# Laboratory Procedure Manual

***Analyte:*** Insulin

***Matrix:*** Serum

***Method:*** Immunoenzymometric Assay
TOSOH AIA-900 Chemistry Analyzer

***As performed by:*** *University of Missouri-Columbia*

***Contact:*** Dr. Randie Little

**Important Information for Users**

The University of Missouri periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

**Public Release Data Set Information**

This document details the Lab Protocol for testing the items listed in the following table:

| File Name | Variable Name | SAS Label |
|---|---|---|
| INS_I | LBXIN | Insulin (µU/mL) |
| | LBDINSI | Insulin (pmol/L) |

I. **Purpose Statement**

a. This assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Insulin (IRI) in human serum on Tosoh AIA System analyzers.
b. Scope: Technical Staff competent to perform the Insulin in Serum Assay on the AIA 900.

II. **Definitions:**

a. Not Applicable

III. **Procedure:**

a. Summary of Test Principle and Clinical Relevance—The AIA-PACK IRI is a two-site immunoenzymometric assay which is performed entirely in the AIA-PACK. This assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of Insulin (IRI) in human serum on Tosoh AIA System analyzers. Insulin present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-PACK. The magnetic beads are washed to remove unbound enzyme-labeled monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the insulin concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

Insulin, the antidiabetic hormone, is produced in the pancreatic β cell as[1,2] a large preproinsulin containing 109 amino acid residues with a molecular weight of approximately 11,500. This peptide is rapidly converted by cleavage to proinsulin consisting of 86 amino acid residues with a molecular weight of approximately 9,000 and is stored within the B cell secretory granules.[3] Equimolar quantities of insulin (51 amino acids with a molecular weight of approximately 6,000) and C-peptide (31 amino acids, molecular weight of approximately 3,000) are produced through proteolytic cleavage and then secreted along with a small amount of proinsulin.[2] Insulin exists in polymeric forms depending on the pH and zinc content. The monometric insulin molecule is composed of two polypeptide chains, "alpha" and "beta" which are connected by two interchain disulfide bridges of cystine.[3] Insulin is degraded in most tissues and has a plasma half-life of 7 - 15 minutes in man. It is also rapidly and completely inactivated in the gastrointestinal tract. Daily production of insulin in a healthy adult is 40 - 50 units. After binding to its specific receptors on target cell membranes, insulin acts as an anabolic and anticatabolic hormone, influencing the rates of carbohydrate, lipid, and protein and electrolyte metabolism.[2] Insulin release is stimulated by glucose. A failure to respond to this glucose stimulus may be one of the fundamental defects in human diabetes.[2] The factors stimulating or

inhibiting insulin release or the factors decreasing the tissue response to insulin have been well documented.

b. Safety Precautions:
    i. Gloves and laboratory coat are required for handling all human blood specimens. Dispose of all waste properly. Waste is segregated according to risk: Regular Trash (non-biohazardous, non-radioactive, non-sharp waste), Sharps Waste (sharp objects such as needles and contaminated glass), Broken Glass (clean, non-contaminated broken glass), Biohazard waste (all plastic tips, sample cups and gloves that contact blood) and washing waste (include diluents and wash solution).
    ii. All work surfaces and instruments are cleaned with a disinfecting detergent or bleach (10% sodium hypochlorite solution) after every run.
    iii. Smoking, eating or drinking is not permitted in work areas.
    iv. Discard all biohazardous waste into properly labeled containers (sharp, non-sharp)
    v. Dispose of washing waste in a sink flashing with large volumes of the water to prevent azide build-up
    vi. BODY SUBSTANCE PRECAUTIONS: All body substances (blood, serum, plasma, urine, etc.) should be treated as potentially infectious. Gloves and lab coat should be worn at all times when handling specimens. Discard contaminated gloves after use: do not touch doors, use phone or computer, nor touch any non-contaminated surface with latex gloves. Wash hands thoroughly after each procedure.
    vii. Hepatitis B vaccines are offered at no charge to the employee. Should a technician become exposed to a potential pathogen, such as an accidental needle stick, contact of blood on an open wound, etc., the Diabetes Lab will arrange for appropriate infectious disease testing (HIV, Hepatitis, etc.)
c. Specimen Collection, Storage, and Handling Procedures: Criteria for Specimen Rejection;
    i. Serum collected in Red Top Tubes is required for the assay. EDTA, heparinized and citrated plasmas SHOULD NOT BE USED. SST or gel tubes have not been validated. No special patient preparation is necessary. A venous blood sample is collected aseptically without additives (Red top tube).
    ii. Sample Stability: Refrigerated: 7days (see in-house stability study performed);[3]

