Laboratory Procedure Manual

Analyte:	**Insulin**
Matrix:	**Serum**
Method:	**Tosoh AIA-900 Two-site Immunoenzymometric Assay**
Revised Date:	***May* 31, 2023**
As performed by:	**Diabetes Diagnostic Laboratory** **University of Missouri School of Medicine** **1 Hospital Dr. Columbia, MO 65212**
Contact:	**Curt Rohlfing, Rhonda Howard, Dr. Randie Little**

Important Information for Users

The University of Missouri, Diabetes Diagnostic Laboratory 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:

Data File Name	Variable Name	SAS Label
INS_L	LBXIN	Insulin (µU/mL)
	LBXINSI	Insulin (pmol/L)

1. 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.

Summary and Explanation of the Test

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.[b] The factors stimulating or inhibiting insulin release or the factors decreasing the tissue response to insulin have been well documented.

2. SAFETY PRECAUTIONS

1. While working in the lab proper Personal Protective Equipment (PPE) is enforced. This includes wearing gloves, lab coats, face masks (as applicable) and protective eye wear such as goggles, and closed toe shoes are required for handling all human blood specimens. Once gloves are removed wash your hands or use hand sanitizer to ensure your hands are clean before leaving the lab.
2. Vials containing human blood are only to be opened in a biological safety cabinet with the sash in the correct position.
3. All plastic tips, sample cups, gloves, etc. that contact blood are considered contaminated and are to be placed in a biohazard waste container.
4. All hoods, telephones, doorknobs and work surfaces are wiped down with Oxyvir disinfectant or 10% bleach at least one time during each work shift. Any area in which blood is spilled is also to be cleaned and disinfected immediately with Oxyvir disinfectant or 10% bleach. Refer to the Lab Safety Manual located in room M764for additional details.
5. All healthcare personnel shall routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any patient is anticipated. All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear appropriate Personal Protective Equipment (PPE), including facial protection, gloves, and protective clothing. Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of analysis. Dispose of all liquid hazardous waste in properly labeled hazardous waste container.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Data are maintained on a secured Microsoft Access / Microsoft SQL server client-server system in a 128-bit authenticated Windows domain environment.

1. Laboratory services are requested through the Westat system operations via an email notification containing a unique manifest list of the samples and sample analysis type (e.g. INL), which confirms that specimens have been shipped to DDL.
2. Each Manifest Form should include and be 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. Once specimens are received and verified the corresponding file is imported electronically into the SQL server database via secure transfer.
4. After analysis the results, date analyzed and tech initials are imported from the instrument into the SQL server database via secure transfer.
5. Data check sheets are printed out and checked against the instrument printouts by the supervisor.
6. After results are cleared by the supervisor a results file in the specified format is exported and uploaded to Westat via secure transfer.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

1. Patient Preparation: Fasting at the request of physician.
2. Specimen Type and stability:
   a. 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. A venous blood sample is collected aseptically without additives (Red top tube).
   b. Sample Minimum – min volume required for analysis directly from collection tube is 300 µL (ideally 1 mL is preferred).
   c. Storage – Refrigerated: 1 day. Frozen: (- 20); (– 70 °C): ≤ 60 days; 10 years
3. Each specimen must arrive in the laboratory with a unique barcode identification number. Unacceptable specimen criteria:
   a. Clotted samples
   b. Specimen types and stability not listed above (e.g. urine, plasma, whole blood). Only accept serum samples.
   c. Unlabeled samples.
   d. Grossly hemolyzed samples
4. If an unacceptable specimen is received notify NHANES and Westat, and add the appropriate comment code in the database.
5. Handling Conditions:
   a. Samples are to be maintained under frozen condition immediately after collection.
   b. Transport under frozen conditions.
   c. Once received samples should be maintained under frozen conditions prior to analysis. After analyzed samples should be stored at – 70 ^{o}C.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

