| **Cortisol** | |
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| **Purpose** | This procedure provides instructions for performing Cortisol on the Abbott Architect i1000SR |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR. |
| **Principle** | ARCHITECT Cortisol is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of cortisol in human serum, plasma or urine on the ARCHITECT iSystem. The ARCHITECT Cortisol assay is intended for use as an aid in the diagnosis and treatment of adrenal disorders.  The ARCHITECT Cortisol assay is a delayed one-step immunoassay for the quantitative determination of cortisol in human serum, plasma or urine using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample and anti-cortisol coated paramagnetic microparticles are combined. The cortisol present in the sample binds to the anticortisol coated microparticles. After incubation, cortisol acridinium-labeled conjugate is added to the reaction mixture. Following a second incubation, the microparticles are washed, and pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of cortisol in the sample and the RLUs detected by the ARCHITECT iSystem optics. |
| **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, cortisolbinding 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 (not performed at Children’s MN) 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). |
| **Instrument** | **PRIMARY METHOD: Abbott Architect**  Backup Method**: Mayo Medical Laboratories** |
| **Sunquest Test Code** | CORTI |
| **Specimen** | **Sample type:** SST (Serum)  Also acceptable: Lithium Heparin plasma (with or without gel), Sodium Heparin plasma, EDTA plasma  **Draw volume: 1.2 mL**  **Minimum processed volume:** 150 µL of serum or plasma (does not allow for repeat or dilution)  **Transport:** Ship and Store refrigerated (2-8°C) to Minneapolis lab  **Stability:**14 days at 2-8°C, 30 days -20°C or colder.  **Rejection criteria:** Unlabeled specimens, incorrect sample type  **Preparation:**   1. Serum specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures prior to analysis. Plasma specimens can be centrifuged immediately. 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. Lipemic samples should be ultrafuged. 4. Specimens should be free of particulate matter. 5. Transfer serum or plasma to a properly labeled Architect sample cup on top of a sendout tube. Minimum labeling on cup and tube includes sample accession ID, and/ or patient name, medical record number, collection date and time. |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | | Cortisol Reagent | 8D1525 | **Store at:** 2 – 8 °C  **Unopened/Opened:** Manufacturer expiration date.  **On-board:** 30 Days | | Cortisol Calibrator | 08D15-02 | **Store at:**  -20 °C  **Unopened:** Manufacturer expiration date.  **Before Use:** Thaw completely at room temperature. Prior to use, mix thoroughly by inversion 5-10 times. Do not allow to stand at room temperature longer than 20 minutes after thawing.  **Opened:** 2 – 8 °C for 90 days after the first thaw. | | Multiasay Diluent | 7D82-50 | Refer to Supply Status on Analyzer | | Pre-Trigger Solution | 06E23-65 | Refer to Supply Status on Analyzer | | Trigger Solution | 06C55-60 | Refer to Supply Status on Analyzer | | Wash Buffer | 06C54-58 | Refer to Supply Status on Analyzer | | Reaction Vessels | 07C15 (-02 or -03) | N/A | |
| **Risk and Safety:** | Reagent contains methylisothiazolones and sodium azide. Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. If all tests have been used from the reagent, it may be disposed of in regular trash. If any tests remain, recap and dispose of in appropriate Hazardous Waste Container. |
| Calibration/ Verification/AMR | |  |  | | --- | --- | | Analytical Measuring Range: | 1. - 59.8 μg/dL | | Reference Material: | Cortisol Calibrator 08D15-02 | | Suggested Calibration Levels | A – 0.0 μg/dL  B – 3.0 μg/dL  C – 5.4 μg/dL  D – 10.7 μg/dL  E – 25.2 μg/dL  F – 59.8 μg/dL | | Verification Scheme: | n=6 | | Verification Frequency: | * For each new lot of reagent * After major maintenance or service, if indicated by quality control results * As indicated in laboratory quality control procedures | | AMR | Verification of AMR is accomplished with each calibration.   * Cal Verification and AMR verification are performed at least once every six (6) months. | |  | | | |