        Frozen (- 20 °C or below): ≤ 60 days

        1. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to room temperature (18-25 $^{o}$C) and mix gently.

iii. For Pathology Samples—Laboratory services are requested through the Pathology Computer terminal Specimens are sent to IR Processing, which routes the sample to the appropriate lab.
    1. Each Transfer List should include:
        a. Patient name
        b. Patient's birthday and sex
        c. Patient ID number
        d. Specimen collection date and time
        e. Name of test
        f. Name of requesting physician
        g. Phone number
        h. Comments (if any)
    2. Each patient aliquot should be labeled with the information listed and verified against each Transfer List;
        a. Patient name, MRN, and Patient Accession (at least 2 two patient identifiers)
        b. Collection Date
        c. Time of Collection
        d. Lab name
        e. Test name

iv. The below should be followed for NHANES Clinical Trials Samples (refer to individual NHANES SOPs for handling the receipt of NHANES specimen and reporting):
    1. Laboratory services are requested through the Weststat system operations via an email notification containing a unique manifest list of the samples and sample analysis type (e.g. Insulin), which confirms that specimens have been shipped to DDL.
    2. Each Manifest Form should include and verified against each sample received:
        a. Patient Sample ID #
        b. Test Name
        c. Date Collected
        d. Shipment ID #
        e. Shipment Date
        f. Lab Name
        g. Lab ID
        h. Survey Year
    3. For other Research Study specimens the minimum labeling is required:
        a. Patient ID and number (two patient identifiers)
        b. Collection date and time

v. Specimen collection and processing:
    1. Serum obtained from venous blood, is required for this procedure.

2. At the draw station – Store at 18-25°C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain serum. Draw off the serum and store in a plastic cryovial at -20°C until the specimen can be transported to the laboratory.
3. Frozen serum specimens should be delivered within 24 hours of collection with a sample volume minimum of 200 µL.

vi. Handling Conditions:
1. Specimens should be transported under frozen conditions to the laboratory in a double-containment system to minimize the chance of leaking or spills. The cryovial containing the frozen serum should itself tightly sealed to the sample from leaking.
2. Upon receipt by the Diabetes Lab, the specimen will be logged in and stored at - 20°C until analysis for 1 week. If the samples are not analyzed within 1 week, store at -70 ° C. Analyzed specimens will be archived at -70°C for one month, after which they may be discarded. Due to sample volume samples are analyzed in batches.

vii. Unacceptable specimen criteria
1. Incorrectly labeled samples not following the above criteria.
2. Samples collected at room temperature conditions or past sample stability.
3. Samples not collect in Red Top Tubes.
4. Sample volume < 200 µL.
5. Conflicting information between specimen labeling and Transfer List.
6. Incorrect Test Name or Lab.
7. Indication of a sample leakage.
8. Hemolyzed samples.
9. Gross Lipemia
10. Specimen from patients who have received preparation of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated values when tested for insulin.
11. The below, must be followed for unacceptable specimen collections;
    a. Record all unacceptable samples in The Unacceptable Pathology Sample Received Log.
    b. Notify requesting physician or nurse station.
    c. If sample rejection is due to IR Processing error, call IR processing (882-1242), notified laboratory supervisor, and return rejected specimen.

d. Computerization: Data System Management:

i. Pathology tests are requested and resulted through the PATHNET system. Each authorized person is assigned a unique, confidential password for log-in purposes. To log onto the System:
    1. Log onto Pathnet.
    2. Click Appbar, the logon screen will show up.
    3. Enter user name and password then click OK.

ii. Receipt verify—each specimen arriving at the Diabetes Diagnostic Laboratory is accompanied by a transport list, and must be verified upon receipt. Verification may be performed by either the patient ID number (for single specimens) or by Transport List number (for multiple specimens).
    1. Click Specimen login icon of the tool bar then choose ACCESSION and click RETRIEVE.
    2. Type in each sample's accession number then press enter. The screen will show the Patient's name and other information.
    3. Make sure the information from the screen and the Transfer List is matching each other.
    4. Click LOG IN to verify the receipt.

