| **Insulin** |
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| **Purpose** | This procedure provides instructions for performing INSULIN on ABBOTT INSTRUMENTATION. The Alinity i Insulin assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of human insulin in human serum or plasma on the Alinity i analyzer.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Architect i1000 or Alinity i at Children’s Minnesota Laboratory in Minneapolis. |
| **Principle** | This assay is a one-step immunoassay for the quantitative determination of human insulin in human serum or plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample, anti-insulin coated paramagnetic microparticles and anti-insulin acridinium-labeled conjugate are combined to create a reaction mixture and incubated. The insulin present in the sample binds to the anti-insulin coated microparticles and to the anti-insulin acridinium-labeled conjugate. 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 insulin 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** | 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. This 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. |
| **Analyzer** | **Minneapolis: Abbott Alinity i (Sunquest method code: MACI)****Backup: Abbott Architect i1000SR (Sunquest method code:AI1)** |
| **Sunquest Test Codes** | **INS** |
| **Specimen** | Sample: Serum or Plasma**Preferred:** SST (gold, marble) or Red No Gel**Alternative:** Potassium EDTA, Sodium EDTA, Sodium Heparin, Sodium Fluoride**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**LOAD SAMPLES IN THE PRIORITY LANES.**Priority Loaded: Sample volume for first test: 74 μL on Alinity, 150 μL Architect Sample volume for each additional test from same sample cup: 24 μLRoutinely loaded: Sample volume for first test: 150 μL (both analyzers) Sample volume for each additional test from same sample cup: 24 μL**Stability when separated from cells/gel:** Analyze fresh specimens if possible. If there will be any delay in testing, freeze immediately after centrifugation.**20 to 25°C** 2 hours**2 to 8°C:** freeze if any delay in testing **-20°C** 7 days Avoid multiple freeze/thaw cycles.**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 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.**Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.**Place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate. Store in upright position. If cartridge does not remain upright during storage, discard the cartridge.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. 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 reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.* 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 i1000SR:**• Do not use reagent kits beyond the expiration date.• Do not pool reagents within a kit or between kits.• 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 instructions, refer to the PROCEDURE, Assay Procedure section of the package insert.• 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:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity i Insulin Reagent Kit  | 04T7520 | **Store at:** 2 to 8°C**Unopened:** Until expiration DateStore in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use**.****On-board**: 30 daysStore in upright position. If cartridge does not remain upright during storage, discard the cartridge.Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance.Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright. |
| Alinity i Insulin Calibrators | 04T7501 | **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.)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. Maximum In-use Stability can be found in the Assay Parameter Report. |
| Abbott Architect Insulin Reagent Kit | 08K41-27 | **Store at:** 2 to 8°C**Unopened:** Manufacturer’s expiration date**Opened expiration:** 30 Days |
| Abbott Architect Insulin Calibrators | 08K4102 | **Store at: 2 to 8°C****Unopened:** Manufacturer’s expiration date**Opened expiration:** Until Manufacturer’s expiration date. Gently mix after removal from the fridge. |

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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:** *Microparticles* **WARNING** Contains 4-Morpholinopropanesulphonic acid\* and sodium azide. Causes mild skin irritation. Contact with acids liberates very toxic gas.**The following warnings and precautions apply to:** *Conjugate* Contains sodium azide. Contact with acids liberates very toxic gas.**The following warnings and precautions apply to:** *CAL A- CAL F* 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** | **Alinity c:**

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| Assay Range: | 1.6 – 300.0 µU/mL |
| Reference Material: | Alinity i Insulin Calibrators |
| Suggested Calibration Levels: | A = 0B = 3C = 10D = 30E = 100F = 300 |
| Calibration Scheme: | 6 Levels |
| Calibration 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 | AMR is verified twice with every calibration, at least every 6 months. |

**Architect i1000SR**:

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| Analytical Measuring Range: | AMR: 1.6 - 300.0 µU/mL |
| Reference Material: | 8K41-02 ARCHITECT Insulin Calibrators |
| Suggested Calibration Levels, in µU/mL | A = 0B = 3C = 10D = 30E = 100F = 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

AMR is verified with every calibration, at least every 6 months. |

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| **Quality Control** | **Both analyzers****QC Material:** Bio-Rad Liquichek Immunoassay Plus Levels 1, 2, 3**Frequency:** Three levels each day of use**Stability:** Stable until the expiration date when stored frozen between -20 and -40°C. Once thawed, opened, and stored tightly capped at 2 to 8°C, this product is stable for 5 days in Minneapolis (due to estradiol in this control). **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour.
* After thawing, the product **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**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.
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| **Interferences** | The specificity of the ARCHITECT Insulin assay was determined by testing sera containing the potentially interfering substances listed below. These substances showed less than 10% interference in the ARCHITECT Insulin assay at the levels indicated.Bilirubin up to ≤ 20 mg/dLHemoglobin up to ≤ 500 mg/dLTotal Protein up to ≤ 12 g/dLTriglycerides up to ≤ 3000 mg/dLProinsulin, C-Peptide and Glucagon did not cross-react with the Architect and Alinity assays. |
| **Reference Intervals** |

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| Age | Range µU/mL |
| 0-1 year | 1-23.5 |
| 1-6 years | 1-40 |
| 6-19 years | 2-40 |
| 19 + | 2-25 |

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| **Critical Values** | None Specified |
| **Limitations** | **WARNING:** The Insulin assay value in a given specimen, as determined with assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the Insulin assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining insulin levels serially is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored.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 which employ mouse monoclonal antibodies. These specimens should not be assayed with the Alinity i Insulin assay. Refer to the section LIMITATIONS OF THE PROCEDURE in the package insert. If the insulin 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.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.Insulin levels may be measured lower in patients with insulin autoimmune syndrome or familial high pro-insulinemia.**Hemolyzed samples should not be used, since enzymatic degradation of insulin may occur and result in lower assay values. However, purified hemoglobin up to 500 mg/dL has been shown not to interfere.**Specimens from patients treated with bovine or porcine insulin may contain insulin antibodies which could show interference in the assay. |
| **Dilutions** |

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| **Alinity c** and **Architect i1000** |
| Max Auto Dilution: | 1:2 |
| Maximum Manual Dilution: | 1:10Add 20 μL of the sample to 180 μL of Alinity i/Architect Insulin Calibrator A. |
| Diluent: | Alinity i Insulin Calibrator A |
| 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 1.6, do not report the result. Rerun and/or investigate for other possible causes of error. |

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| **Result Reporting** | **Both analyzers:*** Results between 1.6 and 300.0 µU/mL without error messages are released
* Results below 1.6 without error messages are reported as < 300.0 µU/mL.
* Results > 300 should be diluted using the onboard automated 1:2 dilution. Release results without error messages following this dilution.
* Results > 600.0 following automated dilution should be manually diluted with the 1:10 manual dilution and reported as > 3000.0 µU/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, but the stability of Insulin is 7 days when frozen within 8 hours of draw, therefore, results are not reliable after 7 days. |
| **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. Abbott Alinity i Insulin Package Insert, Abbott Labs, Abbott Park, IL Revised April 2018
4. Abbott Alinity i Insulin Calibrators Package Insert, Abbott Labs, Abbott Park, IL Revised May 2018
5. Bio-Rad Lyphochek Immunoassay Plus Package Insert 1536-00 May 2017, Bio-Rad Laboratories, Irvine, CA
6. [CALIPER reference studies](http://www.sickkids.ca/Caliperproject/index.html), accessed 4/20/2018
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Elauteria Earnhardt | May 11, 2020 | New Procedure for Abbott analyzers |
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