Total T₄

Customer Support Center
United States: 1-877-4ABBOTT
International: Call your Abbott Representative

Note Changes Highlighted

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

- **REF**: List Number
- **LOT**: Lot Number
- **IVD**: For In Vitro Diagnostic Use
- **STANDARD CAL**: Standard Calibrator (A-F)
- **CONTROL**: Control Low, Medium, High (L,M,H)
- **REAGENT PACK**: Reagent Pack
- **CAUTION**: Handle human sourced materials as potentially infectious. Consult instructions for use. (Infection Risk)
- **REAGENT VESSELS**: Reaction Vessels
- **MASTER CAL**: Master Calibrator (1, 2)
- **SAMPLE CUPS**: Sample Cups
- **ASSAY NO.**: Assay No.
- **CHECKSUM**: Checksum
- **Expiry Date**: Expiration Date
- **Consult Instructions for use**
- **Authorized Representative**
- **Legal Manufacturer**
- **MASTER CALIBRATION BARCODE**: Master Calibration Barcode

See REAGENTS section for a full explanation of symbols used in reagent component naming.
tests such as hTSH, FT4, Total T3, FTI and clinical evaluation by the status should be determined in conjunction with other thyroid function. To ensure maximum diagnostic accuracy, the final definition of thyroid...

Consequently, T4 levels may be elevated with increased concentrations T3-thyrotoxicosis. T4 levels are altered by physiological or pathological changes in TBP capacity. Thyroxine binding globulin (TBG) capacity has a pronounced effect on the concentration of thyroid hormones. Consequently, T4 levels may be elevated with increased concentrations of TBG, such as in pregnancy, administration of oral contraceptives or estrogen, infectious and chronic active hepatitis, biliary cirrhosis or congenital increase in TBG levels. Conversely, when TBG levels are decreased, such as in nephrotic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness or congenital decrease of TBG, T4 may be reduced.

Drugs which compete for protein binding sites, such as phenylbutazone, diphenylhydantoin or salicylates, can result in a depressed T4 measurement. Serum T4 levels in neonates and infants are higher than values in the normal adult, due to the increased concentration of TBG in neonate serum.

While in many cases T4 values give good indications of thyroid status, T4 values should be normalized for individual variations in thyroxine binding protein (TBP) capacity. The Free Thyroxine Index (FTI) is conventionally used to achieve this measurement. For more information, refer to the calculation of the AxSYM FTI in the Procedure Section of the AxSYM T4-Labellet assay package insert.

To ensure maximum diagnostic accuracy, the final definition of thyroid status should be determined in conjunction with other thyroid function tests such as nTSH, FT4, Total T3, and clinical evaluation by the physician.

BIological PRINCIPLES OF THE PROCEdure
AxSYM Total T4 is based on Fluorescence Polarization Immunoassay (FPIA) technology. The AxSYM Total T4 Reagents and sample are pipetted in the following sequence:

SAMPLING CENTER
Sample and all AxSYM Total T4 reagents required for one test are pipetted by the Sampling Probe into various wells of a reaction vessel (RV).

• Sample, Pretreatment Solution, T4 Antiserum (antibody), and buffer are pipetted into one well of the RV.

• An aliquot of the predilution mixture is transferred to the cuvette along with the T4 Fluorescent Tracer.

• The analyte (T4) and labeled tracer compete for the sites on the antibody molecule.

• The intensity of polarized fluorescent light is measured by the FPIA optical assembly. For further information, refer to the AxSYM System Operating Manual, Section 3.

REAGENTs

1. Bottle (4.8 mL) T4 Pretreatment Solution. Surfactant in TRIS buffer. Preservative: Sodium Azide. (Reagent Bottle 1)
2. 1 Bottle (3.5 mL) T4 Antiserum (Mouse, Monoclonal) in phosphate buffer with protein stabilizers. Preservative: Sodium Azide. (Reagent Bottle 2)
3. 1 Bottle (10 mL) T4 Fluorescent Tracer in TRIS buffer containing surfactant. Preservative: Sodium Azide. (Reagent Bottle 3)  
4. AxSYM Total T4 Reagent Pack. 100 Tests and reaction vessels (100 each).  

