**Alinity i Total $T_4$-24**

**Prepared by:** Yusra Othman /Director/Supervisor-Chem **Date:** May/26/2024
signature/title

**Reviewed by:** Jordan Dillard /Instructor **Date:** June 26 2024
signature/title

**Approved by:** Sanford N. Bauch, M.D /Chairman **Date:** June 28 2024
signature/title

**BIENNIAL REVIEW:**

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# INTENDED USE

The Alinity i Total $T_4$ ($TT_4$) assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of thyroxine (Total $T_4$) in human serum and plasma on the Alinity i analyzer.

The Alinity i Total $T_4$ assay is to be used as an aid in the assessment of thyroid status.

# SUMMARY AND EXPLANATION OF THE TEST

Thyroxine ($T_4$) is an iodine-containing hormone which has a molecular weight of approximately 777 daltons and is secreted by the thyroid gland. $T_4$ and its associate thyroid hormone $T_3$ are responsible for regulating diverse biochemical processes throughout the body

which are essential for normal metabolic and neural activity.[1]

Although $T_3$ has greater biologic potency[2], $T_4$ is normally present in human serum in approximately 50-fold excess of circulating $T_3$ and accounts for more than 90% of the circulating protein-bound iodine. $T_4$ is 99.9% bound to serum thyroxine binding proteins (TBP). The hormone is transported bound primarily to thyroxine binding globulin (TBG) and secondarily by thyroxine binding prealbumin (TBPA) and albumin.[3] Less than 0.05% of the total circulating $T_4$ is unbound and therefore biologically active.[4, 5] Clinically, $T_4$ measurements have long been recognized as an aid in the assessment and diagnosis of thyroid status. Elevated $T_4$ values are characteristically seen in patients with overt hyperthyroidism, while $T_4$ levels are generally depressed in patients with overt hypothyroidism. Normal $T_4$ levels accompanied by high $T_3$ values are seen in patients with $T_3$- thyrotoxicosis.[6] $T_4$ levels are altered by physiological or pathological changes in TBP capacity.[3, 4] Thyroxine binding globulin (TBG) capacity has a pronounced effect on the concentration of thyroid hormones. Consequently, $T_4$ 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.[7, 8, 9] Conversely, when TBG levels are decreased, such as in nephrotic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness or congenital decrease of TBG, $T_4$ may be reduced.

Drugs which compete for protein binding sites, such as phenylbutazone, diphenylhydantoin or salicylates, can result in a depressed $T_4$ measurement.[7, 8, 9] Serum $T_4$ levels in neonates and infants are higher than values in the normal adult, due to the increased concentration of TBG in neonate serum.[10]

While in many cases $T_4$ values give good indications of thyroid status, $T_4$ 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.[11, 12]

To ensure maximum diagnostic accuracy, the final definition of thyroid status should be determined in conjunction with other thyroid function tests such as TSH, Free $T_4$, Total $T_3$, FTI and clinical evaluation by the physician.

# BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of thyroxine (Total $T_4$) in human serum and plasma using **chemiluminescent microparticle immunoassay (CMIA) technology.**

Sample and anti-$T_4$ coated paramagnetic microparticles are combined and incubated. Bound $T_4$ is removed from the binding sites on thyroxine binding globulin, prealbumin and albumin. The $T_4$ present in the sample binds to the anti-$T_4$ coated microparticles. The mixture is washed. $T_3$ acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of Total $T_4$ in the sample and the RLUs detected by the system optics.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

# REAGENTS

## Kit Contents

Alinity i Total $T_4$ Reagent Kit 07P95

Volumes (mL) listed in the table below indicate the volume per cartridge.

| REF | 07P9520 | 07P9530 |
|---|---|---|
| Tests per cartridge | 100 | 600 |
| Number of cartridges per kit | 2 | 2 |
| Tests per kit | 200 | 1200 |
| MICROPARTICLES | 6.6 mL | 32.1 mL |
| CONJUGATE | 6.1 mL | 31.6 mL |

MICROPARTICLES Anti-$T_4$ (sheep) coated microparticles in TRIS buffer with sheep IgG stabilizers. Minimum concentration: 0.05% solids. Preservative: sodium azide.

CONJUGATE $T_3$ acridinium-labeled conjugate in MES buffer with NaCl and Triton X-100 stabilizers. Minimum concentration: 0.2 ng/mL. Preservative: ProClin 300.

## Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

### Safety Precautions

**CAUTION:** This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[13], [14], [15], [16]

| The following warnings and precautions apply to: CONJUGATE | |
|---|---|
|  | |
| **WARNING** | Contains methylisothiazolones. |

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |
| **Prevention** | |
| P261 | Avoid breathing mist / vapors / spray. |
| P272 | Contaminated work clothing should not be allowed out of the workplace. |
| P280 | Wear protective gloves / protective clothing / eye protection. |
| **Response** | |
| P302+P352 | IF ON SKIN: Wash with plenty of water. |
| P333+P313 | If skin irritation or rash occurs: Get medical advice / attention. |
| P362+P364 | Take off contaminated clothing and wash it before reuse. |
| **Disposal** | |
| P501 | Dispose of contents / container in accordance with local regulations. |

| The following warnings and precautions apply to: MICROPARTICLES | |
|---|---|
| Contains sodium azide. | |
| EUH032 | Contact with acids liberates very toxic gas. |
| P501 | Dispose of contents / container in accordance with local regulations. |

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 8.**

**Reagent Handling**

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
    - Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.

- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

## Reagent Storage

| | Storage Temperature | Maximum Storage Time | Additional Storage Instructions |
|---|---|---|---|
| **Unopened** | 2 to 8°C | Until expiration date | Store in upright position.<br>If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use. |
| **Onboard** | System Temperature | 30 days | |
| **Opened** | 2 to 8°C | Until expiration date | Store in upright position.<br>If cartridge does not remain upright during storage, discard the cartridge.<br>Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance. |

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

## Indications of Reagent Deterioration

Deterioration of the reagents may be indicated when:

- a calibration error occurs
- a control value is out of the specified range

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**

# INSTRUMENT PROCEDURE

The Alinity i Total $T_4$ assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 2.**

For information on printing assay parameters, **refer to the Alinity ci-series Operations Manual, Section 5.**

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

## Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

| Default Result Unit | Conversion Factor | Alternate Result Unit |
|---|---|---|
| **µg/dL** | 12.87 | nmol/L |

# SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

## Specimen Types

The specimen types listed below were verified for use with this assay.

| Specimen Types | Collection Tubes |
|---|---|
| Serum | Serum |
| | Serum separator |
| Plasma | Sodium heparin |
| | Lithium heparin |
| | Lithium heparin plasma separator |
| | Potassium EDTA |

## Specimen Conditions

- Do not use:
  - heat-inactivated specimens
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

## Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

### Specimen Storage

| Specimen Type | Temperature | Maximum Storage Time | Special Instructions |
|---|---|---|---|
| Serum/Plasma | 2 to 8°C | 6 days | If testing will be delayed more than 6 days, specimens should be frozen at -10°C or colder. |

If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.

Specimens stored frozen at -10°C or colder for 6 days showed no performance difference.

Avoid multiple freeze/thaw cycles.

### Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

## PROCEDURE

### Materials Provided

07P95 Alinity i Total $T_4$ Reagent Kit

### Materials Required but not Provided

- Alinity i Total $T_4$ assay file
- 07P9501 Alinity i Total $T_4$ Calibrators
- 07P9510 Alinity i Total $T_4$ Controls or other control material
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, **refer to the Alinity ci-series Operations Manual, Section 1.**

For information on materials required for maintenance procedures, **refer to the Alinity ci-series Operations Manual, Section 9.**

### Assay Procedure

For a detailed description of how to run an assay, **refer to the Alinity ci-series Operations Manual, Section 5.**

- If using primary or aliquot tubes, **refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.**
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

Maximum number of replicates sampled from the same sample cup: 10

- Priority:
    - Sample volume for first test: 74 µL
    - Sample volume for each additional test from same sample cup: 24 µL
- ≤ 3 hours on the reagent and sample manager:
    - Sample volume for first test: 150 µL
    - Sample volume for each additional test from same sample cup: 24 µL
- > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity i Total $T_4$ calibrator package insert and/or Alinity i Total $T_4$ control package insert for preparation and usage.
- For general operating procedures**, refer to the Alinity ci-series Operations Manual, Section 5.**
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, **Section 9. Perform maintenance more frequently when required by laboratory procedures.**

## Sample Dilution Procedures

Samples with a Total $T_4$ value exceeding **24.00 µg/dL** (308.88 nmol/L) are flagged with the code "> 24.00 µg/dL" (">308.88 nmol/L") and may be diluted with the Manual Dilution Procedure.

**Manual Dilution Procedure**

Suggested dilution: **1:2**

It is recommended that dilutions not exceed 1:2.

Add 75 µL of the sample to 75 µL of Alinity i Total $T_4$ Calibrator A.

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 3.00 µg/dL (38.61 nmol/L), rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

## Calibration

For instructions on performing a calibration, **refer to the Alinity ci-series Operations Manual, Section 5.**

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.

Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance, as described in the Quality Control Procedures section of this package insert.

- **If statistically-based quality control limits are not available**, then the calibration should not exceed **a 30-day** limit for recalibration frequency.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

## Quality Control Procedures

The recommended control requirement for the Alinity i Total $T_4$ assay is that a single sample of each control level be tested once every day testing performed.