1. Equipment:
   Automated Enzyme Immunoassay Analyzer (AIA-900); ST IRI Test Code 058.
   Refer to Operator's Manual for Startup, Operation, and Shut down procedures.
2. Materials

REF	CONTENT
22930	AIA-900 System
20968	AIA-PACK Substrate Set II
20360	AIA-PACK IRI Calibrator Set
20560	AIA-PACK IRI Sample Diluting Solution
20955	AIA-PACK Wash Concentrate Set
20956	AIA-PACK Diluents Concentrate
20970	AIA-PACK Detector Standardization Test Cups
20971	AIA-PACK Sample Treatment Cup

   Reagent Preparation:
   Refer to Package Insert for instruction for use details.
3. Calibrators (Standards) – Calibrators are handled and prepared following manufacturer's instructions.
4. Controls
   a. At least two levels of controls are purchased Bio-rad Laboratories; Lyphochek® Immunoassay Plus Controls. Refer to manufacturer's package insert for processing and handling details.
   b. In-house controls once thawed are ready for use – Controls were prepared by collecting one unit each of whole blood from three or more non-diabetic volunteers. Transfer 500 µL aliquots to polypropylene storage tubes. Cap tightly and freeze at -70^{o}C or colder.
   c. Controls once thawed to room temperature should be immediately used for testing. Controls should be kept under refrigerated conditions until the results are checked by a supervisor.
   d. Controls are discarded after daily use.
5. Adjustable Variable Volume Pipettes in 0.5-10, 2-20, 20-200, and 100-1000 µL volumes.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

1. When using new calibrator lots, enter the calibrator concentration values and lot number into the software test file (Refer to the Tosoh AIA System Operator's Manual for details).
   i. Calibrators for ST AIA-PACK IRI are lyophilized and require reconstitution prior to use.
   ii. Load the appropriate amount of ST AIA-PACK IRI test cups on the instrument.
   iii. Add the appropriate amount of each calibrator to sample cups.
   iv. The ST AIA-PACK IRI Calibrator Set contains assigned concentrations of insulin. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 0.5 to 320 µU/mL of insulin.
   v. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
   vi. Calibrators should be run in triplicate measurements.
   vii. Press the CALIB button to order calibration.
   viii. Press the UP or DOWN button to select #IRI on the screen.
   ix. 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.
   x. Move the cursor to CAL.
   xi. 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.
   xii. Press EXIT button back to HOME screen.
   xiii. 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.
   xiv. Press START button to start the assay. The display will return to the HOME screen.

2. Calibration verification is performed at a minimum when a system is first placed in service and every 90 days. Recalibration is required (regardless of the length of time since last performed) immediately if any of the following occurs:

   a. A change of reagent lots for chemically or physically active or critical components, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results or the range used to report patient/client test data.
   b. If QC materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.
   c. After major maintenance or service. The Laboratory Director must determine what constitutes major maintenance or service.
   d. When recommended by the manufacturer.

3. Calibration Acceptability Criteria
   a. The mean rate for the zero calibrator should be <3.0 nM/sec.
   b. Since there is a direct relationship between concentration and rate, the rates should increase as the concentration increases.
   c. The replicate values should be within a 10% range.
4. Calibration Review and Acceptance
   a. Upon completion of the assay, review the calibration curve carefully using the criteria listed above.
   b. Edit the calibration if necessary, then accept the calibration curve by following the below steps:
      - Press the (SUB MENU) button on the HOME screen to display the SUB MENU screen.
      - Press the (CALIBRATION) button to display the CALIBRATION REVIEW (PENDING) SCREEN.
   - Select the calibration to be reviewed and press the REVIEW button.
   - Once the assay results have been confirmed, press the (CALCULATE) button, the Calibration Curve Graph will appear on the screen.
   - Press the (ACCEPT) button to accept the calibration. If the calibration curve of the same lot already exists, the previous curve will be overwritten.
   - Press the (PRINT) button to print the calibration curve.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

