| **Cortisol** |
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| **Purpose** | This procedure provides instructions for performing CORTISOL on ABBOTT INSTRUMENTATION. The Abbott Cortisol assays are chemiluminescent microparticle immunoassays (CMIAs) used for the quantitative determination of cortisol in human serum or plasma on the Alinity i and Architect i1000SR analyzers. They are intended to be used as an aid in the diagnosis and treatment of adrenal disorders.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Architect i1000 or Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | This assay is a delayed one-step immunoassay for the quantitative determination of cortisol in human plasma, serum or urine using chemiluminescent microparticle immunoassay (CMIA) technology. Sample and anti-cortisol coated paramagnetic microparticles are combined and incubated. The cortisol present in the sample binds to the anti-cortisol coated microparticles. Cortisol acridinium-labeled conjugate is added to the 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 indirect relationship between the amount of cortisol 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** | Cortisol is the major glucocorticoid hormone secreted by the adrenal cortex. Its physiological functions include regulation of carbohydrate metabolism and electrolyte and water distribution. Cortisol also has immunosuppressive and anti-inflammatory activity. In normal individuals, cortisol levels are regulated through a negative feedback loop in which the adrenal cortex responds to increased adrenocorticotropic hormone (ACTH) levels by increasing cortisol secretion, and the pituitary responds to elevated cortisol levels by down-regulation of ACTH production. Plasma cortisol levels are highest in the morning, and concentrations decrease by about half toward evening. Pregnancy or estrogen treatment markedly elevates cortisol levels. Other stimuli such as severe stress may also lead to increased cortisol production.Cortisol measurements are used as a direct monitor of adrenal status and an indirect measure of pituitary hyper or hypofunction. Elevated cortisol levels are associated with adrenal tumors, pituitary tumors or ectopic ACTH-producing tumors. Subnormal cortisol concentrations may indicate generalized adrenal hypofunction or a defect in the metabolic pathway for cortisol biosynthesis. The majority of cortisol in plasma is bound to proteins and approximately 1% is excreted unchanged into the urine. Urinary cortisol is generally thought to reflect the level of unbound (free) plasma cortisol, which is biologically active. In cases of cortisol overproduction, cortisol-binding globulin becomes saturated, such that unbound plasma cortisol increases disproportionately, as does urinary excretion. The measurement of urinary cortisol is a sensitive means of determining adrenocortical hyperfunction such as Cushing’s syndrome. Urinary cortisol from 24-hour collections represent integration over a full day and are not affected by the diurnal variation evident in plasma cortisol levels. Cortisol measurements are often performed in conjunction with certain “challenge” tests designed to measure whether regulation of the hypothalamic-pituitary-adrenal axis is intact. These include the dexamethasone suppression test (DST), ACTH stimulation test and insulin tolerance test. Such challenge tests aid in the differential diagnosis of Cushing’s syndrome (cortisol overproduction) and the assessment of Addison’s disease (cortisol underproduction). |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup: Abbott Architect i1000SR (Sunquest method code: AI1)** |
| **Sunquest Test Codes** | **CORTI** |
| **Specimen** | Sample: Serum or Serum Separator Tube (SST)**Preferred: SST****Alternative:** Sodium heparin, lithium heparin, Potassium EDTA, plasma separator tube with lithium heparin**Minimum sample volume:** 1.0 mL blood, 0.25 mL serum/plasmaOn **Alinity I** and **Architect i1000**:Priority loaded: Sample volume for first test: 70 μL Sample volume for each additional test from same sample cup: 20 μLRoutinely loaded: Sample volume for first test: 150 μL Sample volume for each additional test from same sample cup: 20 μL**Stability when separated from cells/gel:** **20 to 25°C:** Not specified. Remove to storage at least every shift.**2 to 8°C:** 14 days**-20°C:** 30 days **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** Upon receipt, gently invert cartridges 5 times, then 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 8 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. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* 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.

**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.  |
|  | **Alinity c and Architect i1000:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Cortisol Reagent Kit | 08P3320 | **Store at:** 2 to 8°C until expiration date**Unopened:** 2 to 8°C until expiration date. If the cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.**On-board**: System temperature for 30 days. Do not invert after reagent has been loaded on the analyzer. If the cartridge does not remain upright, discard the cartridge. Do not reuse containers, original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance. |
| Alinity i Cortisol Calibrators | 08P3301 | **Store at:** -10°C or colder until manufacturer’s printed expiration date**Unopened:** -10°C or colder until expiration dateTo Use: allow to thaw at room temperature for 45-60 minutes. Prior to each use, mix thoroughly by gentle inversion 5-10 times.**Opened expiration:** 2 to 8°C up to 90 days after thawing. After use, return to refrigerated storage and tightly cap with new replacement cap. The analyzer will track In-use Stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer. The analyzer will not allow the use of the calibrator if the In-use Stability has been exceeded. |
| Abbott Architect Cortisol Reagent Kit | 08D1525 | **Store at:** -10°C or colder until manufacturer’s printed expiration date**Unopened:**  -10°C or colder until expiration date**Opened expiration:** 2 to 8°C up to 90 days after thawing. After use, return to refrigerated storage and tightly cap with new replacement cap |
| Abbott Architect Cortisol Calibrator Kit | 08D1502 | **Store at:** -10°C or colder until manufacturer’s printed expiration date**Unopened:** -10°C or colder until expiration date**Opened expiration:** Thaw completely at room temperature (15-30°C) for 45-60 minutes. Prior to use, mix thoroughly by inversion 5-10 times. After each use, immediately return the thawed calibrators to refrigerated storage (2-8°C) for up to 90 days after thaw. |

