The CEDIA T4 assay is for the quantitative determination of total thyroxine (T4) in serum and plasma on automated clinical chemistry analyzers. T4 measurements are used in the diagnosis and treatment of thyroid disorders.

### Summary

Thyroxine (T4) and triiodothyronine (T3) are thyroid hormones which are essential in the regulation of various metabolic functions. They are synthesized in the thyroid gland from iodine and the amino acid tyrosine, and their synthesis and release is a complex, coordinated process mediated through a complex negative feedback system involving the hypothalamus, pituitary, and thyroid glands. The hypothalamus releases thyrotropin releasing hormone (TRH) which stimulates the pituitary to release thyroid stimulating hormone (TSH). TSH in turn causes the thyroid to secrete T3 and T4. Increased levels of thyroid hormones then act on the anterior pituitary to decrease TSH release, creating a self-regulating feedback system.

Approximately 70-100 µg of T4 are secreted into the circulation daily. The more potent T3 is released in much smaller amounts, but an additional 35-50% is formed from the deiodination of T4 at the cellular level.

Once in circulation, T4 is found free or bound to plasma proteins. Approximately 98% of circulating T4 is bound to thyroxine-binding prealbumin and in some cases, albumin. Less than 1% of thyroxine is found free or unbound in plasma.

In increases in thyroxine are seen in hyperthyroidism, a condition caused by an excess of circulating hormone, and decreased levels are seen in hypothyroidism. The quantitation of total thyroxine levels, along with clinical history and other thyroid function tests such as T Uptake, is a valuable tool in the evaluation of thyroid function.

### Intended use

The CEDIA T4 assay is based on the bacterial enzyme β-D-galactosidase, which has been engineered into two inactive fragments. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.

NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the reagent compartment of the analyzer or into refrigerated storage (2-8°C) immediately prior to preparation of the working solutions.

### Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

The reagents contain less than 1% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

### Reagent handling

See below for reagent handling instructions for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.

Prepare the working solutions using cold reagents and buffers. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the working solutions.

Prepare the solutions in the following order to minimize possible contamination.

R1: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2.

R2: Connect Bottle 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at 15-25°C. Mix again.

Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

R1: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard.

Cap Bottle 1 and let stand approximately 5 minutes at 15-25°C. Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

Calibrate: Reconstitute Bottle 3 (Low Calibrator) and Bottle 4 (High Calibrator) with 20 ml distilled or deionized water. Swirl gently at 15-25°C for 15 minutes. Avoid the formation of foam. Ensure complete dissolution before use. Record the reconstitution date on the bottle labels.

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### Storage and stability

Unopened kit components: up to the expiration date at 2-8°C. Do not freeze.

R1 and R2: 45 days opened and refrigerated on the analyzer or at 2-8°C. Do not freeze.

Reconstituted Bottle 3 (Low Calibrator) and Bottle 4 (High Calibrator): 45 days at 2-8°C. Do not freeze.

To ensure reconstituted EA reagent stability, protect from prolonged continuous exposure to bright light.

### Specimen collection and preparation

Collect serum using standard sampling tubes. Sodium EDTA or sodium or lithium heparin plasma may be used.

Stability: 10 days capped at 2-8°C

6 months capped at -20°C

Avoid repeated freezing and thawing. Do not induce foaming of specimens. Centrifuge samples containing precipitate before performing the assay.

### Testing procedure

Materials provided

- Working Solutions as described above
- Low and High Calibrators as described above
- Additional materials required
- Controls as indicated below
- Distilled or deionized water and volumetric pipet for calibrator reconstitution

### Assay

Refer to the instrument-specific application sheet for analyzer specific assay instructions. For other analyzers, refer to the instrument-specific application sheet.

The performance of applications not validated by Microgenics is not warranted and must be defined by the user.
Calibration
S1: CEDIA T4 Low Calibrator
S2: CEDIA T4 High Calibrator

Calibration frequency
See below for calibration frequency recommendations for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.
Recalibration is recommended
• as blank calibration every 12 hours
• as 2 point calibration every 5 days if the reagent bottles are on analyzer for more than 5 days
• as 2 point calibration after reagent bottle change
• as 2 point calibration after reagent lot change
• as 2 point calibration as required following quality control procedures
Calibration verification: Not necessary.