CALIBRATIONs

AxSYM Total T4 Master Calibration (7A55-3D)
2 Bottles (5 mL Master Calibrator 1; 4 mL Master Calibrator 2) of AxSYM Total T4 Calibrators. Master Calibrator 1 contains processed human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, and Master Calibrator 2 contains thyroxine prepared in processed human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, to yield the following concentrations:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>T4 Concentration (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MASTER CAL 1</td>
<td>0.0</td>
</tr>
<tr>
<td>MASTER CAL 2</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

AxSYM Total T4 Standard Calibrators (7A55-01)
6 Bottles (5 mL each B-F) of AxSYM Total T4 Standard Calibrators. Calibrator A contains processed human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, and Calibrators B-F contain thyroxine prepared in processed human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2, to yield the following concentrations:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>T4 Concentration (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD CAL A</td>
<td>0.0</td>
</tr>
<tr>
<td>STANDARD CAL B</td>
<td>3.0</td>
</tr>
<tr>
<td>STANDARD CAL C</td>
<td>6.0</td>
</tr>
<tr>
<td>STANDARD CAL D</td>
<td>12.0</td>
</tr>
<tr>
<td>STANDARD CAL E</td>
<td>18.0</td>
</tr>
<tr>
<td>STANDARD CAL F</td>
<td>24.0</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

The calibrators are matched to a set of Abbott internal reference standards. These internal reference standards are manufactured by gravimetric methods using USP reference L-Thyroxine.
Controls

AxSYM Total T4 Controls (7A55-10)
3 Bottles (8 mL each) of AxSYM Total T4 Controls contain thyroxine prepared in processed human serum, nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-ARC and anti-HIV-1/HIV-2, to yield the following concentration range:

<table>
<thead>
<tr>
<th>Bottle</th>
<th>T4 Concentration Range (µg/dL)</th>
<th>(nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL A</td>
<td>15.0</td>
<td>160.88 - 225.23</td>
</tr>
<tr>
<td>CONTROL B</td>
<td>8.0</td>
<td>84.94 - 120.98</td>
</tr>
<tr>
<td>CONTROL C</td>
<td>4.5</td>
<td>42.47 - 73.36</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

The AxSYM Total T4 reporting unit is factory set to µg/dL. An alternate result unit (nmol/L) may be selected for reporting results (Assay Parameter 44).

Storage Instructions

The AxSYM Total T4 Reagent Pack must be stored at 2-8°C (do not freeze). The AxSYM Total T4 Calibrators and Controls must be stored at 2-8°C. The AxSYM Total T4 Reagent Pack, Calibrators and Controls may be used immediately after removing them from the refrigerator. Calibrators and Controls should be returned to 2-8°C storage immediately after use.

Reagents are stable until the expiration date when stored and handled as directed.

The AxSYM Total T4 Reagent Pack may be on-board the AxSYM System for a maximum of 112 cumulative hours; for example, 14 eight hour shifts. After 112 hours the reagent pack must be discarded. Refer to the AxSYM System Operations Manual, Appendices, for further information on tracking on-board time.

The AxSYM Probe Cleaning Solution and Solution 4 (Line Diluent) must be stored at 15-30°C.

End of Controls Section.
Refer to the AxSYM System Operations Manual for a detailed description of Instrument Procedures.

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

- Serum (including serum collected in separator tubes) or plasma (collected in sodium heparin, tripotassium EDTA, or potassium oxalate/sodium fluoride) may be used in the AxSYM Total T4 Assay. Follow the manufacturer’s processing instructions for serum or plasma collection tubes.

- The AxSYM System does not provide the capability of verifying sample type. It is the responsibility of the operator to verify the correct sample type(s) is (are) used in the Total T4 assay.