e. Materials; Equipment and Instrumentation

Tosoh Materials- refer to the Tosoh's Operators' Manual for the AIA-900 system components.

i. OTHER MATERIALS (not provided by Tosoh)
    1. Waterproof markers (for labeling tubes)
    2. Pipette Stand
    3. Class A 20 mL Volumetric Pipette, calibrated "To Deliver" (Fisher Scientific, St. Louis, MO)
    4. Latex examination gloves
    5. Disinfecting detergent (hospital distribution).
    6. Ethyl Alcohol (Fisher Scientific)

ii. Instrumentation
    1. AIA-900
    2. Eppendorf Tip Ejector Fixed Volume Pipettes (300, 100 mL in volume, Fisher Scientific, St. Louis, MO).
    3. Eppendorf Repeater Pipet (range from 1 mL to 50 mL, precision to 0.1%, Fisher Scientific, St. Louis, MO).
    4. Combitips for Eppendorf Repeater with Adapter (2.5 mL tip graduated in 50 mL increment, Fisher Scientific, St. Louis, MO).
    5. Pipet Aid XP (Drummond scientific company)
    6. Pipetman Adjustable Pipet (200-1000 mL, Rainin Instrument, Woburn, MA)
    7. Milli-Q Plus Ultra-Pure Water System (Millipore, Bedford, MA)
    8. Volumetric pipets (1 mL, 5 mL)

iii. Warnings and Precautions
    1. The AIA-PACK IRI is intended for in vitro diagnostic use only.

2. Test cups from different lots should not be mixed within a tray.
3. Do not use beyond the expiration date.
4. The AIA-PACK IRI has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Samples with insulin concentrations between 320 and 30000 uU/mL will read > 320 uU/mL. The "hook effect" phenomenon may occur at insulin concentrations >30000 uU/mL.
5. The materials provided by Tosoh contain solution azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
6. Human sera is not used in the preparation of this product, however, since human specimens will be used for samples, standard laboratory safety procedures should be used in handling all specimens and controls.

f. Storage and Stability—All unopened materials are stable until expiration date on the label when stored at the specific temperature.
    i. Refrigerator Temperature (2 – 8°C)
        1. ST AIA-PACK IRI
        2. AIA-PACK IRI Calibrator Set
        3. AIA-PACK IRI Sample Diluting Solution
        4. AIA-PACK Substrate Set II
        5. AIA-PACK Wash Concentrate
        6. AIA-PACK Diluents Concentrate
    ii. Room Temperature (18 – 25 °C)
        1. AIA-PACK Detector Standardization Test Cups
        2. AIA-PACK Sample Treatment Cup
    iii. ST AIA-PACK IRI test cups may be stored for up to 24 hours at a room temperature of 18° - 25° C. Calibrators must be kept tightly sealed and refrigerated at 2° - 8° C. After opening, calibrators should be used within 7 days. After opening, Sample Diluting Solution is stable for up to 90 days refrigerated at 2° - 8° C. Reconstituted substrate solution is stable for 3 days at 18-25°C or 30 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at room temperature (18° - 25° C). Reagents should not be used if they appear cloudy or discolored.

g. Preparation of Reagents, Calibrators (Standards), and Controls:
    i. Reagent labeling
        1. Reagents, calibrators, controls, and solutions should be traceably identified to indicate the following:
            a. Content and quantity, concentration or titer
            b. Storage requirements.
        2. The below should be followed for reagents used daily
            a. Preparation date or opened date and the identity of the preparer.

b. Tech's initials.

ii. Reagent Preparation—Bring all reagents to room temperature before using. Mix all reagents thoroughly before use. When new reagents are received, they need to be initialed and dated by technician who received them. Opened and working reagents should be labeled with the opened/ expiration date, and tech's initials.