Quality control
For quality control use Preciclot-N and Preciclot-A, Precinorm TDM or other suitable control material.
The control intervals and limits must be adapted to the individual laboratory and country-specific requirements.
Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calculation
Hitachi systems automatically calculate the T4 concentration of each sample.
Conversion factor: µg/dl x 12.9 = nmol/l

Limitations - interference
Criterion: recovery ≥ 90% of initial value at concentrations <5 µg/dl or ≥100% of initial value at concentrations >5 µg/dl.
Lipemia: No significant interference from lipoprotein up to an L index of 60 (approximate triglyceride concentration: 2000 mg/dl).
Hemolysis: Significant negative interference from hemoglobin above an H index of 550 (approximate hemoglobin concentration: 550 mg/dl).
Lupus (Intrinsic): No significant interference up to an L index of 1000 (approximate triglyceride concentration: 2000 mg/dl).
There is poor correlation between turbidity and triglycerides concentration.
No significant interference from total protein up to 12 g/dl or rheumatoid factor up to 96 IU/ml.
The incidence of patients with antibodies to E. coli β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high T4 results that do not fit the clinical profile. If this occurs, contact Customer Technical Support.
As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely elevated results.

Measuring/reportable range
0.5 - 20 µg/dl
Specimen dilution
Manually dilute samples above the measuring range 1 + 1 with CEDIA T4 Low Calibrator and re assay. Multiply the result by 2 and subtract the concentration of the low calibrator to obtain the specimen value.

Expected values
4.5 - 12.0 µg/dl
This range was determined using a population of 301 blood donors. The samples were run on a Hitachi 704 analyzer using Microgenics T4 reagents.
The following published results may also be used as a reference until a laboratory has established its own normal range:

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Normal Range (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chopra, et al</td>
<td>4.8 - 13.0</td>
</tr>
<tr>
<td>Tietz</td>
<td>5.0 - 12.0</td>
</tr>
<tr>
<td>Meyers, et al</td>
<td>5.0 - 12.0</td>
</tr>
<tr>
<td>Keffer</td>
<td>4.5 - 11.5</td>
</tr>
<tr>
<td>Lee, et al 4</td>
<td>4.5 - 13.2</td>
</tr>
</tbody>
</table>

Elevated levels of T4 can result from increased hormone synthesis, increased hormone release from thyroid cells, and increased binding capacity of plasma proteins, in particular thyroxine-binding globulin (TBG). Examples of hyperthyroidism include Graves’ disease, toxic multinodular goiter, and toxic thyroid adenoma. T4 levels will also be increased in pregnancy and in women taking oral contraceptives containing estrogen.
Decreased levels of T4 occur in primary, secondary, and tertiary hypothyroidism.
These include myxedema, cretinism, and nephrosis.
There are a number of compounds which can interfere directly or indirectly with the synthesis of thyroid hormones, thereby affecting thyroxine levels. These major inhibitors can be classified as follows:
1. Antithyroid drugs which interfere with hormone synthesis.
2. Iodine inhibitors which block the iodide transport mechanism.
3. Iodide which in high concentrations can suppress the thyroid.
4. Radioactive iodine which damages the thyroid gland with ionizing radiation.

The free thyroxine index (FTI) is a means of normalizing the effects of thyroxine-binding proteins on total T4 levels. The FTI yields a value which is related to the biologically active free T4 concentration.

The FTI may be calculated as follows:

FTI = T4 x T Uptake Ratio
where T Uptake Ratio is defined as:

T Uptake Ratio = Mean Normal T Uptake Value

Mean Normal T Uptake Value in the above equation refers to the mean value of the normal range study. A mean normal T Uptake value of 31.7% was used in these calculations. The normal range of FTI values as defined by the mean ± two standard deviations is 4.6 to 11.7.

The FTI may also be calculated as follows:

FTI = Total T4 x T Uptake

Using this calculation, the normal range of FTI values is 1.5 - 3.8. Total thyroxine tests should be used in conjunction with a T Uptake test to determine the free thyroxine index. It is recommended that the Microgenics T4 Reagents be used with the Microgenics T Uptake Reagents for FTI determinations.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.
For diagnostic purposes, T4 results should always be assessed in conjunction with the patient’s medical history, clinical examinations and other findings.

