**Alinity i TSH-26**

**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:** Stanford W. Bandy, M.D /Chairman **Date:** June 28 2024
signature/title

**BIENNIAL REVIEW:**

| | | |
|---|---|---|
| **REVIEWED** | signature/title | Date |
| **REVIEWED** | signature/title | Date |
| **REVIEWED** | signature/title | Date |
| **REVIEWED** | signature/title | Date |
| **REVIEWED** | signature/title | Date |
| **REVIEWED** | signature/title | Date |

| | | |
|---|---|---|
| **REVISED** | signature/title | Date/Page/Paragraph |
| **REVISED** | signature/title | Date/Page/Paragraph |
| **REVISED** | signature/title | Date/Page/Paragraph |
| **REVISED** | signature/title | Date/Page/Paragraph |
| **REVISED** | signature/title | Date/Page/Paragraph |

**SUPERSEDES: Procedure titled** _____

# INTENDED USE

The Alinity i TSH assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of human Thyroid Stimulating Hormone (TSH) in human serum and plasma on the Alinity i analyzer.

# SUMMARY AND EXPLANATION OF THE TEST

Human Thyroid Stimulating Hormone (TSH) or thyrotropin is a glycoprotein with a molecular weight of approximately 28 000 daltons, synthesized by the basophilic cells (thyrotropes) of the anterior pituitary.[1] TSH is composed of two non-covalently linked

subunits designated alpha and beta. Although the alpha subunit of TSH is common to the luteinizing hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG), the beta subunits of these glycoproteins are hormone specific and confer biological as well as immunological specificity. Both alpha and beta subunits are required for biological activity.[1] TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine ($T_4$) and triiodothyronine ($T_3$), by interacting with a specific receptor on the thyroid cell surface.[2] $T_3$ and $T_4$ are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development and metabolic and neural activity.

The synthesis and secretion of TSH is stimulated by thyrotropin releasing hormone (TRH), the hypothalamic tripeptide, in response to low levels of circulating thyroid hormones.[3], [4] Elevated levels of $T_3$ and $T_4$ suppress the production of TSH via a classic negative feedback mechanism. Other evidence also indicates that somatostatin and dopamine exert inhibitory control over TSH release, suggesting that the hypothalamus may provide both inhibitory and stimulatory influence on pituitary TSH production.[5] Failure at any level of regulation of the hypothalamic-pituitary-thyroid axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of $T_4$ and/or $T_3$.

In cases of primary hypothyroidism, $T_3$ and $T_4$ levels are low and TSH levels are significantly elevated.[6] In the case of pituitary dysfunction, either due to intrinsic hypothalamic or pituitary disease; i.e., central hypothyroidism, normal or marginally elevated basal TSH levels are often seen despite significant reduction in $T_4$ and/or $T_3$ levels. These inappropriate TSH values are due to a reduction in TSH bioactivity which is frequently observed in such cases. Routine TRH stimulation is advised to confirm the diagnosis in such cases. Secondary hypothyroidism typically results in an impaired TSH response to TRH, while in tertiary hypothyroidism the TSH response to TRH may be normal, prolonged or exaggerated.[7], [8], [9]

Primary hyperthyroidism (e.g., Grave's Disease, nodular goiter) is associated with high levels of thyroid hormones and depressed or undetectable levels of TSH.[10] The TRH stimulation test has been used in diagnosis of hyperthyroidism. Hyperthyroid patients show a subnormal response to the TRH test.[11] In addition, large doses of glucocorticoids, somatostatin, dopamine and replacement doses of thyroid hormones reduce or totally blunt the TSH response to TRH.[11], [12]

Earlier assays for serum TSH lacked the sensitivity to be used as a primary test of thyroid function.[13] Sensitive TSH assays now available, with increased ability to clearly distinguish between euthyroid and hyperthyroid populations, are changing thyroid function testing. Analytical sensitivity, as a means of assessing low concentration accuracy, is being replaced by functional sensitivity.[14] The American Thyroid Association has formally recommended the use of functional sensitivity as the means to quantify the sensitivity of TSH assays,[15] although analytical sensitivity is still widely used. Third generation TSH assays exhibit 20% interassay CVs at < 0.02 µIU/mL and are useful in the discrimination of patients with true hyperthyroidism from those with TSH suppression seen in subclinical hyperthyroidism and some non-thyroidal illnesses.[16] Other thyroid tests (Free $T_4$ estimate, Total $T_4$, T-Uptake, and Total $T_3$) combined with the ability to accurately measure low levels of TSH, improve the efficiency of thyroid diagnosis.[17]

The Alinity i TSH assay is to be used as an aid in the assessment of thyroid status, diagnosis of thyroid disease, and treatment of thyroid disease.

# BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is an automated two-step immunoassay for the quantitative determination of human Thyroid Stimulating Hormone (TSH) in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-β TSH antibody coated paramagnetic microparticles, and TSH assay diluent are combined and incubated. The TSH present in the sample binds to the anti-TSH antibody coated microparticles. The mixture is washed. Anti-α TSH 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 a direct relationship between the amount of TSH 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 TSH Reagent Kit 07P48

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

| REF | **07P4820** | **07P4830** |
|---|---|---|
| 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 |
| ASSAY DILUENT | 6.6 mL | 33.6 mL |

MICROPARTICLES Anti-β TSH (mouse, monoclonal) coated microparticles in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.07% solids. Preservatives: antimicrobial agents.

CONJUGATE Anti-α TSH (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizers. Minimum concentration: 60 ng/mL. Preservative: antimicrobial agent.

ASSAY DILUENT TRIS buffer. Preservatives: antimicrobial agents.

## 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 and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.[18, 19, 20, 21]

| The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE | |
|---|---|
|  | |
| Contains polyethylene glycol octylphenyl ether. | |
| H401* | Toxic to aquatic life. |
| H411 | Toxic to aquatic life with long lasting effects. |
| **Prevention** | |
| P273 | Avoid release to the environment. |
| **Response** | |
| P391 | Collect spillage. |
| **Disposal** | |
| P501 | Dispose of contents / container in accordance with local regulations. |

* Not applicable where regulation EC 1272/2008 (CLP) has been implemented.

| The following warnings and precautions apply to: ASSAY DILUENT* | |
|---|---|
| **WARNING** | |
| H316 | Causes mild skin irritation. |
| P332+P313 | If skin irritation occurs: Get medical advice / attention. |

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

Follow local chemical disposal regulations based on your location along with recommendations and content in the Safety Data Sheet to determine the safe disposal of this product.

For the most current hazard information, see the product Safety Data Sheet.

Safety Data Sheets are available at www.corelaboratory.abbott 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 TSH 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 |
|---|---|---|
| **µIU/mL** | 1 | mIU/L |

# SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

### Specimen Types

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

Other specimen types and collection tube types have not been verified with this assay.

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

## Specimen Conditions

- 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 specimens are centrifuged before a complete clot forms, the presence of fibrin or particulate matter may cause erroneous results. Centrifuge specimens containing fibrin, red blood cells, or particulate matter. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
- If proper specimen collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter. Aliquots poured versus pipetted from specimen tube types that do not include serum separators are at higher risk of including particulates and generating depressed results.
- Failure to follow these instructions may result in depressed specimen 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.
- Insufficient processing of sample, or disruption of the sample during transportation may cause depressed results.

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 | 7 days | If testing will be delayed more than 7 days, specimens should be stored frozen at -10°C or colder. |
| | -10°C or colder | 6 months | Specimens stored frozen at -10°C or colder for 6 months showed no performance difference. |

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

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.

Do not exceed the storage limitations listed above.

## PROCEDURE

### Materials Provided

07P48 Alinity i TSH Reagent Kit

### Materials Required but not Provided

- Alinity i TSH assay file
- 07P4801 Alinity i TSH Calibrators
- 07P4810 Alinity i TSH Controls or other commercially available controls
- 09P1540 Alinity i Multi-Assay Manual Diluent

- 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: 9

Priority:

- Sample volume for first test: 163 µL
- Sample volume for each additional test from same sample cup: 113 µL

≤ 3 hours on the reagent and sample manager:

- Sample volume for first test: 163 µL
- Sample volume for each additional test from same sample cup: 113 µL

> 3 hours on the reagent and sample manager:

- Replace with a fresh aliquot of sample.

- Refer to the Alinity i TSH calibrator package insert and/or Alinity i TSH 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 TSH value exceeding 100.0000 µIU/mL (100.0000 mIU/L) are flagged with the code ">100.0000 µIU/mL" (> 100.0000 mIU/L) and may be diluted with either the Automated Dilution Protocol or the Manual Dilution Procedure.

### Automated Dilution Protocol

The system performs a **1:5** dilution of the sample and automatically calculates the concentration by multiplying the result by the dilution factor.

**Manual Dilution Procedure**

Suggested dilution: **1:10**

It is recommended that dilutions not exceed 1:10.

Add 30 µL of the sample to 270 µL of Alinity i Multi-Assay Manual Diluent.

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 0.0083 µIU/mL (0.0083 mIU/L), do not report the result. 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 TSH 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.[22]

- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- 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.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

**Quality Control Guidance**

Refer to "Basic QC Practices" by James O Westgard, Ph.D. for guidance on laboratory quality control practices.[23]

**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 TSH 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.

## Reportable Interval

Based on representative data for the limit of quantitation (LoQ) and the limit of detection (LoD), the ranges over which results can be reported are provided below according to the definitions of CLSI EP34.[24]

| | **µIU/mL (mIU/L)** |
|---|---|
| Analytical Measuring Interval (AMI)[a] | 0.0083 – 100.0000 |
| Extended Measuring Interval (EMI)[b] | 100.0000 – 1000.0000 |
| Reportable Interval[c] | **0.0036 – 1000.0000** |

[a] AMI: The AMI extends from the LoQ to the upper limit of quantitation (ULoQ). This is determined by the range of values in µIU/mL (mIU/L) that demonstrated acceptable performance for linearity, imprecision, and bias.