1. Substrate Set II Solution
    a. Bring all reagents to room temperature before preparing the working reagents.
    b. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.
    c. Mix thoroughly. Let stand for 20 minutes to dissolve contents completely.
    d. Label the bottle with the preparation date, tech's initials, and expiration date.
    e. The reconstituted Substrate Reagent is stable for 3 days at room temperature and 30 days in the refrigerator. Always store AIA-PACK Substrate Set II under refrigerated conditions (2 – 8 °C).
2. Wash Concentrate Set Solution
    a. Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water.
    b. Mix thoroughly and adjust the final volume to 2.5 L.
    c. Let it sit overnight at room temperature before use.
    d. Label the bottle with the preparation date, lot number, and expiration date.
    e. Once prepared the wash solution is stable for 30 days at room temperature. Always store the AIA-PACK Wash Concentrate Set under refrigerated conditions when not in use.
3. Diluent Concentrate Set
    a. Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I or NCCLS (CLSI) Type I Reagent Grade water.
    b. Mix well, and adjust the final volume to 5.0 L.
    c. Let it sit overnight before use.
    d. Label the bottle with the preparation date, lot number, and expiration date.
    e. Once prepared the working diluent is stable for 30 days at room temperature.
4. IRI Sample Diluting Solution

a. The AIA-PACK IRI Sample Diluting Solution is provided ready to use.
b. Always store the Sample Diluting Solution in an upright position at 2-8°C when not in use.

5. Unit Dose AIA Test Cups Reagent Cups
    a. Plastic test cups containing lyophilized magnetic beads coated with anti-insulin mouse monoclonal antibody and mouse monoclonal antibody (to human insulin) conjugated to bovine alkaline phosphates with 0.1% sodium acid as a preservative.

iii. Calibration

1. Using a volumetric pipette, reconstitute the lyophilized calibrators accurately to the volume of 1 mL with CAP Class I or NCCLS Type I Reagent Grade water.
2. Allow the lyophilized material to fully dissolve.
3. Mix calibrators gently, but thoroughly prior to performing the calibration.
4. Bring calibrator to room temperature prior to use.
5. Always store the Calibrator Set in an upright position at 2° - 8° C when not in use.
6. When stored unopened and refrigerated at 2° - 8° C, the AIA-PACK IRI Calibrator Set is stable until the expiration date on the label. After opening, the calibrators should be used within 7 days.

iv. Quality Control:

1. Commercially Available Controls –Three levels of lyophilized controls are purchased from Bio-Rad Laboratories; Lyphochek Immunoassay Plus Control Level 1, 2, and 3
    a. Allow one box to reach room temperature.
    b. Using a volumetric pipet, reconstitute each control with 5.0 mL distilled water or deionized water.
    c. Replace the stopper and allow the control stand for approximately 15 minutes swirling occasionally.
    d. Gently swirl the vial several times to ensure homogeneity.
    e. When controls are completely dissolved, combine the four bottles of same level (identical Lot numbers) into a beaker.
    f. Swirl mixture gently.
    g. Transfer 500 µL aliquots into polypropylene storage tubes and cap tightly.
    h. Label (each control level with techs name, prep and expiration date) and freeze at -70$^{o}$C. Enter information into the Biorad Controls Log Book/Diary Sheets. Thaw each aliquot one time only.
2. In-house Control—The In-House control is prepared by collecting one unit each of whole blood from three or more non-diabetic

volunteers. All blood is screened for HIV and Hepatitis. Serum is separated from red blood cells and serum from all donors is pooled. Reconstitution is not required for In-House control

a. Transfer 500 µL aliquots to polypropylene storage tubes. Cap tightly and freeze at -70$^{o}$C or colder.
b. Reconstitution of the In-House control is not required.
c. Thaw each aliquot one time only. Enter information into the In-House Control Log Book/Diary Sheets.