- Ensure that complete clot formation has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before complete clot forms, the presence of fibrin may cause erroneous results.

- For optimal results, samples should be free of fibrin, red blood cells or other particulate matter.

- Patient samples should be mixed and centrifuged after any freeze-thaw cycle or to remove red cells or particulate matter.

- Multiple freeze-thaw cycles should be avoided. Samples must be mixed thoroughly after thawing, by LDW speed vortexing or by gently inverting, and centrifuged prior to use to remove particulate matter and to ensure consistency in the results.

- Samples may be stored for up to 24 hours at 2-8°C prior to being tested. If testing will be delayed more than 24 hours, serum or plasma should be separated from the clot or red blood cells and stored frozen (-10°C or colder). Samples stored frozen at -10°C or colder for 12 months showed no performance difference.

- To minimize the effects of evaporation, all samples (patient samples, controls and calibrators) should be sealed within 3 hours of being placed on-board the AxSYM System. Refer to the AxSYM System Operations Manual, Section 5, for a more detailed discussion of on-board sample storage constraints.

- Inspect all samples for bubbles. Remove bubbles prior to analysis.

- When shipped, samples must be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical samples and etiologic agents.

- When testing is completed, it is recommended that samples and the AxSYM TOTAL T4 Reagent Pack are removed from the Sampling Center to minimize the on-board reagent pack usage. Store at 2-8°C.

SAMPLE DILUTION PROCEDURES

Automated Dilution Protocol

Total T4 samples cannot be diluted automatically on the System.

Manual Dilution Protocol

Patient samples with Total T4 concentrations reported as greater than 24 µg/dL or flagged results with background intensity greater than 21,000 AU may be manually diluted. The patient sample should be diluted with an equal amount of the AxSYM Total T4 Standard Calibrator A (0.0 µg/dL) or AxSYM Total T4 Master Calibrator 1 (0.0 µg/dL). Repeat the test using this manually diluted sample. The concentration reported by the AxSYM System must be multiplied by the manual dilution factor to obtain the final sample concentration.

Final Sample

Concentration = Reported Concentration x Manual Dilution Factor

Materials Provided

- 7A55-99 AxSYM Total T4 Reagent Kit, containing:
  - AxSYM Total T4 Reagent Pack
  - REAGENT VESSELS

AxSYM TOTAL T4 PROCEDURE

Materials Provided

- 7A55-10 AxSYM Total T4 Controls
- 7A55-01 AxSYM Total T4 Standard Calibrators or AxSYM Total T4 Master Calibrators
- 8A46 SOLUTION (LINE DILUENT)
- 8A35-05 AxSYM PROBE CLEANING SOLUTION
- 8A76-01 Pipettes/pipette tips (optional) to deliver the volumes specified on the Order screen.

CAUTION:

- When manually dispensing sample in the sample cups, verify that the dispensing equipment does not introduce cross contamination and that it delivers the specified sample volume. Use a separate pipette tip for each sample. Use accurately calibrated equipment.

- For optimal performance it is important to follow the routine maintenance procedures defined in the AxSYM System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Assay Procedure

Sections 5 and 6 of the AxSYM System Operations Manual contain detailed steps for performing assay calibration and sample testing procedures.

Prior to ordering tests, confirm that the System inventory of bulk solutions and waste levels are acceptable.

The Orderlist Report contains sample placement information and sample cup volume requirements for all ordered tests. It is recommended that this report be referenced when loading samples into sample segments. When using Host Order Query, the Order screen information and the Orderlist Report is not available. Refer to the AxSYM System Operations Manual, Section 5, for a description of the Host Order Query option.

CAUTION: When operating the AxSYM System, always observe the following:

- The System status must be WARMING, PAUSED, READY or STOPPED before adding or removing sample segments, reagent packs or reaction vessels (RIVs).