Specific performance data
The data determined using a Hitachi system are given below. Refer to the instrument specific application sheet for specific performance data.

Imprecision
Reproducibility was determined using controls in an internal protocol. Within run imprecision was determined by assaying 21 replicates in a single run. Between day imprecision was determined by single point quantitation in 21 separate runs. The following results were obtained.

Analytical sensitivity (lower detection limit)
Detection limit: 0.5 µg/dl
The lower detection limit represents the lowest T4 concentration that can be distinguished from zero. It is calculated as two standard deviations of 21 replicates of the Low Calibrator.

Method comparison
A comparison using the Microgenics CEDIA T4 assay (y) on a Hitachi system with a commercially available fluorescence polarization immunoassay (x) gave the following correlation (µg/dl):

Deming’s Linear regression

y = -0.02 + 1.05 x
ty = 0.06 + 1.04 x
rf = 0.990
Sy.x = 0.44
Sy.x = 0.62

Number of samples measured: 114
The sample concentrations were between 1.2 and 17.7 µg/dl.

Lineararity
To assess the linearity of the assay, a high sample was diluted with the CEDIA T4 Low Calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

<table>
<thead>
<tr>
<th>% Recovery</th>
<th>Expected Value (µg/dl)</th>
<th>Assayed Value (µg/dl)</th>
<th>High Sample</th>
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<tr>
<td>100.0</td>
<td>19.70</td>
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</tr>
<tr>
<td>87.5</td>
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<td>75.0</td>
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<td>37.5</td>
<td>7.49</td>
<td>7.67</td>
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</tr>
<tr>
<td>25.0</td>
<td>5.22</td>
<td>5.26</td>
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<tr>
<td>12.5</td>
<td>3.06</td>
<td>2.86</td>
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</tr>
<tr>
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<tr>
<td>0.0</td>
<td>0.45</td>
<td>0.45</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Recovery

To assess the recovery of the assay, thyroxine in the form of high (spiked) patient sample was added to a low patient sample. The percent recovery was then determined by dividing the assayed value by the expected value.

<table>
<thead>
<tr>
<th>% Expected</th>
<th>Assayed</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value (µg/dl)</td>
<td>Value (µg/dl)</td>
<td></td>
</tr>
<tr>
<td>100.0</td>
<td>----</td>
<td>20.60</td>
</tr>
<tr>
<td>87.5</td>
<td>18.43</td>
<td>18.54</td>
</tr>
<tr>
<td>75.0</td>
<td>16.27</td>
<td>16.68</td>
</tr>
<tr>
<td>62.5</td>
<td>14.10</td>
<td>14.12</td>
</tr>
<tr>
<td>50.0</td>
<td>11.93</td>
<td>11.91</td>
</tr>
<tr>
<td>37.5</td>
<td>9.77</td>
<td>9.85</td>
</tr>
<tr>
<td>25.0</td>
<td>7.64</td>
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</tr>
<tr>
<td>12.5</td>
<td>5.43</td>
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</tr>
<tr>
<td>0.0</td>
<td>----</td>
<td>3.26</td>
</tr>
</tbody>
</table>

Specificity

The CEDIA T4 assay is highly specific, having very low cross-reactivity to similar amino acids and drugs. Cross-reactivity was 90% for D-Thyroxine and is clinically insignificant for the following compounds:

- 3,3',5-Triiodothyroacetic acid
- 3,3',5-Triiodo-L-thyronine (T3)
- 3,3',5 - Triiodo-D-thyronine (D-T3)

There was negligible cross-reactivity (< 0.1%) with the following compounds:

- Acetylsalicylic acid
- Methimazole
- Phenylbutazone
- Salicylic acid
- 5,5'-Diphenylhydantoin
- DL-Tyrosine
- 6-n-Propyl-2-thiouracil
- 3,5-diiodo-L-thyronine
- 3-iiodo-L-tyrosine

Instrument settings

Refer to the instrument specific application sheet for additional operating information.

References