| **Quality Control** | BioRad Lyphochek Immunoassay Plus Levels 1,2 and 3  **Frequency:** Three levels each day of use.  **Stability:** 3 Days at 2-8°C.  **Preparation**: Reconstitute with exactly 5.0 mL of DI water. Let vials sit for 15 minutes and gently swirl to ensure homogeneity. Do not allow to stand at room temperature longer than 20 minutes.  **Sunquest Control names:** Level 1 = C-LYIP1, Level 2 = C-LYIP2, Level 3= C-LYIP3  **Acceptable ranges:**   * Ranges are current in Sunquest and the instrument. Refer to the Quality Control in Chemistry procedure for QC exception codes. * If a control value is outside the confidence interval, the determination must be repeated. If the repeat determination confirms the deviation, a new reference curve should be established. * Do not release patient results until the cause of deviation has been identified and corrected * When a new lot of assayed control is received, validate the manufacturer’s insert range by running the new lot in parallel with the current lot, and confirming that the results obtained are within the stated range * When a new lot of unassayed control is received, verify new ranges by running the new lot in parallel with the current lot 30 times, and calculate a new range using the method mean ± 3 SD. Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. |
| **Interferences** | **•** 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.   * Specimens should be free of red cells and other particulate matter (fibrin.)   **•** Patients receiving fludrocortisone, prednisolone or prednisone (which is converted to prednisolone *in vivo*) may show artificially elevated cortisol values due to cross-reactivity. Please refer to the package insert for detailed cross-reactivity 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 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. |
| **Reference Range** | **AM/PM Window Reference Ranges:**   |  |  |  | | --- | --- | --- | |  | **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:**   |  |  | | --- | --- | | **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 |   Results in Sunquest will flag according to the AM Reference Intervals. |
| **Critical Values** | None specified |
| **Limitations** | * The instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages. * 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. * 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. |
| **Dilutions** | * Specimens with a cortisol value exceeding 59.8 μg/dL are flagged with the code “> 59.8” will be automatically diluted on board with a dilution of 1:2. * Specimens with a cortisol value exceeding 119.6 μg/dL are flagged with the code “>119.6” when run using the Automated Dilution Protocol. These specimens may be diluted by following the Manual Dilution Procedure.   Manual Dilution Procedure:   1. The suggested and maximum dilution is 1:4 2. Prior to diluting the specimen, dispense approximately 7 drops of ARCHITECT Cortisol Calibrator A into a clean tube for use in the next step. 3. Add 150.0 μL of ARCHITECT Cortisol Calibrator A from the test tube prepared in the prior step into another clean test tube and add exactly 50.0 μL of the patient specimen. 4. The operator must enter the dilution factor in the Patient order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result. The result should be > 3.0 μg/dL before the dilution factor is applied. Refer to the [Abbott Architect Operating Procedure](https://starnet.childrenshc.org/References/labsop/chem/procedure/ch5.106-abbott-architect-operating-procedure.pdf), under programming sample data, for assistance programming the manual dilution factor. |
| **Result Reporting** | * Cortisol will flag according to the AM ranges, and PM ranges will be appended. * Results <1 μg/dL will be reported as <1 μg/dL rather than the numerical value. * Results >59.8 μg/dL will trigger an autodilution on the analyzer. * Results > 119.6 μg/dL should be manually diluted 1:4 * Results that are >239.2 μg/dL will be reported as >239.2 μg/dL rather than the numerical value * Specimens drawn outside the times should be compared with the Random Cortisol ranges. * All results will autofile unless they are greater than or less than AMR. |
| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Architect reagent cartridge insert sheet Abbott Laboratories, Abbott Park, IL, 60064. Revised Date November 2015. 2. Abbott Architect calibrator insert sheet Abbott Laboratories, Abbott Park, IL 60064. Revised January 2015. 3. Abbott Architect Safety Data Sheet, Abbott Diagnostics, Abbott Park, IL 60064. Revised July 2015. 4. Bio-Rad Lyphochek Immunoassay Plus Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 May 2017 5. [CALIPER Reference Studies](http://www.sickkids.ca/Caliperproject/index.html), accessed 4/20/2018. |
| **Historical Record** | |  |  |  |  | | --- | --- | --- | --- | | **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** | | 1 | Kelsi Brown/Erin Bartos | May 5, 2018 | New Procedure | |  |  |  |  | |  |  |  |  | |