[b] EMI: The EMI extends from the ULoQ to the ULoQ x dilution factor. The value reflects a 1:10 manual dilution factor.

[c] The reportable interval extends from the LoD to the upper limit of the EMI.

## LIMITATIONS OF THE PROCEDURE

- Specimens MUST be processed according to the specimen test tube manufacturer's instruction. Insufficient processing including deviations from recommended clotting times, centrifugation times, centrifugation speed and sample preparation techniques may cause inaccurate results.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the Alinity i TSH results are inconsistent with clinical evidence, additional testing is recommended to confirm the result.
- Suspected hyperthyroidism based on low or undetectable TSH levels should be confirmed with additional thyroid function testing along with other clinical information.
- Specimens 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 either falsely elevated or depressed values when tested with assay kits such as Alinity i TSH that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.*25*, *26*
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.*27*

# 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.

The manufacturer provided refrence range will be adopted and effort made to verify.

A normal range of **0.35 µIU/mL to 4.94 µIU/mL (99% confidence interval)** was obtained by testing serum specimens from 549 individuals defined as normal by the 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 utilize 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 TSH Reagent Kit, 1 lot of the Alinity i TSH Calibrators, and 1 lot of the Alinity i TSH Controls and 1 instrument. Three buffer based panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.*28*

| | | Mean µIU/mL | Within-Run (Repeatability) | | Within-Laboratory (Total)[a] | |
|---|---|---|---|---|---|---|
| **Sample** | **n** | **(mIU/L)** | **SD** | **%CV** | **SD** | **%CV** |
| Panel 1 | 119 | 0.0949 | 0.00141 | 1.5 | 0.00187 | 2.0 |
| Panel 2 | 120 | 6.1453 | 0.07995 | 1.3 | 0.08970 | 1.5 |
| Panel 3 | 119 | 30.2624 | 0.47546 | 1.6 | 0.62563 | 2.1 |

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

Reproducibility

A study was performed to estimate within-laboratory precision and reproducibility. Testing was conducted using 1 lot of the Alinity i TSH Reagents, a minimum of 1 lot of the Alinity i TSH Calibrators, 1 lot of the Alinity i TSH Controls, and 3 instruments. Three levels of Alinity i TSH Controls (low, medium, and high) were assayed in a minimum of 3 replicates (from separate sample cups), 2 times per day (separated by a minimum of two hours), on at least 5 different days, for a minimum of 30 required measurements per instrument. The performance is shown in the following table.

| | | Mean µIU/mL | Repeatability | | Within-Laboratory[a] | | Reproducibility[b] | |
|---|---|---|---|---|---|---|---|---|
| **Sample** | **n** | **(mIU/L)** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| Panel 1 | 120 | 0.1023 | 0.00155 | 1.5 | 0.00170 | 1.7 | 0.00201 | 2.0 |

| Sample | n | Mean µIU/mL (mIU/L) | Repeatability SD | Repeatability %CV | Within-Laboratory[a] SD | Within-Laboratory[a] %CV | Reproducibility[b] SD | Reproducibility[b] %CV |
|---|---|---|---|---|---|---|---|---|
| Panel 2 | 119 | 5.7049 | 0.08793 | 1.5 | 0.10324 | 1.8 | 0.11247 | 2.0 |
| Panel 3 | 119 | 28.6698 | 0.62608 | 2.2 | 0.76105 | 2.7 | 0.85730 | 3.0 |

[a]Within-Laboratory variability contains repeatability (within-run), between-run, and between-day variance components.

[b]Reproducibility contains repeatability (within-run), between-run, between-day, and between-instrument variance components.

### 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 TSH 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.*29*

| | µIU/mL | mIU/L |
|---|---|---|
| LoB[a] | 0.0026 | 0.0026 |
| LoD[b] | 0.0036 | 0.0036 |
| LoQ[c] | **0.0083** | 0.0083 |

[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 20 %CV was met.

### Linearity

A study was performed based on guidance from CLSI EP06-A.*30*

This assay is linear across the measuring interval **of 0.0083 to 100.0000** µIU/mL (0.0083 to 100.0000 mIU/L).

### Analytical Specificity

This study was performed on the ARCHITECT i System.

