INTENDED USE

The kit has been designed for the quantitative determination of Triiodothyronine (T3) in human serum. The method can be used for samples over the range of 0.06-10.0ng/ml. The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 3000 and Maglumi 4000).

SUMMARY AND EXPLANATION OF THE TEST

In healthy subject, the thyroid secretes approx. 5-10μg triiodothyronine per day. Circulating 3,5,3’-triiodothyronine (T3) is, however, for the most part produced by peripheral deiodination so that the overall daily secretion rate of total T3 amounts to approx. 20μg. In serum, the thyroid hormones are bound to carrier proteins, and only their free fraction is physiologically active.

The clinical relevance of quantitative T3 determination in suspected thyroid disease lies mainly in the diagnosis and evaluation of hyperthyroidism. Particularly in isolated T3 hyperthyroidism, elevated T3 concentrations with concomitant normal T3 and T4 levels are observed. Following surgical resection of the thyroid gland and therapy with iodine-131, T3 concentrations may - in contrast to those of T4 remain at an elevated level or even rise (recurrence of hyperthyroidism). Occasional elevations of triiodothyronine levels are also found in approx. 50% of patients with autonomous adenoma associated with hyperthyroidism. Such elevations may also occur in the early stages of hyperthyroidism, in endocrine ophthalmopathy associated with latent hyperthyroidism, during treatment of hyperthyroidism (thyrostatics), in iodine deficiency with and without goitre and in Hashimoto’s thyroiditis, whereby the metabolic state may be normal.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay: Use an anti-T3 monoclonal antibody to label ABEI, and use a purified T3 antigen to label Nano Magnetic Microbeads. Sample, Calibrator, or Control, ABEI Label, and Buffer are mixed thoroughly and incubated at 37°C. Then add Nano Magnetic Microbeads and incubated, the Sample and Nano Magnetic Microbeads competitively binding the ABEI Label, forming an immuno-complex. After sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of T3 present in samples.

KIT COMPONENTS

Material Supplies

<table>
<thead>
<tr>
<th>Material Supplies</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with purified T3 antigen</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Calibrator Low: bovine serum, 0.2%NaNO₃</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Calibrator High: bovine serum, 0.2%NaNO₃</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Buffer: containing BSA, 0.2%NaNO₃</td>
<td>4.5ml</td>
</tr>
<tr>
<td>ABEI Label: anti-T3 monoclonal antibody labeled ABEI, containing BSA, 0.2% NaNO₃</td>
<td>6.5ml</td>
</tr>
</tbody>
</table>

All reagents are provided ready-to-use.
Reagent Vials in kit box

<table>
<thead>
<tr>
<th>Internal Quality Control: containing BSA, 0.2% NaN3 (target value refer to Quality Control information date sheet)</th>
<th>2.0ml</th>
</tr>
</thead>
</table>

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own standards and knowledge.

Accessories Required But Not Provided

- MAGLUMI Reaction Module REF: 630003
- MAGLUMI Starter T+2 REF: 130299004M
- MAGLUMI Wash Concentrate REF: 130299005M
- MAGLUMI Light Check REF: 130299006M

Please order accessories from SNIBE or our representative.

Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!)

Remove the sealing and turn the small wheel of the magnetic stirrers to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.

Keep upright for storage.

Keep away from sunlight.

CALIBRATION AND TRACEABILITY

1) Traceability

To perform an accurate calibration, we have provided test calibrators standardized against the USP Reference Material liothyronine.

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI.

If controls are beyond the expected range.

- The room temperature has changed more than 5 °C (recommendation).

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect 5.0ml venous blood into Blood Collection Tube. Standing at room temperature, centrifuging, separating serum part.

WARNING AND PRECAUTIONS FOR USERS
For use in IN-VITRO diagnostic procedures only.
Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach. A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens 1910.1030. This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual reagents may dry on the kit surface, please pay attention the solid film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

| 40μl | Sample, calibrator |
| +40μl | ABEI Label |
| +20μl | Buffer |
| 5 min | Incubation |
| +20μl | Nano Magnetic Microbeads |
| 10 min | Incubation |
| 400μl | Cycle washing |
| 3 s | Measurement |

DILUTION

Sample dilution by analyzer is not available in this reagent kit. Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Normal T3 concentrations do not necessarily reflect a normal-thyroid state. Certain thyroid disorders (such as latent hyper- or hypothyroidism, compensatory T3 over secretion in iodine deficiency, T3Q over secretion) may also be associated with euthyroid T3 levels. Furthermore, the clinical evaluation of serum findings must take into consideration both age- or pregnancy-related differences as well as a potential influence of exogenously administered thyroid hormones, contraceptive steroids, salicylates, diphenhydantoin or other drugs as well as changes of the binding capacities of serum proteins for thyroid hormones.

Serum T3 levels alone give no evidence of the presence or absence of thyroid disease. They must always be interpreted in context with the clinical picture and other diagnostic procedures.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin < 35mg/dl, haemoglobin < 2000mg/dl or triglycerides < 1800mg/dl.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the T3 concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.
- Conversion factor: 1 ng/ml = 1.54 nmol/L

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were: 0.69-2.15ng/ml
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision
Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

### Intra-assay precision

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>1.08</td>
<td>0.06</td>
<td>5.41</td>
</tr>
<tr>
<td>Level 2</td>
<td>2.25</td>
<td>0.12</td>
<td>5.12</td>
</tr>
<tr>
<td>Level 3</td>
<td>4.55</td>
<td>0.22</td>
<td>4.73</td>
</tr>
</tbody>
</table>

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

### Inter-assay precision

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>1.12</td>
<td>0.10</td>
<td>9.34</td>
</tr>
<tr>
<td>Level 2</td>
<td>2.35</td>
<td>0.21</td>
<td>8.73</td>
</tr>
<tr>
<td>Level 3</td>
<td>4.08</td>
<td>0.37</td>
<td>9.14</td>
</tr>
</tbody>
</table>

2) Analytical Sensitivity

The sensitivity is defined as the concentration of T3 equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.06 ng/ml.

3) Specificity

The specificity of the T3 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/ml)</th>
<th>Cross reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>300</td>
<td>0.1</td>
</tr>
<tr>
<td>rT3</td>
<td>100</td>
<td>0.5</td>
</tr>
</tbody>
</table>

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% - 110%.

<table>
<thead>
<tr>
<th>Expected</th>
<th>Mean Measuring</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.634 ng/ml</td>
<td>7.039 ng/ml</td>
<td>106%</td>
</tr>
</tbody>
</table>

5) Linearity

Use T3 calibrator to prepare the six-point standard curve, measuring all points’ RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient (r) should be bigger than 0.9800.

<table>
<thead>
<tr>
<th>Calibrator Point</th>
<th>Concentration (ng/ml)</th>
<th>Absolute linear correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.25</td>
<td>r = 0.9923</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

6) Method comparison

A comparison of MAGLUMI T3(y) with a commercially available T3(x) using clinical samples gave the following correlations (ng/ml): Linear regression:

\[ y = 1.0381x + 0.0665 \]

\[ r = 0.9881 \]

Number of samples measured: 100

The sample concentrations were between 0.89-10.00 ng/ml

References


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