|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **INSULIN** | | | | | | |
| **Purpose** | This procedure provides instructions for performing INSULIN on the Abbott Architect i1000SR | | | | | |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR | | | | | |
| **Principle** | The ARCHITECT Insulin assay is a one-step immunoassay to determine the presence of human insulin in human serum or plasma, using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample, anti-insulin coated paramagnetic microparticles, and anti-insulin acridinium-labeled conjugate are combined. Insulin present in the sample binds to the anti-insulin coated microparticles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are then added to the reaction mixture; the resulting Chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of insulin in the sample and the RLUs detected by the ARCHITECT *i*1000SR optical system. For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3. | | | | | |
| **Clinical Significance** | Insulin is a polypeptide hormone (MW 6000) composed of two nonidentical chains, A and B, which are joined by two disulfide bonds. Insulin is formed from a precursor, proinsulin (MW 9000), in the beta cells of the pancreas. In proinsulin, the A and B chains are joined by a connecting peptide, referred to as the C-peptide. Both insulin and C-peptide are stored in secretory granules of the islet cells of the pancreas and are then secreted. Insulin secretion follows two basic mechanisms, tonic secretion and biphasic secretion. The basal or tonic secretion is independent of stimulation by exogenous glucose but is modulated by the fluctuations in physiological levels of glucose. The biphasic secretion is primarily a direct response from stimulation by exogenous glucose. Stimulation of insulin secretion can be caused by many factors including hyperglycemia, glucagon, amino acids, and by complex mechanisms involving growth hormone or catecholamines. Increased levels of Insulin are found with obesity, Cushing’s Syndrome, oral contraceptives, acromegaly, insulinoma and hyperthyroidism. Decreased levels of insulin are found in overt diabetes mellitus (although this may not be clearly expressed in early stages of the condition) and by part of a complex mechanism involving catecholamines. “Immunoreactive insulin” (IRI) is a term often used to refer to the component of circulating insulin and insulin-like biological activity which can be measured using antibodies against insulin. Insulinomas may produce various forms of insulin and proinsulin-like material and show total immunoreactive insulin at normal or elevated levels. Since proinsulin and insulin both contain A and B polypeptide chains, there is a possible cross-reactivity with antibodies generated against insulin. The ARCHITECT Insulin assay shows no cross-reactivity with proinsulin (≤ 0.1% at 106 pg/mL). Another possible interference is brought about by insulin antibodies which develop in patients treated with bovine or porcine insulin.  Immunoassays for insulin have been widely used to provide supplementary information, first, for the diagnosis of diabetes mellitus and, second, for differential diagnosis of fasting hypoglycemia to discriminate between insulinoma and factitious hypoglycemia. In these applications, the ratio of immunoreactive insulin to blood glucose (I/G) may be more valuable than the insulin level alone.  Furthermore, a single random blood sample may provide insufficient information due to wide variations in the time responses of insulin levels and blood glucose which are found among individuals and various clinical conditions. Other uses of insulin assays have been suggested by the finding of an increase in risk factors for coronary artery disease among healthy persons with hyperinsulinemia and normal glucose tolerance. | | | | | |
| **Instrument** | **PRIMARY METHOD:** Abbott Architect i1000SR  Backup Method**:**  Mayo Medical Laboratories | | | | | |
| **Test Code** | INS | | | | | |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | | Insulin Reagent kit | 08K4127 | **Store at:** 2 – 8 °C  **Unopened/Opened:** Manufacturer expiration date.  **On-board:** 30 Days | | Insulin Calibrator | 08K41-02 | **Store at:**  2 – 8°C  **To Use**: Gently mix after removal from fridge  **Unopened**: Manufacturer expiration date.  **Opened**: Store at 2 – 8 °C until manufacturer | | 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 | | Bio-Rad Lyphochek Immunoassay Plus (LYIP) | Level 1 - 371  Level 2 - 372  Level 3 - 373 | **Unopened storage:** ≤-20°C  **To Use:** Thaw at Room Temperature  **Once Opened, Store:** 2-8°C  **Stability:** 30 Days | | | | | | |
| **Sample** | **Container**:  **Preferred**: SST (gold, marble) or Red no gel  Acceptable: K2EDTA (purple top), or Sodium Heparin (NaHep)  **Draw Volume**:  Preferred: 1.2 mL blood. Minimum: 0.5mL  **Processed Volume:**  Preferred: 0.4mL serum or plasma  Minimum: 0.15 mL serum or plasma  Note: Minimum volume does not permit repeat analysis or dilution  **Sample Stability**:  7 days when frozen at -20º within 8 hours of draw  **Sample Processing**:  Centrifuge specimen within one hour of collection. Remove serum/EDTA plasma into a screw-capped round bottom plastic vial (sendout tube). For minimum volume use the ARCHITECT Sample Cups on top of a sendout tube.  **Sample Rejection Criteria**:  Specimens not separated within one hour of collection.  mislabeled or unlabeled specimen  grossly hemolyzed specimens | | | | | |
