. Typical clinical symptoms are over-excitability, weight loss, diffuse struma, endocrine ophthalmic symptoms (in 40% of patients), tachycardia (in some cases cardiac insufficiency), coma. The symptoms can vary from just perceptible to life-threatening crises. Patients frequently tend to relapse, but spontaneous remission or an unspaced transition to hypothyroidism is also possible. From a serological point of view, autoantibodies against TSH receptors of the thyroid are considered to be diagnostic markers. Where normal values are found, the diagnosis can be supported by the determination of microsomal antibodies. Additional autoantibodies against thyroglobulin are frequently found. Since there are associations with other autoimmune diseases, it is likely that further autoantibodies are found (e.g. AMA in approximately 30% of cases, AMA, ASMA, PCA).

Hashimoto's autoimmune thyroiditis is a form of hypothyroidism, the main symptoms being tiredness, muscle weakness, weight increase and, in some cases, struma. In clinical terms, the disease frequently starts with a diffuse goiter. From a serological point of view, antibodies against TPO can be detected; antibodies against thyroglobulin are initially elevated (prevalence 90 to 100%). Autoimmune thyroiditis is characterized by a subtotal destruction of the thyroid and is seen as a risk factor for the development of coronary heart diseases. For differential diagnosis, a malignant struma must be ruled out. Hashimoto's thyroiditis frequently occurs in combination with other autoimmune diseases (e.g. hypothyroidism, pernicious anemia, atrophic gastritis).

In other rare autoimmune thyroid diseases, the determination of antibodies against TPO is helpful; the value of antibody titers against thyroglobulin for diagnostic differentiation is limited.

Literature references