To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying **a minimum of 20 replicates over several (3-5) days** and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.[17]

- If quality control results do not meet the acceptance criteria defined by laboratory QC procedure, sample results may be suspect. Follow the established quality control

procedures to troubleshoot. Recalibration may be necessary. For troubleshooting information, **refer to the Alinity ci-series Operations Manual, Section 10.**

- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

### Quality Control Guidance

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[18]

### Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

# RESULTS

## Calculation

The Alinity i Total $T_4$ assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

## 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 Alinity ci-series Operations Manual, Section 5.

## Measuring Interval

Measuring interval is defined as the range of values in µg/dL (nmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i Total $T_4$ assay is **3.00 to 24.00 µg/dL** (38.61 to 308.88 nmol/L).

# LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other thyroid tests, clinical impressions, etc.
- If the Total $T_4$ results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Performance of this test has not been established with neonatal specimens.

# EXPECTED VALUES

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Manufacturers provided reference range adopted, effort made to verify.

A normal range of **4.87 to 11.72 µg/dL** (central 95% interval) was obtained by testing serum specimens from 437 individuals determined as normal by AxSYM Ultrasensitive hTSH II and AxSYM Free $T_4$ assays.

# SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilized the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

## Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2. Testing was conducted using 1 lot of the Alinity i Total $T_4$ Reagent Kit, 1 lot of the Alinity i Total $T_4$ Calibrators, and 1 lot of the Alinity i Total $T_4$ Controls and 1 instrument. Three human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[19]

| | | | Within-Run (Repeatability) | | Within-Laboratory (Total)[a] | |
|---|---|---|---|---|---|---|
| **Panel** | **n** | **Mean (µg/dL)** | **SD** | **%CV** | **SD** | **%CV** |
| 1 | 120 | 4.79 | 0.099 | 2.1 | 0.200 | 4.2 |
| 2 | 120 | 8.50 | 0.161 | 1.9 | 0.378 | 4.4 |
| 3 | 120 | 16.87 | 0.483 | 2.9 | 0.604 | 3.6 |

[a] Includes within-run, between-run, and between-day variability.

| | | | Within-Run (Repeatability) | | Within-Laboratory (Total)[a] | |
|---|---|---|---|---|---|---|
| **Panel** | **n** | **Mean (nmol/L)** | **SD** | **%CV** | **SD** | **%CV** |
| 1 | 120 | 61.71 | 1.275 | 2.1 | 2.573 | 4.2 |
| 2 | 120 | 109.37 | 2.070 | 1.9 | 4.866 | 4.4 |

| Panel | n | Mean (nmol/L) | Within-Run (Repeatability) | | Within-Laboratory (Total)[a] | |
|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV |
| 3 | 120 | 217.17 | 6.218 | 2.9 | 7.771 | 3.6 |

[a] Includes within-run, between-run, and between-day variability.

## Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2. Testing was conducted using 3 lots of the Alinity i Total $T_4$ Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.*20*

| | µg/dL | nmol/L |
|---|---|---|
| LoB[a] | 0.28 | 3.60 |
| LoD[b] | 0.55 | 7.08 |
| LoQ[c] | **2.17** | 27.93 |

[a]The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b]The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 10 %CV was met.

## Linearity

A study was performed based on guidance from CLSI EP06-A.*21*

This assay is linear across the measuring interval of **3.00 to 24.00 µg/dL** (38.61 to 308.88 nmol/L).

## Analytical Specificity

This study was performed on the ARCHITECT i System.

Analytical specificity was evaluated with the ARCHITECT Total $T_4$ assay and determined to have a mean analytical specificity of ≤ 3.2% cross reactivity with triiodothyronine ($T_3$) at a concentration of 100 µg/dL in a sample containing approximately 3 µg/dL of Total $T_4$ as confirmed by a study based on guidance from CLSI document EP7-A.*22*

## Interference

This study was performed on the ARCHITECT i System.

A study was performed based on guidance from CLSI document EP7-A. Potential interference was assessed with the ARCHITECT Total $T_4$ assay and the mean interference was determined to be < 10% for hemoglobin, bilirubin, triglycerides, and protein at the levels

indicated below.[22]

| Potentially Interfering Substance | Interferent Level |
|---|---|
| Hemoglobin | ≤ 500 mg/dL |
| Bilirubin | ≤ 20 mg/dL |
| Triglycerides | ≤ 3000 mg/dL |
| Protein | ≥ 4.5 and ≤ 12 g/dL |

### Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[23]

| | | Units | n | Correlation Coefficient | Intercept | Slope | Concentration Range |
|---|---|---|---|---|---|---|---|
| Alinity i Total $T_4$ vs ARCHITECT Total $T_4$ | Serum | µg/dL (nmol/L) | 112 | 1.00 | -0.01 (-0.10 ) | 0.98 | 3.16-21.24 (40.61-273.30) |

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