h. Calibration and Calibration Procedures
    i. Calibration curve
        1. The calibrators for use with the AIA-PACK IRI have been standardized against WHO 1st IRP 66/304 (1974).
        2. The calibration curve for the AIA-PACK IRI is stable for up to **90 days**.
        3. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and AIA System maintenance according to the manufacturer's instructions.
        4. Recalibration may be necessary more frequently if controls are out of the established range for this assay or if certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, or detector lamp adjustment or change) or the Test cup lot number is changed. Refer to AIA System Operator's Manual for additional instructions.
    ii. Calibration Procedure
        1. Press the ORDER (NON-BAR) button on the HOME screen
        2. Press the CALIB button to order calibration.
        3. Press the UP or DOWN button to select #IRI on the screen.
        4. Display the Numeric keypad screen in order to enter the lot No. or use the barcode scanner reading the lot No. from the barcode label.
        5. Move the cursor to CAL. Repeat step 4 in order to enter the concentration values until all the calibrator values are verified.
        6. If the correct lot No. and concentration values are displayed, press OK button. The calibration program for the analyte will appear on the ORDER (NON-BARCODE) screen. Each calibrator is run in triplicate.
        7. Press EXIT button back to HOME screen
        8. After placing the sample racks loaded with calibrators and test cups on the sample loader, press the (ASSAY START-NON) button on the HOME screen. The END OF REQUEST will display the number of calibrator.
        9. Press START button to start the assay. The display will return to the HOME screen.
    iii. Calibration Acceptability Criteria

1. The mean rate for the zero calibrator should be <3.0 nM/sec.
2. Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
3. The replicate values should be within a 10% range.

iv. Calibration Review and Acceptance
1. Upon completion of the assay, review the calibration curve carefully using the criteria listed above.
2. Edit the calibration if necessary, then accept the calibration curve by following the below steps:
   a. Press the (SUB MENU) button on the HOME screen to display the SUB MENU screen.
   b. Press the (CALIBRATION) button to display the CALIBRATION REVIEW (PENDING) SCREEN.
   c. Select the calibration to be reviewed and press the REVIEW button.
   d. Once the assay results have been confirmed, press the (CALCULATE) button, the Calibration Curve Graph will appear on the screen.
   e. Press the (ACCEPT) button to accept the calibration. If the calibration curve of the same lot already exists, the previous curve will be overwritten.
   f. Press the (PRINT) button to print the calibration curve.

i. Quality Control (QC) Procedures—Two types of quality control systems are used in this analytical method.
   i. Sample QC: Five percent of specimens are randomly selected and analyzed either within-assay or between-assay for quality assurance purposes.
   ii. Batch QC: quality control specimens are placed before and after all specimens analyzed. The bench quality control consists of four levels of controls, which cover the spectrum of Insulin ranges for both normal and diabetic populations. Three are commercial lyophilized serum controls purchased from Bio-Rad Laboratories (Irvine, CA). The other control is prepared in-house and stored in -70$^{o}$C or colder. One vial of each is thawed and used in each assay.
   iii. If the stock of these controls becomes low, another batch is ordered or prepared in time to analyze it concurrently with the current quality control materials. The new controls are used only after their means and the ranges are established after twenty characterization runs.
   iv. The bias limit is set at 1 SD or the 67% limit; the warning limit (WL) is the 2 SD or the 95% limit and the control limit (CL) is the 3 SD or the 99% limit.
   v. QC Guidelines—The three levels of commercial controls were used to established control ranges using 20 inter-assay observations. The in-house controls were established using 20 inter-assay observations.

vi. Refer to the Quality Controls' Manual for the current QC materials' used and acceptable ranges. QC acceptable ranges are also located at the bench where the assay is being performed. Please refer to the current QC acceptable ranges regularly if there are concerns prior to releasing patient results or refer to the supervisor for guidance.
    1. After each assay run, all control data are recorded on the Tosoh Insulin Diary Sheet.
    2. For Pathology Samples:
        a. QC should be treated the same as patient specimens.
        b. In the event of damage to packaging, contact the local Bio-Rad Laboratories Technical Services
        c. If QC falls outside the limits of the established range above notify the Supervisor.
        d. If QC is still unacceptable, recalibrate the assay and repeat the QC. If it is still unacceptable, try fresh QC material and/or calibrator, or new reagent. Notify the department supervisor and if needed, consult Bio-Rad technical service at 210-748-2199.
        e. Refer to section 12 for Calibration and QC remedial action.
    3. The analysis is judged to be accepted or rejected following the guidelines established by the CDC for National Health And Nutrition Examination Survey (NHANES III, 9/21/89) with a slight modification on the determination of a trend.
        a. The quality of an assay is assessed by two types of quality chart plots (Levy-Jennings). The first chart plots the mean of all the replicate determinations in a run. It is then compared with the target mean which is the overall mean established by the twenty characteristic runs.
            i. The NHANES guideline declares a system "out-of-control" if any of the following events occur for any one of the quality control materials:
                1. The mean from a single control falls outside the 99% (3SD) confidence limits;
                2. The means from two controls fall outside the 95% (2SD) confidence limits; or
                3. The daily means of one control from eight successive runs lie either all above +1SD or all below -1SD.
        b. The second type of quality control chart plots the range of the replicates (the difference between the highest and lowest value of a single control within a run). It is compared with the target range which is the overall mean of daily ranges established by the twenty characteristic runs.

