Alinity i Free T_4-13

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i Free T_4 assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of free thyroxine (free T_4) in human serum and plasma on the Alinity i analyzer. The Alinity i Free 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) circulates in the blood as an equilibrium mixture of free and serum protein bound hormone. Thyroxine binding globulin (TBG), albumin and pre-albumin bind approximately 75%, 10% and 15% of the total circulating T_4 respectively.[1], [2], [3] The binding of T_4 by these proteins is such that less than 0.03% is present in the circulation as unbound, free T_4.[4] This small percentage of the total T_4 represents the physiologically available

hormone which is biologically active. Once the free T_4 is absorbed by the target cells, the equilibrium reestablishes circulating free T_4 levels. The equilibrium results in the maintenance of a constant level of free T_4 when alterations occur in either the concentration or affinity of the serum binding proteins. Therefore, in a variety of normal (pregnancy)[4] and abnormal (Familial Dysalbuminemic Hyperthyroxinemia, FDH)[5], [6], [7] states, or as a result of the administration of certain drugs (e.g., furosemide[8], [9] and fenclofenac[10], [11], [12]), the target tissues are assured of receiving the required amount of hormone. Free T_4 values may, therefore, provide the best indication of thyroid dysfunction, since free T_4 is less sensitive to changes in the serum binding proteins.

Historically, the diagnosis of thyroid function has involved performing a total T_4 assay[13], [14] in addition[15] to a Thyroxine Uptake (TU) assay of the same sample. The mathematical combination of these two assays produces a Free Thyroxine Index (FTI) which provides an indirect proportional estimate for free T_4.[16]

Alternatively, direct assays have been developed using equilibrium dialysis,[17], [18] ultrafiltration,[19], [20] RIA,[21] and solid-phase EIA technology[22] to measure free T_4. In these methods, separation of free and bound tracer is achieved either with a membrane, or by binding free T_4 to a solid phase antibody. This extraction step removes an amount of T_4 which is proportional to the original amount of free T_4 present in the patient sample. Provided that the extracted T_4 is less than approximately 5% of the T_4 in the sample, a true estimation of the free T_4 content can be obtained.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a two-step immunoassay for the quantitative determination of free thyroxine (free 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. The free 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 free 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 Free T_4 Reagent Kit 07P70

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

REF	07P7020	07P7030

REF	07P7020	07P7030
Tests per cartridge	100	600
Number of cartridges per kit	2	2
Tests per kit	200	1200
MICROPARTICLES	5.4 mL	24.8 mL
CONJUGATE	4.9 mL	24.3 mL

MICROPARTICLES anti-T_4 (sheep) coated microparticles in TRIS buffer with sheep IgG stabilizer. 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.

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.[23], [24], [25], [26]

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. 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. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the 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 Free 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
ng/dL	12.87	pmol/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

- When serial specimens are being evaluated, the same type of specimen should be used throughout the study.
- Liquid anticoagulants may have a dilution effect resulting in lower concentration values for individual specimens.

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. Ensure centrifugation is adequate.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- 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 gently inverting.
- 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	Specimens may be stored on or off the clot, red blood cells, or separator gel. If testing will be delayed more than 24 hours, remove serum or plasma from the clot, red blood cells, or separator gel. If testing will be delayed more than 6 days, specimens should be frozen at -10°C or colder. Follow the manufacturer's processing instructions for serum or plasma collection tubes if a removal time of less than 24 hours is specified.

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

07P70 Alinity i Free T_4 Reagent Kit

Materials Required but not Provided

- Alinity i Free T_4 assay file
- 07P7001 Alinity i Free T_4 Calibrators
- 07P7010 Alinity i Free 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: 84 µL
- Sample volume for each additional test from same sample cup: 34 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: 34 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i Free T_4 calibrator package insert and Alinity i Free 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 cannot be diluted for the Alinity i Free T_4 assay. Samples with a free T_4 concentration of > 5.00 ng/dL (> 64.35 pmol/L) are flagged as "> 5.00 ng/dL (> 64.35 pmol/L)" and need to be reported as such.

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 Free T_4 assay is that a single sample of each control level be tested once every day testing performed.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

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.[27]

- 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.[28]

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 Free 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 ng/dL (pmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i Free T_4 assay **is 0.42 to 5.00 ng/dL** (5.41 to 64.35 pmol/L).

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other thyroid tests, clinical impressions, etc.
- If the free T_4 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

EXPECTED VALUES

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

This study was performed on the ARCHITECT i System.

A normal range of **0.70 to 1.48 ng/dL** (9.01 to 19.05 pmol/L) (central 99% interval) was obtained by testing serum specimens from 411 individuals determined as normal by AxSYM Ultrasensitive hTSH II and AxSYM Free T_4 assays.

The manufacturers provided reference range adopted, effort made to verify locally.

Caliper (https://caliperdatabase.org) Suggestive pediatrics reference ranges:

Reference Intervals (Female and Male)

Age	Lower Limit	Upper Limit	Sample Size	Lower Confidence Intervals	Higher Confidence Intervals
0 to <1 Year	0.81	1.46	56	(0.78, 0.84)	(1.37, 1.56)
1 to <19 Years	0.77	1.11	156	(0.76, 0.80)	(1.09, 1.16)

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 Free T_4 Reagent Kit, 1 lot of the Alinity i Free T_4 Calibrators, and 1 lot of the Alinity i Free 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.[29]

Panel Member	n	Mean (ng/dL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
1	120	0.66	0.015	2.2	0.017	2.6
2	121	1.16	0.019	1.7	0.023	2.0
3	118	2.61	0.078	3.0	0.080	3.1

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

Panel Member	n	Mean (pmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
1	120	8.50	0.186	2.2	0.218	2.6
2	121	14.92	0.252	1.7	0.296	2.0
3	118	33.57	1.003	3.0	1.029	3.1

[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 Free 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.[30]

	ng/dL	pmol/L
LoB[a]	0.22	2.83
LoD[b]	0.28	3.60
LoQ[c]	**0.42**	5.41

[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.[31]

This assay is linear across the measuring interval of **0.42 to 5.00 ng/dL** (5.41 to 64.35 pmol/L).

Analytical Specificity

This study was performed on the ARCHITECT i System.

The Free T_4 assay is designed to have a mean analytical specificity of ≤ 0.0035% cross reactivity with triiodothyronine (T_3) at a concentration of 12 000 ng/dL in a sample containing 0.5 ng/dL of free T_4.

Interference

This study was performed on the ARCHITECT i System.

The Free T_4 assay is designed to have a mean potential interference from hemoglobin, bilirubin, triglycerides, and protein of < 10% at the levels indicated below.

Potentially Interfering Substance	Interferent Level
Hemoglobin	≤ 500 mg/dL
Bilirubin	≤ 20 mg/dL
Triglycerides	≤ 3000 mg/dL
Protein	≤ 12 g/dL

Method Comparison

A study was performed based on guidance from CLSI document EP09-A3 using the Passing-Bablok regression method.[32]

Assay	Sample Type	Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i Free T_4 vs ARCHITECT Free T_4	Serum	ng/dL	116	0.99	-0.06	1.01	0.92-4.34
	Serum	pmol/L	116	0.99	-0.81	1.01	11.78-55.79

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