| **TSH** |
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| **Purpose** | This procedure provides instructions for performing TSH testing on ABBOTT INSTRUMENTATION. The Alinity i and Architect TSH assays are 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 and Architect i1000SR analyzers. |
| **Policy Statements** | This procedure applies to all personnel responsible for operating Alinity i or Architect i1000SR at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a 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. |
| **Clinical Significance** | 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. 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. TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by interacting with a specific receptor on the thyroid cell surface. T3 and T4 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. Elevated levels of T3 and T4 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. Failure at any level of regulation of the hypothalamic-pituitary-thyroid axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T4 and/or T3.In cases of primary hypothyroidism, T3 and T4 levels are low and TSH levels are significantly elevated. 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 T4 and/or T3 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.Primary hyperthyroidism (e.g., Grave’s Disease, nodular goiter) is associated with high levels of thyroid hormones and depressed or undetectable levels of TSH. The TRH stimulation test has been used in diagnosis of hyperthyroidism. Hyperthyroid patients show a subnormal response to the TRH test. In addition, large doses of glucocorticoids, somatostatin, dopamine and replacement doses of thyroid hormones reduce or totally blunt the TSH response to TRH.Earlier assays for serum TSH lacked the sensitivity to be used as a primary test of thyroid function. 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. The American Thyroid Association has formally recommended the use of functional sensitivity as the means to quantify the sensitivity of TSH assays 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. Other thyroid tests (Free T4 estimate, Total T4, T-Uptake, and Total T3) combined with the ability to accurately measure low levels of TSH, improve the efficiency of thyroid diagnosis.The Alinity i and Architect TSH assays are to be used as an aid in the assessment of thyroid status, diagnosis of thyroid disease, and treatment of thyroid disease. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACI)****Backup: Architect i1000 (Sunquest method code: AI1)** |
| **Sunquest Test Codes** | **TSH** |
| **Specimen** | Sample: Plasma or Serum (with or without gel barrier)**Preferred:** Lithium Heparin**Alternative:** SST, Sodium Heparin, EDTA**Minimum sample volume:** 0.75 mL blood, 0.25 mL serum/plasma**Run the sample in the priority lane on Alinity ci RSM.**Priority loaded: Sample volume for first test: 163 μL Sample volume for each additional test from same sample cup: 113 μLRoutinely loaded: Sample volume for first test: 163 μL Sample volume for each additional test from same sample cup: 113 μL**Stability when separated from cells/gel:** **20 to 25°C:** not specified. Place in refrigerated storage within 8 hours.**2 to 8°C:** 7 days**-20°C:** 6 months**NOTE:** If testing will be delayed more than 24 hours, remove serum orplasma from the clot, red blood cells, or separator gel.**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**Preparation:** 1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Specimens should be free of particulate matter.
4. Transfer serum or plasma directly to a properly labeled pilot tube.
5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required.
6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Reagents** | **Reagent Handling** **Alinity c** 1. 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.
2. Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
3. After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
4. 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.
5. 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

**Architect i1000:**• Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing: • Invert the microparticle bottle 30 times.• Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.• If the microparticles do not resuspend, DO NOT USE • Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.• To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.• Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.• Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.\* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded. * Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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|  | **Both analyzers:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i TSH Reagent Kit | 07P4820 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**On-board**: 30 days |
| Alinity i TSH Calibrator Kit | 07P4801 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**Opened expiration:** Until expiration date*(Store tightly capped with new replacement cap.**Return to refrigerated storage after use.)* |
| Architect TSH Reagent Kit | 7K62-25 | **Store at:** 2 to 8°C**Unopened:** Until expiration date**Opened expiration:** 30 days onboard. May be stored on or off the ARCHITECT system, which tracks time onboard. |
| Architect TSH Calibrator Kit | 7K62-01 | **Store at:** 2 to 8°C**Unopened/Opened:** Until expiration date |

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| **Risk and Safety** | **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**The following warnings and precautions apply to:** *assay diluent***WARNING** Contains TRIS hydroxymethyl aminomethane and tromethamine hydrochloride. Causes serious eye irritation. Causes skin irritation. May cause respiratory irritation.**The following warnings and precautions apply to:** *Cal 1 and Cal 2* Contains sodium azide. Contact with acids liberates very toxic gas.No special disposal indicated. Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** | **Both analyzers:**