i. The NHANES guideline declares a system as "out-of-control" if any of the following events occur for any one of the quality control materials:
    1. The daily range from a single control falls above the 99% confidence limit;
    2. The daily ranges from two controls fall above the 95% confidence limits; or
    3. The daily ranges of one control from eight successive runs all fall above +1SD.
4. If the system is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the cause of the problem before any analysis of specimens.

j. Procedure Operating Instructions; Calculations; Interpretation of Results
    i. Preliminaries
        1. Allow frozen samples and controls (three levels of commercial lyophilized serum controls and one level of pooled non-diabetic serum In-House control) to reach room temperature. Invert gently to mix.
        2. Bring the substrate reagent, calibrators if necessary and test cups to room temperature.
        3. Label sample cups (provided by Tosoh) using a fine-point permanent marker with sample ID or control ID.
        4. Check the Diluent solution and wash solution bottle level. Fill the bottles as needed.
        5. Empty the waste bottle and solid waste container.
    ii. Instrument set-up
        1. Place Substrate in substrate compartment. Power on. Wait for Log On screen.
        2. Logon by pressing OK to retain current operator ID. For new operator, press (OPERATER) button then (MODIFY) button to enter your ID. After enter your ID, press OK. Your ID will be saved on user list. Press OK again and the following screen will appear with your user ID.
        3. Press OK again and daily check Screen will appear. Following the screen to do the daily, weekly and monthly maintenance.
        4. Place a cup adapter rack with a Standardization Cup (STD) in position 2 to the instrument. Press OK. Automated maintenance will begin.
        5. Record results of the Substrate Background Measurement on the Substrate Background form and keep the printed result for troubleshooting.
        6. Press (RT.OPEN) button (orange color) to refill tip rack. Then press OK.

iii. Processing Samples:
    1. Using an Eppendorf pipette, transfer the required volume of controls and samples into the corresponding sample cups. Remove any bubbles. For each run one set of controls is placed in front and 2nd set is placed at the end.
    2. Put sample cup into the sample rack with test cup. Place the rack on the instrument using a maximum of eight racks at a time. Put end marker tube in the last hole of the last rack so that the instrument will stop.
    3. Press ASSAY START (NON) button on HOME screen. The number 0 will be displayed in the box of the END OF REQUEST.
    4. Press START to start the assay.
    5. To run more samples repeat step 2 and 4.

iv. Procedural Notes
    1. If other number other than 0 displayed in box of the END OF REQUEST, press the cell to display the NUMERIC KEYPAD screen. Enter 0 than press OK.
    2. The AIA-900 is provided with a camera which reads the analytic name and lot number printed on the test cup. It enables the Instrument to automatically distinguish one specimen from another and also to recognize the assay to be done for each specimen.
    3. Any changes to procedure must be documented. Major changes to the SOP may include the way a procedure is performed or calculations and requires the approval from the Medical Director. Minor changes include typo graphical errors or other minor corrections that do not change the way the procedure or calculation is performed and do not require approval of the laboratory director. Major SOP changes must be reviewed by the Lab Director prior to SOP update.
    4. Any changes to the SOP will be communicated to technical staff via verbal communication and email notification. Technical Staff after reading the changes made to the SOP will sign and date their SOP review.

v. Printing after Calibration
    1. The AIA System performs all sample and reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically converts the rate to insulin concentration in µU/mL then both the rate and concentration are printed out.
    2. If the calibration curve is undetermined before the analysis, use the steps below to recalculate results after a calibration curve is determined.
        a. Press RESULTS button in HOME screen.