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| **Risk and Safety** | No special disposal of reagents or calibrators indicated.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** | **Alinity c and Architect i1000:**

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| Assay Range: | 1.0 – 59.8 µg/dL |
| Reference Material: | Abbott Alinity i or Architect Calibrator Kits |
| Suggested Calibration Levels: | CAL A: 0.0CAL B: 3.0CAL C: 5.4CAL D: 10.7CAL E: 25.2CAL F: 59.8 |
| Calibration Scheme: | 6 Levels |
| Calibration Frequency: | With every new lot number, after maintenance on or service of major instrument parts, as indicated by quality controls, and as directed by field service representatives. |
| AMR | AMR is verified with every calibration and at least every 6 months. |

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| **Quality Control** | **Alinity c and Architect i1000:****QC Materials:** 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 of Estradiol in the control) at 2-8°C**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** | **Alinity c and Architect i1000:**A study based on guidance from the CLSI Protocol EP07-A2. Serum specimens with cortisol levels between 5.1 and 34.2 μg/dL were supplemented with the following potentially interfering compounds.The average amount of interference observed during the study ranged from -7.8% to 13.2%.Bilirubin 20 mg/dLHemoglobin 500 mg/dLTotal Protein (Low) 3 g/dLTotal Protein (High) 10 g/dLTriglycerides 2000 mg/dLSee Limitations section for more information. |
| **Reference Intervals** | **AM/PM Window Reference Ranges:**

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|  | **Age** | **Reference Range** |
| **Timeframe:** 0500-1100 am | 0-24 months | 0.8-28.6 μg/dL |
|  | 2-11 years | 0.8-27.7 μg/dL |
|  | 11-18 years | 0.8-23.5 μg/dL |
|  | >18 years | 4.2-21.0 μg/dL |
| **Timeframe:** 1700-2300 pm | 0-24 months | 0.8-25.5 μg/dL |
|  | 2-11 years | 0.8-20.2 μg/dL |
|  | 11-18 years | 0.8-18.5 μg/dL |
|  | >18 years | 2.5-13.4 μg/dL |

**For results drawn outside of the AM and PM windows, use random cortisol ranges.****Random Cortisol Ranges:**

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| **Age** | **Reference Range** |
| 2-14 days | 0.5-12.3 μg/dL |
| 15 days to <1 year | 0.5-16.6 μg/dL |
| 1 year to <9 years | 1.7-10.8 μg/dL |
| 9 years to <14 years | 2.2-12.7 μg/dL |
| 14 years to <17 year | 2.8-16.4 μg/dL |
| 17-19 years | 3.5-18.3 μg/dL |
| Adult | 2.9 -19.4 μg/dL |

Results in Sunquest will flag according to the AM Reference Intervals. |
| **Critical Values** | None Specified |
| **Limitations** | Due to the diurnal variation of cortisol levels in normal subjects, all serum/plasma cortisol measurements should be referenced to the time of day of sample collection.Patients receiving fludrocortisone, prednisolone or prednisone (which is converted to prednisolone *in vivo*) may show artificially elevated cortisol values due to cross-reactivity. Cross-reactivity to endogenous and synthetic steroids is reported in the SPECIFIC PERFORMANCE CHARACTERISTICS, Specificity section in the package insert.If the cortisol results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.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 that employ mouse monoclonal antibodies. Assay results that are not consistent with other clinical observations may require additional information for diagnosis.Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. The presence of heterophilic antibodies in a patient specimen may cause anomalous values to be observed. Additional information may be required for diagnosis.The concentration of cortisol in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity. |
| **Dilutions** |

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| **Alinity c and Architect i1000:** |
| Max Auto Dilution: | 1:2 |
| Maximum Manual Dilution: | 1:4Add 50 μL of the sample to 150 μL of Cortisol 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. The result should be > 3.0 μg/dL before the dilution factor is applied. |
| Diluent: | **Alinity c or Architect i1000** Cortisol Calibrator A |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) or [Architect Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/procedure/ch5.106-abbott-architect-i1000-operating-procedure.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 1, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | **Alinity c and Architect i1000:*** Results between 1.0 and 59.8 µg/dL without error messages are released
* Results below 1.0 without error messages are reported as < 1.0 µg/dL.
* Results > 59.8 should be diluted using the onboard automated 1:2 dilution. Release results without error messages following this dilution.
* Results > 119.6 should be diluted using the onboard manual 1:4 dilution. Release results without error messages following this dilution.
* Results > 239.2 following automated dilution are reported as > 239.2 µg/dL.
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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 Cortisol Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
2. Abbott Alinity i Cortisol Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018
3. Abbott Architect Cortisol Reagent Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised November 2015
4. Abbott Architect Cortisol Calibrator Kit Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised January 2015
5. Bio-Rad Lyphochek Immunoassay Plus Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 May 2017
6. CALIPER Reference Interval Studies, accessed 10/27/2020.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Michelle Anton |  | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | October 28, 2020 | Added both analyzers, AMR, ref intervals, references, reagents/calibrators, etc. for Alinity analyzer |