1. Preliminaries
   a. Allow frozen samples and controls to reach room temperature. Invert gently to mix.
   b. Bring the substrate reagent, calibrators if necessary and test cups to room temperature.
   c. Label sample cups (provided by Tosoh) using a fine-point permanent marker with sample ID or control ID.
   d. Check the Diluent solution and wash solution bottle level. Fill the bottles as needed.
   e. Empty the waste bottle and solid waste container.
2. Instrument set-up – (Refer to Chapter 5 of the Operator's Manual for **start-up** procedure, Chapter 8 for the **Operations** procedure, and Chapter 9 for **SHUT Down** procedure).
   a. Place substrate in substrate compartment. Power on. Wait for Log On screen.
   b. Log on by pressing OK to retain current operator ID. For new operator, press (OPERATOR) 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.

c. Press OK again and daily check screen will appear. Following the screen to do the daily, weekly and monthly maintenance.
d. Place a cup adapter rack with a Standardization Cup (STD) in position 2 to the instrument. Press OK. Automated maintenance will begin.
e. Record results of the Substrate Background Measurement on the Substrate Background form and keep the printed result for troubleshooting.
f. Press (RT.OPEN) button (orange color) to refill tip rack. Then press OK.

3. Processing Samples:

a. 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.
b. 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.
c. Press ASSAY START (NON) button on HOME screen. The number 0 will be displayed in the box of the END OF REQUEST.
d. Press START to start the assay.
e. To run more samples repeat steps 2 and 4.

4. Procedural Notes

a. 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.
b. 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.
c. 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 Medical Director prior to approval.
d. Any changes to the SOP will be communicated to technical staff via verbal communication, email notification and changes to procedures are tracked in Navex. Technical Staff after reading the changes made to the SOP will sign and date their SOP review.

5. Printing after Calibration

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.

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 g to d but select option PRINT instead of RECALC then press OK. The result will be printed on the printer tape.

6. Replacement and periodic maintenance of key components
   a. 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.
   b. Clean the B/F Wash Probe tip weekly with a cotton ball moistened with 70% ethanol.
   c. Wash the Substrate line weekly.
   d. Replace the wash probe tip on the end of the B/F wash probe monthly.
   e. Clean diluents and wash tanks tri-monthly with 1:100 dilution of hypochlorous acid solution then rinse reservoirs with DI water.
   f. 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 be replaced soon.

7. Calculations
   a. The AIA Systems perform all sample reagent handling operations automatically. The AIA Systems read the rate of fluorescence produced by the reaction and automatically converts the rate of insulin concentration to µU/mL.

8. Recording of Data
   a. 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.
   b. Analytical Results—When the assay is accepted, record the results.
   c. Refer to the NHANES study sample handling and reporting procedure in Navex for additional details.
   d. During the data entry process, check the sample accession number. Enter any comments associated with the specimen in the comment field.

e. Print a data check sheet with results. Results are 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.

9. REPORTABLE RANGE OF RESULTS

The AMR is 0.5– 320 µU/mL The AMR is verified every 3 months when the assay is calibrated.

a. Limit of Detection (LOD) – 0.5 µU/mL
b. Reportable/Non-reportable values:
   Results less than 0.5 µU/mL are reanalyzed for confirmation and then reported as "<0.5" (less than 0.5). Insulin results that exceed 320 µU/mL should be 1:10 (or 1:100 depending on the re-assayed result) diluted with the ST AIA-PACK IRI Sample Diluting Solution, and reassayed so the diluted specimen reads between 0.5 to 320 µU/mL. Insulin results that exceed 320 µU/mL are reported as “>320.0” (greater than 320.0) with the added below comment, “Insulin concentration above the linear range for this assay, sample was re-analyzed as a 1:10 (or 1:100 [add the correct dilution factor] that was used) dilution and result was multiplied by 10 (or 100) to yield a final result of (add final result) µU/mL.”