b. Place the cursor line under the first sequence number desired.
c. Press SELECT button.
d. Use the down arrow key to move the cursor to the last sequence number desired.
e. Press SELECT again. A green > sign will be on left of the result selected.
f. Press FUNCTION button, the screen will appear with 4 options. Select option RECALC then press OK.
g. Repeat step 3 to 6 but select option PRINT instead of RECALC then press OK. The result will be printed on the printer tape.

vi. Recording of Data

1. Quality Control Data—All replicate values of quality control data plus all pertinent assay information are recorded in the Tosoh Insulin Assay Log Database located on the network drive. Print out the Tosoh Insulin Diary Sheet.
2. Analytical Results—When the assay is accepted, record the results.
    a. For NHANES and Research Samples:
        i. Enter results on the corresponding test request form and then enter the result in the corresponding Database located on network drive.
        ii. During the data entry process, check the lab accession number. Enter any comments associated with the specimen in the comment field.
        iii. Print a data check sheet with test result. Test result is checked against the instrument print out by the supervisor. A copy of the data check sheet is kept in the appropriate book at the Diabetes Diagnostic Laboratory.
3. For Pathology samples—Test results are recorded in the Pathology Log book. Results are then entered into PATHNET via the Department of Pathology computer system.
    a. Result Entry:
        i. Log on to the system (Refer to Section 4).
        ii. Click the Batch Result Entry icon from tool bar.
        iii. In the Procedure box, Type the test name (insulin) then click the icon beside the box. A group test name will be show on the screen. Choose the exact test listed on the specimen order.
        iv. For test site box, type “uh dd man” in then click OK.

v. Enter the results then click TASK to choose Print screen.
vi. Click Perform to finish result entry.
vii. Insulin results are checked against the original printout by Lab supervisor prior to release of results.

b. Result Verification (**Supervisor Only**) – Check and Release Result
i. Refer to Steps 1 – 3 above.
ii. Check the result then Click Verify.

vii. Evaluation of Results
1. Quality Control—Frequency
a. In order to monitor and evaluate the precision of the analytical performance, QC should is included with every run.
b. After calibration, three levels of controls are run in order to accept the calibration curve.
c. Controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change)
d. If one or more control sample value(s) is out of the established acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

viii. Replacement and periodic maintenance of key components
1. Perform the Daily maintenance on the day of assay by following the DAILY MAINTENANCE schedule form. Discard the used sample cups when the analysis is complete. Turn off the instrument once every 24 hours.
2. Clean the B/F Wash Probe tip weekly with a cotton ball moistened with 70% ethanol.
3. Wash the Substrate line weekly.
4. Replace the wash probe tip on the end of the B/F wash probe monthly.
5. Clean diluents and wash tanks tri-monthly with 1:100 dilution of hypochlorous acid solution then rinse reservoirs with DI water
6. AIA-900 performs a substrate background measurement each time daily maintenance is run and the results are automatically printed out. If the substrate background measurement is within specifications, an OK will be displayed next to 4MU Background. If the substrate background is too high a “BH” (blank high) error flag will be printed and Substrate Replacement will be incomplete. Prime or replace the substrate and repeat daily maintenance. If

the lamp intensity level is within specifications an OK will be displayed next to Lamp Intensity Level. If the lamp intensity level is too low an “LL” (lamp low) error flag will be printed. The “LL” is warning that the lamp will need to replace soon.

ix. Calculations
    1. Any specimen with a concentration > 250 µU/mL should be diluted with the AIA-PACK IRI Sample Diluting Solution and re-assayed according to the Assay Procedure. It is desirable to dilute the sample so the diluted sample reads within the established linear range. The result measured from the diluted sample should be multiplied by the dilution factor prior to reporting. Linearity is verified every 6 months.
    2. The low detection limit for all specimens with insulin values less than 1.0 µU/mL are reanalyzed for confirmation and then reported as "<1.0".

k. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria:
    i. When the QC results fail to meet the acceptable criteria, check the sample cup containing the QC specimen for bubbles and reanalyze the QC specimen.
    ii. If the QC results meet the acceptable criteria, accept the run and report the results. Otherwise, troubleshoot the system to locate probable cause of the problem. If a cause can be identified and corrected, notify the supervisor.
    iii. If no obvious cause of a problem can be identified, reject the run. Patient results are considered invalid and the results from the run should not be reported. The run should be repeated and QC results accepted prior to reporting of patient results.
    iv. Document the problem and actions taken, if any, on the daily worksheet comment section

l. Limitations of Method; Interfering Substances and Conditions:
    i. Interferences
        1. Free Bilirubin (up to 17 mg/dL) and conjugated Bilirubin (up to 19 mg/dL) do not interfere with the assay
        2. Lipemia, as indicated by triglyceride concentrations (up to 1,660 mg/dL), does not interfere with the assay. [3]
        3. Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.
        4. Protein, as indicated by human albumin concentrations (up to 5g/dL), does not interfere with the assay.
        5. Hemoglobin may interfere with the assay, particular at the low range. Hemolyzed sample should not be used for this assay because hemolysis may falsely lower the value due to insulin degrading enzyme in red blood cells. [1,3]
        6. Refer to Product Insert for additional details.

m. Reference Ranges (Non-Diabetic Values):

i. Tosoh suggested mean fasting levels for healthy individuals is below 17 uU/mL.

ii. Reference ranges for insulin were updated at the Diabetes Diagnostic Laboratory in January 2013 by combining results from two volunteer groups. A 360 caloric standard meal (Boost™) challenge was performed in Februrary 2009 for fasting and 120 minutes on non-overweight, non-diabetic subjects (n=15, mean age=34, M:F=8:7). A 360 caloric standard meal (Boost™) challenge was performed in October 2012 for fasting, &120 minutes on non-obese, non-diabetic subjects (n=29, mean age =39, M:F=20:9). All participants fasted overnight for at least 10 but no more than 15 hours. Any subject with a BMI greater than 25 $kg/m^2$ and fasting glucose greater than 100mg/dl were excluded from the calculation. Refer to validation study binder.[3] The means and observed ranges are:

| **Insulin Reference Range: µU/mL** | | |
|---|---|---|
| | **Fasting** | **120 min** |
| N | 44 | 44 |
| Mean | 6 | 14 |
| Range | 1 - 14 | 1 - 35 |

iii. Proper interpretation of the Insulin results can be difficult: values are affected by many factors, such as body mass index, age and state of nutrition. Results that are outside of these reference ranges do not necessarily mean the abnormal test result is of clinical significance. This should only be determined by a physician after careful evaluation of the individual person's health record.

n. Critical Call Results (“Panic Values”)—Since test values vary depending on the individual's health record, all values are reported to the physician with no further action taken. Specimens may be repeated for verification upon request of the physician.

o. Alternate Methods for Performing Test or Storing Specimens if Test System Fails—If the analytical system fails, all specimens are returned to storage at -70°C. The specimens are re-analyzed when the system is back in control (repeat thaw should be avoided).

p. Test Result Reporting System—After the assay has been accepted by the tech, the instrument prints outs, QC results and Laboratory worksheets are printed for supervisor overview. The supervisor ensures QC results pass within acceptable ranges and verifies instrument print out against each patient accession listed on the Laboratory Worksheet. Results are entered into the LIS data base, further verified and released. (Refer to Section J. vi. Procedure Operating Instructions; Calculations; Interpretation of Results).

IV. **Attachments:**

a. Not Applicable

V. **References:**

1. Insulin ST AIA-PACK IRI Product Insert.
2. AIA 900 Operator's Manual.
3. AIA C-peptide and Insulin Validation Study Binder.

VI. **Summary Statistics and QC Graphs**

See next page

## 2015-2016 Summary Statistics and QC Chart for Insulin (µU/mL)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| 40283 | 33 | 14JAN15 | 26AUG15 | 156.003 | 4.423 | 2.8 |
| 40281 | 33 | 14JAN15 | 26AUG15 | 16.799 | 0.956 | 5.7 |
| 40282 | 33 | 14JAN15 | 26AUG15 | 53.836 | 1.970 | 3.7 |
| IH13 | 105 | 14JAN15 | 31JAN17 | 77.791 | 2.515 | 3.2 |
| 40303 | 72 | 02SEP15 | 31JAN17 | 180.586 | 4.226 | 2.3 |
| 40301 | 72 | 02SEP15 | 31JAN17 | 17.914 | 0.823 | 4.6 |
| 40302 | 72 | 02SEP15 | 31JAN17 | 54.943 | 1.843 | 3.4 |