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| Assay Range: | 0.01 to 100.0000 ng/mL |
| Reference Material: | Alinity i or Architect TSH Calibrator |
| Suggested Calibration Levels: | CAL 1: 0 ng/mLCAL 2: 40 ng/mL |
| Calibration Scheme: | 2 Levels |
| Calibration Frequency: | With every new lot number, after maintenance or service of major instrument parts, as indicated by quality controls, and as directed by field service representatives. |
| AMR | AMR is verified twice annually using the Audit Microcontrols K833M-5 by running all applicable levels in triplicate. Assay results are submitted to Audit Microcontrols or EP Evaluator for compilation. Results are reviewed and approved by the Technical Specialist. Questionable results are investigated and corrective actions documented. |

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| **Quality Control** | **Both analyzers:****QC Material:** BioRad Liquichek Immunoassay Plus Levels 1,2 and 3 **Frequency:** Three levels each day of use. **Stability:** 5 Days at 2-8°C (due to the inclusion and use of Estradiol in this control)**Preparation**: Let vials thaw for 30 minutes at room temperature and gently swirl to ensure homogeneity. Do not allow to stand at room temperature longer than 20 minutes after completely thawed. **Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.

Do not load or release patients until QC is acceptable in Unity Real Time.  |
| **Interferences** | **Both analyzers:**The ARCHITECT TSH assay is designed to have a potential interference from hemoglobin, bilirubin, triglycerides and protein of ≤ 10% at the levels indicated below:Hemoglobin ≤ 500 mg/dLBilirubin ≤ 20 mg/dLTriglycerides ≤ 3000 mg/dLProtein ≤ 2 g/dL and 12 g/dLSee Instructions for Use for other hormones that were tested by Abbott for cross reactivity. None were identified. |
| **Reference Intervals** | Birth to 5 days: 0.7 to 15.2 µIU/mL6 days to <6 Months: 0.73 - 4.77 µIU/mL 6 Months to < 14 Years: 0.7 - 4.17 µIU/mL14 to < 19 Years: 0.47 - 3.41 µIU/mL Adult: 0.35 to 4.94 µIU/mL  |
| **Critical Values** | None specified |
| **Limitations** | **•** 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 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.**•** 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. |
| **Dilutions** |

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| **Both analyzers:**  |
| Max Auto Dilution: | 1:5 |
| Maximum Manual Dilution: | None specified. |
| Diluent: | Onboard diluent |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 0.01, do not report the result. Rerun and/or investigate for other possible causes of error. |
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| **Result Reporting** | **Alinity c:*** Results between 0.01 and 100.00 without error messages are released
* Results below 0.01 without error messages are reported as < 0.01 µIU/mL
* Results > 100.0000 should be diluted using the onboard automated 1:5 dilution. Release results without error messages following this dilution.
* Results > 500.0000 following automated dilution are reported as > 500.0000 µIU/mL
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity I TSH Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity I TSH Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised December 2017
3. Abbott Architect TSH Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised November 2015
4. Abbott Architect TSH Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised November 2015
5. [CALIPER Reference Interval Studies](https://caliper.research.sickkids.ca/#/login;next=search;queryParams=%7B%7D), accessed 10/27/2020.
6. BioRad Liquichek Immunoassay Plus Quality Control Package Insert, BioRad Laboratories, Irvine, CA. Revised April 2020
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Elauteria Earnhardt |  | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added all Architect information, reference intervals, references, result reporting, dilutions, qc material, etc for new testing on Alinity and Architect  |
|  | 2 | Erin Bartos | January 26, 2021 | Added newborn reference range after consultation with Endocrine dept. |
|  | 2 | Matt Johnson | 9/17/2021 | Minor update. Revised time before sample separation from gel/red cells to match IFU.  |
|  | 3 | Matt Johnson | 11/1/2022 | Corrected reportable range to reflect actual practices. Low changed from 0.0083 to 0.01 |