10. QUALITY CONTROL (QC) PROCEDURE

1. QC is used to ensure that a test system is performing accurately. QC aliquots are tested in the same manner as patient specimens and by the same personnel performing patient testing. QC is used to monitor the analytic performance of the testing being performed at DDL and to certify that all results reported by the lab are accurate.
2. For the insulin assay two types of quality control (QC) systems are used in this analytical method: 1) "sample QC" and 2) "batch QC". For sample QC, 2% of specimens are randomly selected and analyzed either within-assay or between-assay for quality assurance purposes. If the difference between duplicates is greater than ± 20% or 1.0 µU/mL(whichever is greater), the specimen is reanalyzed.
   Batch QC: The bench quality control consists of at least three levels of controls, which cover the spectrum of insulin ranges for both normal and diabetic populations. At least two levels of commercial controls are purchased from Bio-Rad Laboratories. Other matrix appropriate controls are prepared in-house and stored in -70 °C or colder. In-house controls are used for quality assurance purposes and do not affect the reporting of results. When available one vial of each level of in-house control is thawed and used in each assay.

The quality control levels for this assay are established from calculating the mean, SD, and CV (for each level) from of at least 20 interassay determinations. The bias ranges of the daily results are set at ±1 SD or the 67% confidence interval (CI); the warning limits (WL) are the ±2 SD or the 95% CI and the control limits (CL) are the ±3 SD or the 99% CI. For manufacturer controls, the established acceptable limits should fall within manufacturer's defined limits for each level of control. If the stock of these controls becomes low, another batch is ordered or prepared in time to analyze it concurrently with the current QC materials. The new controls are used only after their means and the ranges have been established by performing 20 characterization runs. Established acceptability limits specific for each level of control (new controls) are approved by the Lab Director prior to implementation.

3. Controls are tested with every run. Control results must fall within the specified ranges prior to the release of results. Control results are reviewed daily by the bench tech and lab supervisor/delegee (prior to the release of results) and examined weekly by the lab supervisor or delegate.
4. QC charts are plotted for the daily results for each control and compares them to the established target ranges. The ranges are approved (monthly) by the Lab Director or delegate.
5. If control results are outside of the acceptable limits, patient results should not be reported. Patient samples should be return to optimal storage conditions until all QC issues are resolved. QC Corrective actions could include repeating of QC (of the same lot), performing a recalibration procedure or changing out the appropriate reagents.
6. QC results must be acceptable prior to releasing patient results. If QC results are still unacceptable after performing corrective actions, call the appropriate technical hotline for additional troubleshooting advice and alert the supervisor or delegate immediately.
7. All Corrective Action/Preventive Action (CAPA) events concerning out of range controls, calibrators, or rejected runs that occur at the time of testing, must be documented (in the daily diary sheet for that particular assay) and immediately communicated (verbally and by email) to the Lab Director or Supervisor. The QC policy found in the Quality Management Program should be used as a guide for additional details.
8. **Beginning of the day QC** is rejected if any of the following events occur for an individual quality control level;
   i. A single control level falls outside the 99% (3SD) confidence limits (1 3S rule);
   ii. Two or more controls fall outside the 95% (2SD) confidence limits (2 2 S rule); or
   iii. Eight sequential values for a control fall either all above or all below the mean, not including values that fall within 1 SD.

10. **End of the day QC** should be performed when the number of samples on the batch run exceeds five samples (n > 5 samples). Results from end of day QC should all fall within ± 3SD of the established acceptable limits.
11. After each assay run, all controls are recorded on the Daily Diary Log Sheet.
12. Refer to the Quality Controls' Manual for the current QC materials used with 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 if there are concerns prior to releasing patient results and consult with the lab manager (or delegate) if you have questions.
13. Quality Control—Frequency