| **Special Safety Precautions** | Follow Children’s Laboratory Safety guidelines when handling patient samples and reagents.  Safety data sheets (MSDS/SDS) available on [Children’s Intranet](http://starnet.childrenshc.org/emergency-and-safety/) | | | | | |
| **Calibration** | |  |  | | --- | --- | | Analytical Measuring Range: | AMR: 1.0 - 300.0 µU/mL | | Reference Material: | 8K41-02 ARCHITECT Insulin Calibrators | | Suggested Calibration Levels, in µU/mL | A = 0  B = 3  C = 10  D = 30  E = 100  F = 300 | | 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 | | | | | | |
| **Analytical Measuring Range (AMR)** | AMR: 1.0 - 300.0 µU/mL  Verification of AMR is accomplished with each calibration at an interval that does not exceed 6 months. | | | | | |
| **Quality Control** | **Bio-Rad** **Lyphochek Immunoassay Plus** Levels 1, 2, and 3  **Frequency:** 3 levels every 24 hours  **Stability:** 7 days at 2-8º C  **Preparation**: Using a volumetric pipette or equivalent, reconstitute each vial with exactly 5.0 mL of deionized water. Replace the stopper and allow this product to stand for approximately 15 minutes, swirling occasionally. Before sampling, allow vials to reach room temperature. Gently swirl prior to use to ensure homogeneity. After sampling, return immediately to refrigerated storage. Do not allow exposure to room temperature to exceed 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, investigate common causes, such as insufficient control material or expired materials. The determination must be repeated. If the repeat determination confirms the deviation, a new calibration curve should be established. * Do not release patient results until the cause of deviation has been identified and corrected. Document ALL troubleshooting actions in Sunquest or in the Architect i1000SR Instrument Maintenance Log notes under the current day's maintenance log. * 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 for a recommended 20 days, and confirming that the results obtained are within the stated range. | | | | | |
| **Dilutions** | **•** Specimens with an insulin value exceeding 300 μU/mL are flagged with the code “>300.0” and should be diluted using first the 1:2 Automated Dilution Protocol, then the 1:10 Manual Dilution Procedure as appropriate.  **•** In the automated dilution, the system performs a 1:2 dilution of the specimen and automatically calculates the concentration of the undiluted specimen and reports the result.  Manual dilutions should be performed as follows:  **•** The maximum manual dilution for the ARCHITECT Insulin assay is 1:10.  **•** For a 1:10 dilution, add 20 μL of the patient specimen to 180 μL of ARCHITECT Insulin Calibrator A (8K41-02).  **•** To avoid contamination of Calibrator A, dispense several drops of Calibrator A into a clean test tube prior to pipetting.  **•** 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 dilution should be performed so that the diluted result reads greater than 3.0 μU/mL.  **•** For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5. | | | | | |
| **Limitations** | Technical range: 1.0 - 3000.0 µU/mL   * Specimens from patients treated with bovine or porcine insulin may contain insulin antibodies which could show interference in the assay. * Insulin levels may be measured lower in patients with insulin autoimmune syndrome or familial high pro-insulinemia. | | | | | |
| **Reference Intervals** | |  |  | | --- | --- | | Age | Range µU/mL | | 0-1 year | 1-23.5 | | 1-6 years | 1-40 | | 6-19 years | 2-40 | | 19 + | 2-25 | | | | | | |
| **Alternate Method** | If the assay does not meet test performance requirements, consult the Technical Specialist or Medical Director and refer testing to Mayo Medical Laboratories. | | | | | |
| **Result Reporting** | Results within the measuring range of 1.0 to 3000.0 (after 1:10 manual dilution) will be reported with the numerical value.   * Results between 1.0 – 100.0 µU/mL without error messages are released automatically   If there is not enough sample to repeat append the code “-UNQ” (Unable to Quantitate Further) to the result   * Result below 1 µU/mL report as < 1.0 µU/mL. * Results above 3,000.0 µU/mL report as >3,000.0 rather than the numerical value. | | | | | |
| **References** | 1. Abbott Architect Insulin Package Insert F5-Y302-2/R02\_Sept2012, Abbott Labs, Abbott Park, IL 2. Abbott Architect Insulin Calibrators Package Insert 8K41-02, April 2015, Abbott Labs, Abbott Park, IL 3. Bio-Rad Lyphochek Immunoassay Plus Package Insert 1536-00 May 2017, Bio-Rad Laboratories, Irvine, CA 4. [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 | S. Gripentrog/E. Bartos | | 04/24/2018 | Initial Version | |