- When only performing FPIA assays, the instrument homes all motors and may display "Error Code 0066 Matrix cell not detected, trip door, processing center". Select OK to proceed with testing the FPIA assays.

- Do not open the Interior Waste Door or the AxSYM Processing Center Cover while any test is in process. If opened, all processing will stop. Tests in process will be terminated and must be repeated.

- When testing is completed, it is recommended that sample cups and the AxSYM TOTAL T4 Reagent Pack are removed from the Sampling Center to minimize the on-board reagent pack usage. Store at 2-8°C.

AxSYM TOTAL T4 PROCEDURE

Materials Provided

- 7A55-99 AxSYM Total T4 Reagent Kit, containing:
  - AxSYM Total T4 Reagent Pack
  - REAGENT VESSELS

AxSYM TOTAL T4 PROCEDURE

Materials Required But Not Provided

- AxSYM System
  - 7A55-10 AxSYM Total T4 Controls
  - 7A55-01 AxSYM Total T4 Standard Calibrators or AxSYM Total T4 Master Calibrators
  - 8A46 SOLUTION (LINE DILUENT)
  - 8A35-05 AxSYM PROBE CLEANING SOLUTION
  - 8A76-01 Pipettes/pipette tips (optional) to deliver the volumes specified on the Order screen.

FTI PROCEDURE

To review the protocol for the calculation of the Free Thyroxine Index (FTI) values, refer to the AxSYM T-Uptake assay package insert.
QUALITY CONTROL PROCEDURES

CALIBRATION

The AxSYM Total T4 Assay must be calibrated using either a Master Calibration (2-point) or a Standard Calibration (6-point) procedure. The use of a particular calibration procedure is dependent on individual laboratory policy:

Master Calibration

Each AxSYM Total T4 Reagent Pack is shipped with a bar coded label “insert” that contains the Master Curve information for that specific lot of reagents. When using a lot number for the first time, the bar coded Master Curve information must be entered into the AxSYM System. Refer to the AxSYM System Operations Manual, Section 6, for additional information on entering Master Curve bar codes. Once this bar code information is entered, a Master Calibration must be performed.

To perform an AxSYM Total T4 Master Calibration, label Master Calibrators 1 and 2 in duplicate. A single sample of all levels of total T4 controls must be tested as a means of evaluating the assay calibration.

Standard Calibration

The Standard Calibration procedure may be used without prior entry of the bar coded Master Curve information. To perform an AxSYM Total T4 Standard Calibration, label Standard Calibrators A, B, C, D, E, and F in duplicate. A single sample of all levels of total T4 controls must be tested as a means of evaluating the assay calibration. Each AxSYM Total T4 calibration is accepted and stored; all subsequent samples may be tested without further calibration unless:

- A reagent pack with a new lot number is used.
- Controls are out of range.

Refer to the AxSYM System Operations Manual, Section 6, for:

- Setting up an assay calibration
- When recalibration may be necessary
- Calibration verification

The AxSYM System verifies that the results of an assay calibration meet the specifications assigned to selected validity parameters. An error message occurs when the calibration fails to meet a specification. Refer to the AxSYM System Operations Manual, Section 10, for an explanation of the corrective actions for the error code. Refer to the AxSYM System Operations Manual, Appendices, for an explanation of the calibration validity parameters that may be used by the AxSYM System.

QUALITY CONTROL

The recommended control requirement for an AxSYM Total T4 Assay is a single sample of all total T4 control levels tested once every 24 hours each day of use. Controls may be placed in any position in the Sample Carousel.

If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow those procedures. Ensure that assay control values are within the concentration ranges specified in the package insert. Refer to the REAGENTS, CONTROLS section of this package insert for AxSYM Total T4 Control ranges.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and require rerunning. Assay recalibration may be indicated. Refer to the AxSYM System Operations Manual, Section 10, Subsection: Observed Problems, for further troubleshooting information.

