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| **ACTH (Adrenocorticotropic Hormone)** |
| **Purpose** | This procedure provides instructions for ACTH (ADRENOCORTICOTROPIC HORMONE) on the Siemens DPC Immulite ® 2000. |
| **Principle** | IMMULITE® 2000 ACTH is a solid-phase, two-site sequential chemiluminescent immunometric assay. The IMMULITE ® 2000 assay is traceable to an internal standard manufactured using qualified materials and measurement procedures. |
| **Policy Statements** | This procedure applies to all personnel who run the Immulite® 2000 and to those who process samples for testing. |
| **Clinical Significance** | Adrenocorticotropic hormone (ACTH) is a polypeptide hormone that exists principally as a chain 39 amino acids long, with a molecular mass of approximately 4,500 Daltons. It is produced in the pituitary and serves to stimulate steroid production by the adrenal cortex. ACTH secretion is in turn controlled by the hypothalamic hormone corticotrophin releasing factor and by negative feedback from cortisol.ACTH determinations are valuable in the differential diagnosis of adrenal insufficiency and hypersecretion. In Addison’s disease (primary adrenal insufficiency), elevated levels are typical, whereas low levels are the rule when adrenal insufficiency is secondary to pituitary dysfunction.ACTH can also help identify cortisol hypersecretion in Cushing’s syndrome. ACTH levels are low when this is due to lesions or hyperplasia of the adrenal cortex, and high when it is due to ectopic ACTH production or hypersecretion of ACTH by the pituitary. |
| **Instrument** | Siemens DPC Immulite® 2000 |
| **Sunquest Test Code** | ADCT (Adrenocorticotropic Hormone) Plasma ACTH pg/mL* Testing is performed within 12 hours of receipt in St. Paul Lab
* Routine testing is performed daily from 6:30 am to 10:00 pm.
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| **Specimen** | * EDTA plasma, collected in tubes without gel barriers (see special instructions for collection and processing of samples)
* Samples should be drawn between 6 AM and 10 AM for peak values.

**Minimum volume:*** 500µL (12x75 tube) preferred
* 200µL (micro) absolute minimum

**Stability:**30 days frozen at –20°CValues increase within 90 seconds of traumatic, repeated, or prolonged venipuncture.**Special instructions for specimen collection:**1. Pre-chill EDTA tube using packed wet ice
2. Collect blood by atraumatic venipuncture
3. Mix 8-10 times by inversion
4. Immediately retun tube to packed wet ice
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| **Specimen (cont)** | **Specimen Processing:**1. Separate plasma from cells as soon as possible after collection using a refrigerated centrifuge.
2. Transfer plasma to labeled **plastic** tube, and freeze immediately at -20°C or lower, unless one of the conditions are met as described below.
3. The use of an ultracentrifuge is recommended to clear lipemic samples with triglyceride concentrations >5000 mg/dL.
4. **Minneapolis STAT specimens**: If a STAT ACTH sample is received prior to 5:15pm, follow the instructions above and send on the next available courier. Call St. Paul to notify them a STAT ACTH sample is coming. If a sample is ordered STAT and is drawn and received between the hours of 5:30 pm and 8:15 pm, obtain packed ice from the breakroom refrigerator ice maker. Place the processed specimen on packed wet ice in the refrigerator for transport to St. Paul on the 8:30 pm courier (maintain specimen at ≤ 4°C on packed wet ice). For samples received after the last courier has left, revert to steps 1 and 2. Notify St. Paul they should follow step 5 for the STAT specimen.
5. Prior to testing, samples should be thawed in an ice bath and kept at or below 4°C.
6. **St. Paul Staff**: If a STAT ACTH is received during the hours that the instrument is not operation, hang the STAT ACTH sign on the Immulite screen so dayshift chemistry knows to start thawing STAT ACTH specimens at 6:30 am.