    a. In order to monitor and evaluate the precision of the analytical performance, QC is tested in the same manner as patient samples and by the same personnel performing patient testing.
    b. QC results must be acceptable prior to releasing patient results. If beginning control results fall outside the established acceptability limits, patient results should not be reported. Patient samples should be returned to optimal storage conditions until all QC issues are resolved. All QC Corrective actions should be documented.
    c. QC corrective action could include retrieving new controls of the same lot, reconstituting of new QC, performing a re-calibration, or performing analytical precision studies to access the integrity of the QC in used against the acceptable limits.
    d. Once beginning QC is considered acceptable, patient samples should be tested, and end of day QC should be performed for quality assurance purposes to verify the acceptable performance of the AIA-system.
    e. End of day (closing) QC should fall within ± 3SD of the established acceptable limits. If closing QC results for each level is not within ± 3SD an immediate QC corrective action (as stated above) should also be performed.
    f. If it is determined that there is a systemic issue with the AIA-system, it is the responsibility of the tech to immediately call the technical service hot line at 1-800-248-6764 for additional troubleshooting advice.
    g. Once all analyzer issues are resolved, and closing QC results are considered acceptable, selected samples from the prior run should be re-assayed and results should be within ± 20 % or 1.0 µU/mL of the previous results.
    h. QC should also be performed after calibration; at least two levels of controls are run in order to accept the calibration curve.
    i. QC is also performed after a PM (sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).
    j. QC should be performed after daily maintenance to verify the overall performance of the analyzer.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

1. 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.
2. If QC is still unacceptable perform a recalibration of the assay and reanalyze the QC. QC must be considered acceptable prior to releasing patient results.
3. If steps above do not result in correction of the "out-of-control" values for QC materials, troubleshoot the instrument and reagents until the system is back “in control”. Refer to the above QC procedure section for additional details.
4. Samples should be placed under optimal storage conditions during instrument and reagent troubleshooting.
5. All corrective actions should be documented.
6. QC must be considered acceptable prior to releasing patient results.
7.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

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. For patients that are being treated with Asfotase Alfa (Strensiq®), elevated test results are likely to occur using this method. Results should not be used for patients receiving Asfotase Alfa treatment.
   Asfotase Alfa is a prescription medicine used for treatment of patients with hypophosphatasia (HPP), a rare genetic metabolic disorder characterized by the abnormal development of bones and teeth because of defective mineralization.

   About HPP, from NORD (National Organization for Rare Disorders);
   ref: http://raredisease.org/rare

   An alternative test method should be used that does not utilize alkaline phosphatase technology. All results should be interpreted with respect to the clinical picture of the patient.

   Refer to Product Insert for additional details.

13. REFERENCE RANGES (NORMAL VALUES)

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 February 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

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.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not Applicable for this Procedure

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are stored at -70°C until analyzed. On the day of analysis, thaw the specimens. Mix thoroughly. Upon completion of analysis, refreeze at -70°C.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

If the instrument is unable is perform the test, the specimens are stored at -70°C until testing is available. NHANES should be notified if the platform will be down for an extended period of time.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

NHANES data files with results are exported from the NHANES database in the specified format and uploaded to Westat via secure transfer weekly.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