The ARCHITECT TSH assay had an analytical specificity of < 10% cross reactivity with the following substances, at the concentration levels listed, in human serum samples containing TSH in the normal range.

| | |
|---|---|
| FSH | ≤ 500 mIU/mL |
| LH | ≤ 500 mIU/mL |

| | |
|---|---|
| Hcg | ≤ 200 000 mIU/mL |

### Interference

This study was performed on the ARCHITECT i System.

The ARCHITECT TSH assay had a potential interference from hemoglobin, bilirubin, triglycerides, and protein of ≤ 10% at the levels indicated below.

| | |
|---|---|
| Hemoglobin | ≤ 500 mg/dL |
| Bilirubin | ≤ 20 mg/dL |
| Triglycerides | ≤ 3000 mg/dL |
| Protein | ≤ 12 g/dL |

Note: As the Alinity i TSH assay does not utilize a biotinylated antibody complex, there is no risk of potential interference to values reported by the assay when analyzing samples containing Biotin.

### Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method.[31]

| | | Units | n | Correlation Coefficient | Intercept | Slope | Concentration Range |
|---|---|---|---|---|---|---|---|
| Alinity i TSH vs ARCHITECT TSH | Serum | µIU/mL (mIU/L) | 114 | 0.99 | 0.30 | 0.94 | 0.0172 - 97.7306 |

# BIBLIOGRAPHY


1. Pierce JG. The subunits of pituitary thyrotropin. Their relationship to other glycoprotein hormones. *Endocrinology* 1971; 89:1331-1344.
2. Rees Smith B, Pyle GA, Petersen VB, et al. Interaction of thyrotropin with the human thyrotropin receptor. *J Endocrinol* 1977;75:391-400.
3. Sterling K, Lazarus JH. The thyroid and its control. *Annu Rev Physiol* 1977;39:349-371.
4. Patel YC, Alford FP, Burger HG. The 24-hour plasma thyrotropin profile. *Clin Sci* 1972;43:71-77.
5. Morley JE. Neuroendocrine control of thyrotropin secretion. *Endocr Rev* 1981;2:396-436.
6. Burger HG, Patel YC. The value of serum thyrotropin measurement in the diagnosis and management of hypothyroidism. *Med J Aust* 1972;2:293-297.
7. Petersen VB, McGregor AM, et al. The secretion of thyrotropin with impaired biological activity in patients with hypothalimic-pituitary disease. *Clin Endocrinol* 1978;8:397-402.

8. Faglia G, Bitensky L, Pinchera A, et al. Thyrotropin secretion in patients with central hypothyroidism: evidence for reduced biological activity of immunoreactive thyrotropin. *J Clin Endocrinol Metab* 1979;48:989-998.
9. Beck-Peccoz P, Amr S, Menezes-Ferreira MM, et al. Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. *N Engl J Med* 1985;312:1085-1090.
10. Wehmann RE, Rubenstein HA, Pugeat MM, et al. Extended clinical uility of a sensitive and reliable radioimmunoassay of thyroid-stimulating hormone. *South Med J* 1983;76:969-976.
11. Lauridsen UB, Deckert T, Friis TH, et al. Estimation of serum thyrotropin (TSH) and stimulation with thyrotropin-releasing hormone (TRH) in thyroid diseases. *Acta Med Scand* 1974;196:171-176.
12. Jackson IMD. Thyrotropin-releasing hormone. *N Engl J Med* 1982;306:145-155.
13. Spencer CA. Clinical uses and limitations of rapid TSH assays. *Medical Laboratory Products* 1988;17-19.
14. Bayer MF. Performance criteria for appropriate characterization of "(highly) sensitive" thyrotropin assays. *Clin Chem* 1987;33:630-631.
15. Hay ID, Bayer MF, Kaplan MM, et al. American Thyroid Association assessment of current free thyroid hormone and thyrotropin measurements and guidelines for future clinical assays. *Clin Chem* 1991;37:2002-2008.
16. The National Academy of Clinical Biochemistry: Standards of Laboratory Practice. *Laboratory Support for the Diagnosis & Monitoring of Thyroid Disease*. NACB, 1996.
17. Hay ID, Klee GG. Linking medical needs and performance goals: clinical and laboratory perspectives on thyroid disease. *Clin Chem* 1993;39:1519-1524.
18. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
19. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
20. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
21. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
22. Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition.* CLSI Document C24-A3. Wayne, PA: CLSI; 2006.
23. Westgard JO. *Basic QC Practices.* 3rd ed. Madison, WI: Westgard Quality Corporation; 2010.

24. Clinical and Laboratory Standards Institute (CLSI). *Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking.* 1st ed. CLSI Guideline EP34. Wayne, PA: CLSI; 2018.

25. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.

26. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.

27. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.

28. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.

29. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition.* CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.

30. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.

31. Clinical and Laboratory Standards Institute (CLSI). *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition.* CLSI Document EP09-A3. Wayne, PA: CLSI; 2013.