The AxSYM System has a capability to generate a Linearity-jenkosky plot of each assay’s quality control performance. Refer to the AxSYM System Operations Manual, Section 5. All the discretion of the laboratory, selected quality control rules may be applied to the quality control data.

RESULTS

The results for each assay are expressed in µg/dL. When selecting the alternate result unit, nmol/L, the conversion factor used by the AxSYM System is 12.87.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the AxSYM System Operations Manual, Sections 1 and 2.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, the AxSYM Total T4 results should be used in conjunction with other data, e.g., symptoms, results of other thyroid tests, clinical impressions, etc.
- Performance of this assay has not been established with neonatal specimens.
- Serum from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.
- Serum containing anti-thyroxine antibodies cannot be measured accurately without interference.

EXPECTED VALUES

The suggested normal range for AxSYM Total T4 is 4.5-12.0 µg/dL. This range represents the Total T4 values obtained by testing serum specimens from 144 apparently healthy individuals. It is recommended that each laboratory establish its own normal range.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-T2. A three member serum based panel was assayed, using a single lot of reagents and a single calibration, in replicates of 2 at two separate times per day for twenty days. Data from this study are summarized in the following tables.

| PANEL MEMBER 1 | | PANEL MEMBER 2 | | PANEL MEMBER 3 |
|-----------------|-----------------|-----------------|-----------------|
|                  | Mean Conc. (µg/dL) | Within Run SD  | Between Run SD  |
|                  | Value           | (% CV)          | (% CV)          |
|                  |                 |                 |                 |
| 1                | 14.13           | 0.30            | 2.16            |
| 2                | 4.44            | 0.27            | 6.07            |
| 3                | 8.46            | 0.27            | 3.25            |
| 4                | 14.13           | 0.30            | 2.16            |

4.44 14.13 80 0.27 5.02 0.20 4.54 0.00 0.00 0.30 6.74
RECOVERY
Known concentrations of T4 were added to 4 human serum samples. The concentration of T4 was determined using the AxSYM Total T4 assay and the resulting percent recovery was calculated.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogenous Value Obtained (µg/dL)</th>
<th>T4 Added (µg/dL)</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.93</td>
<td>3.44</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>7.07</td>
<td>3.44</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>4.55</td>
<td>3.44</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>6.22</td>
<td>3.44</td>
<td>86</td>
</tr>
</tbody>
</table>

Average Percent Recovery: 95.3%

SENSITIVITY
The sensitivity of the AxSYM Total T4 assay was calculated to be 1.25 µg/dL (<0.5 units). The sensitivity is defined as the concentration at two standard deviations from the AxSYM Total T4 Calibrator A (0.0 µg/dL) and represents the lowest measurable concentration of T4 that can be distinguished from zero.

SPECIFICITY
The specificity of the AxSYM Total T4 assay was determined by studying interference in plasma volume in some collection tubes. A change in T4 values may be due to the volume increase and not interference in assays used to measure T4. Normal ranges for samples collected with potassium oxalate may be as much as 10% lower than serum or samples collected with other anticoagulants.

ACCURACY BY LINEAR REGRESSION
A study was performed where specimens were tested using the AxSYM Total T4 assay and the ARCHITECT Total T4 assay. Data from this study were analyzed using Least Squares and Passing-Bablok regression methods and are summarized in the following table.

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Specimens</th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Least Squares</td>
<td>278</td>
<td>0.94</td>
<td>0.92</td>
<td>0.945</td>
</tr>
<tr>
<td>Passing-Bablok</td>
<td>278</td>
<td>0.10</td>
<td>0.93</td>
<td>0.945</td>
</tr>
</tbody>
</table>

In this evaluation, specimens tested ranged from 1.49 to 23.23 µg/dL, with the AxSYM Total T4 assay and from 2.34 to 23.23 µg/dL, with the ARCHITECT Total T4 assay.

BIBLIOGRAPHY

AxSYM and ARCHITECT are registered trademarks of Abbott Laboratories.

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October, 2005