**Rejection criteria**: Unlabeled, grossly hemolyzed, improper storage, other than EDTA plasma, specimen stored in glass. |
| **Reagents** | Components are a matched set. Labels inside the box are needed for the assay.**ACTH Bead Pack (L2AC12)**With barcode. 200 beads, coated with monoclonal murine anti-ACTH. Stable at 2-8°C until expiration date. **L2KAC2:** 1 pack.**ACTH Reagent Wedge (L2ACA2)**With barcode. 2 reagents: 11.5 mL protein buffer/serum matrix; 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal rabbit anti-ACTH in buffer, with preservative. Stable at 2-8°C until expiration date.* Before use, tear off the top of the label at the perforations without damaging the barcode. Remove the foil seal from the top of the wedge; snap the sliding cover down into the ramps on the reagent lid.
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| **Calibration** |

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| Assay Range: | **5.0-1250 pg/mL** |
| Suggested Adjustor Levels: | **ACTH Adjustors (LACL, LACH)**Two vials (Low and High) of lyophilized ACTH in a bovine protein-based matrix with preservative.1. Reconstitute each vial with **4.0 mL reagent grade water.**
2. Let stand **30 minutes**
3. Mix by gentle swirling or inversion.
4. Aliquot, label with supplied barcode labels, and freeze.
5. Stable at -20°C for 2 months after reconstitution.
6. Prior to testing, adjustors should be thawed in an ice bath and kept at or below 4°C

**L2KAC2:** 2 sets. |
| Adjustor Frequency: | 1. 4 week curve stability
2. Each new lot of reagent (kit)
3. When QC repeatedly fails
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| **Analytical Measuring Range (AMR)** | * Once every 6 months verify the Analytical Measuring Range by running a minimum of 3 levels of DPC Calibration material (Calibrators A, F, and 90% of H), or by add-mixing a low and high calibrator as the 3rd level.
* Run in triplicate as a ***Cal verifier.***
* File the result printout in Adjustor Log under AMR.
* Values must agree ±10% of target, or within manufacturer’s stated range, and target values must be <50% of lower reportable range value, and <10% of upper reportable range value.
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| **Quality Control** | **Product:** Immulite ACTH Controls, LACCM, 2 levels containing lyophilized ACTH in a protein-based buffer matrix containing human serum, with preservatives.**Preparation:** No more than ***30 minutes before use:***1. Reconstitute each vial with **2.0 mL reagent grade water.**
2. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved.
3. Place the controls in ice slurry immediately.
4. Aliquot and freeze the remaining volume.
5. Prior to testing, controls should be thawed in an ice bath and kept at or below 4°C.

**Frequency:** Two levels once each day of patient testing.**Stability:** Stable at -20°C for 2 months. Once thawed, do not refreeze.**Sunquest Control Names:** Level 1 = C-ACTH1, Level 2 = C-ACTH2**Acceptable Ranges:** Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. |
| **Interferences** | **Bilirubin:** The presence of conjugated or unconjugated bilirubin in concentration up to 20 mg/dL was shown to have no effect on results, within the precision of the assay.**Hemolysis:** Presence of hemoglobin in concentrations up to 512 mg/dL has no effect on results, within the precision of the assay. However, the effect of traumatic puncture must be considered.**Lipemia:** Presence of triglycerides in concentrations up to 5000 mg/dL has no effect on results, within the precision of the assay.Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings. |
| **Reference Range** | 10 – 60 pg/mL |
| **Critical Values** | None specified. |
| **Limitations** | Plasma levels of ACTH exhibit diurnal variation. It is therefore important to standardize the time of collection. ACTH is also affected by stress. ACTH results are best interpreted in conjuction with plasma cortisol results. |
| Dilutions | N/A |
| **Result Reporting** | * Results between 5.0-1250 pg/mL without error messages are released.
* Results below 5.0 pg/mL: report as <5.0 pg/mL instead of the numerical value.
* Results greater than 1250 pg/mL are reported as >1250 pg/mL
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| **Specimen Storage** | As soon as possible after testing, stopper tested specimen and store frozen in dated biohazard bags.Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. Burtis, CA, Ashwood, ER, Bruns, DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th Edition, Philadelphia: W.B. Saunders, 2006.
2. Jacobs and DeMott Laboratory Test handbook, 5th Edition, Lexi-comp Inc. 2001
3. Siemens Immulite® 2000 ACTH Product Insert, PIL2KAC-17, 2015-07-16
4. Siemens Immulite® ACTH Control Module Product Insert, PILACCM-23, 8/24/09
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | L. Lichty | April 26, 2010 | Initial Version |
| 2 | D. Helfinstine | April 1, 2011 | New format |
|  | 3 | L. Lichty | March 17, 2014 | Define turnaround time |
|  | 4 | Erin Bartos | June 30, 2017 | Changed hours of operation, updated IFU, biennial review |
|  | 5 | Kelsi Brown | October 31,2017 | Updated operating days/hours. Added specimen processing for STAT ACTH. |
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