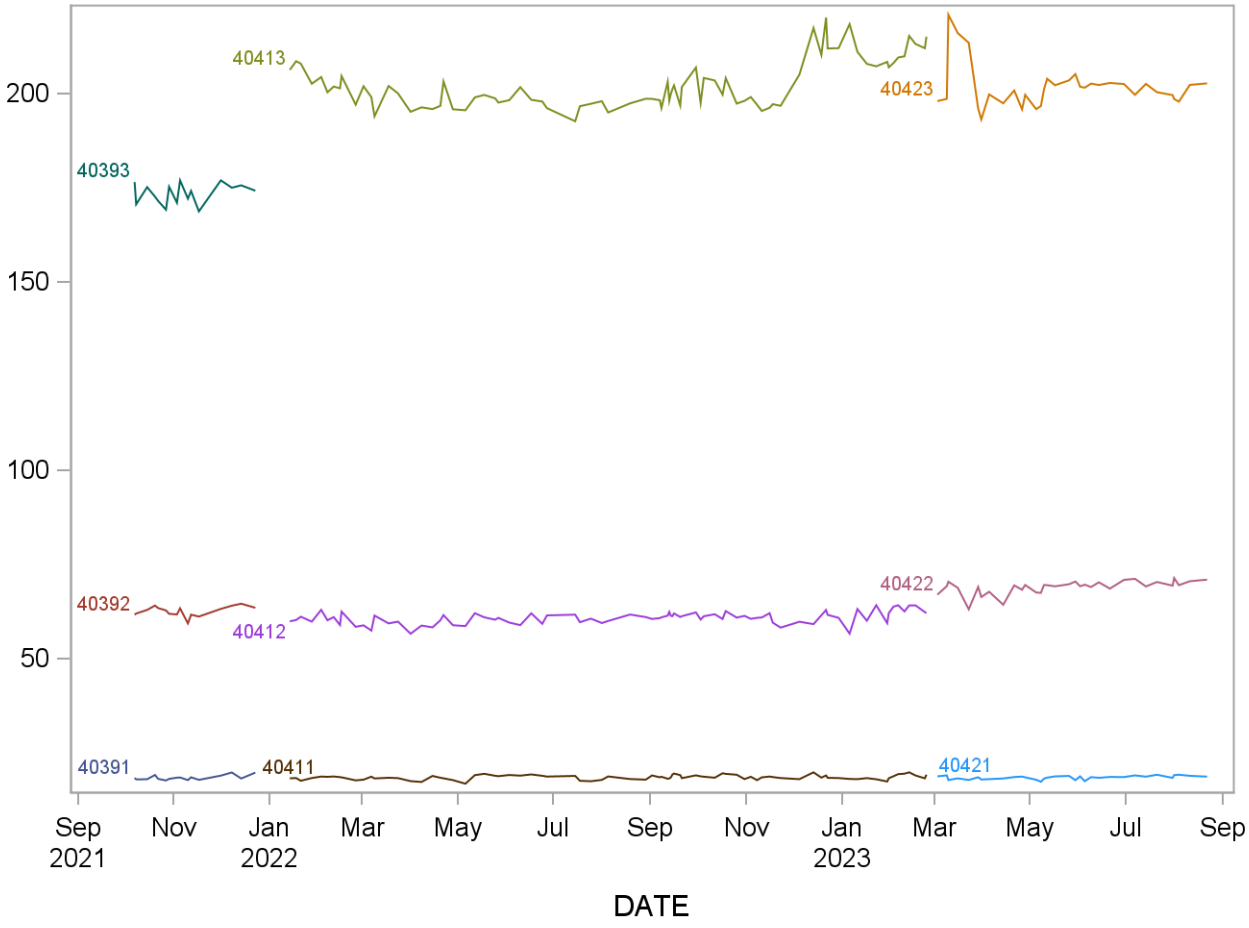
All shipments are recorded on the NHANES Shipping Log upon receipt. Actions taken during the course of analysis, result reporting, and specimen retention are also recorded on the log. Specimens are stored frozen at -70 °C or colder after analysis; specimen locations are recorded according to sequential DDL accession number and box number. After one year, specimens may be shipped to the SriSai Biorepository for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Please see following page

2021-2022 Summary Statistics and QC Chart
LBXIN (Insulin (uU/mL))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
40393	16	07OCT21	23DEC21	173.498	2.703	1.6
40391	16	07OCT21	23DEC21	18.259	0.657	3.6
40392	16	07OCT21	23DEC21	62.475	1.337	2.1
40413	81	14JAN22	24FEB23	202.314	6.352	3.1
40411	81	14JAN22	24FEB23	18.339	0.605	3.3
40412	81	14JAN22	24FEB23	60.695	1.629	2.7
40423	33	03MAR23	22AUG23	201.737	5.666	2.8
40421	33	03MAR23	22AUG23	18.268	0.490	2.7
40422	33	03MAR23	22AUG23	68.891	1.815	2.6



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