Laboratory Procedure Manual

Analyte: Insulin

Matrix: Serum

Method Human Insulin Immunoassay
Using ROCHE ELECSYS 2010

as performed by: Fairview-University Medical Center
University Campus
Collaborative Studies Clinical Laboratory
Minneapolis, Minnesota

Contact: Dr. Michael Steffes

Important Information for Users

The University of Minnesota 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 items in the following table:

Data File Name	Variable Name	SAS Label
GLU_F	LBXIN	Insulin (μU/mL)
	LBDINSI	Insulin (pmol/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Insulin is a peptide hormone with a molecular weight of approximately 6000 daltons. It is secreted by the B-cells of the pancreas and passes into circulation via the portal vein and the liver. Insulin is generally released in pulses, with the parallel glucose cycle normally about 2 minutes ahead of the insulin cycle.

The insulin molecule consists of two polypeptide chains, the α-chain with 21 and the β-chain with 30 amino acids. Biosynthesis of the hormone takes place in the β-cells of the islets of Langerhans in the form of single-chain preproinsulin, which is immediately cleaved to give proinsulin. Specific proteases cleave proinsulin to insulin and C-peptide which pass into the bloodstream simultaneously. About half of the insulin, but virtually none of the C-peptide, is retained in the liver. Circulating insulin has a half-life of 3-5 minutes and is preferentially degraded in the liver, whereas inactivation or excretion of proinsulin and C-peptide mainly takes place in the kidneys.

The amino acid sequence of insulin has remained surprisingly constant during evolution, with the result that prior to the development of genetically engineered human insulin it was possible to successfully use porcine or bovine insulin in the therapy of diabetes mellitus.

The action of insulin is mediated by specific receptors and primarily consists of facilitation of the uptake of sugar by the cells of the liver, fatty tissue and musculature; this is the basis of its hypoglycemic action.

Serum insulin determinations are mainly performed on patients with symptoms of hypoglycemia. They are used to ascertain the glucose/insulin quotients and for clarification of questions concerning insulin secretion, e.g. in the tolbutamide test and glucagon test or in the evaluation of oral glucose tolerance tests or hunger provocation tests.

Although the adequacy of pancreatic insulin synthesis is frequently assessed via the determination of C-peptide, it is still generally necessary to determine insulin. For example, therapeutic administration of insulins of non-human origin can lead to the formation of anti-insulin antibodies. In this case, measurement of the concentration of serum insulin shows the quantity of free - and hence biologically active - hormone, whereas the determination of C-peptide provides a measure of the patient's total endogenous insulin secretion.

A disorder in insulin metabolism leads to massive influencing of a number of metabolic processes. A too low concentration of free, biologically active insulin can lead to the development of diabetes mellitus. Possible causes of this include destruction of the β-cells (type I diabetes), reduced activity of the insulin or reduced pancreatic synthesis (type II), circulating antibodies to insulin, delayed release of insulin or the absence (or inadequacy) of insulin receptors.

On the other hand, autonomous, non-regulated insulin secretion is generally the cause of hypoglycemia. This condition is brought about by inhibition of gluconeogenesis, e.g. as a result of severe hepatic or renal failure, islet cell adenoma, or carcinoma. Hypoglycemia can, however, also be facilitated intentionally or unintentionally (factitious hypoglycemia).

In 3% of persons with reduced glucose tolerance, the metabolic state deteriorates towards diabetes mellitus over a period of time. Reduced glucose tolerance during pregnancy always requires treatment. The clearly elevated risk of mortality for the fetus necessitates intensive monitoring.

The Elecsys Insulin assay employs two monoclonal antibodies which together are specific for human insulin.

Sandwich principle. Total duration of assay: 18 minutes.
1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex (Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$) form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of insulin in the sample.
Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

2. SAFETY PRECAUTIONS

Follow all procedures and policies listed the Fairview-University Medical Center Laboratory Safety Manual. Consider all specimens, control materials, and calibrator materials as potentially infectious.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT RESULT ENTRY

NHANES Insulin results are entered unto a spreadsheet provided electronically by WESTAT, Inc for NHANES.

To access the spreadsheet click on My Computer → Z drive → User → Dep Labs → Collab Studies → NHANES → Insulin 023.

Choose the file named with the corresponding box number.

Enter the analysis date, run number, technologist's initials, insulin result, and result comment code.

The spreadsheet will be sent electronically by the contact person.

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

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes or tubes containing separating gel.
Heparin, EDTA, and sodium citrate plasma.
Hemolysis interferes, as insulin-degrading peptidases are released from erythrocytes.[6]
Twenty (20) ☐L of each sample of serum or plasma is required to test each specimen singly per assay, as well as a dead volume of 200 ☐L.
Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within <± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure

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

Equipment
Cat. No. 12017504, Insulin CalSet, for 4 x 1 mL
General laboratory equipment
Elecsys 1010/2010, MODULAR ANALYTICS E170 or C**obas E** analyzer, Roche Diagnostics Corporation, Indianapolis, IN 46250

Accessories for Elecsys 1010/2010 and **Cobas e** 411 analyzers:
Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
Cat. No. 11662970, Clean Cell, 6 x 380 mL measuring cell cleaning solution
Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL wash water additive
Cat. No. 11933159, Adapter for SysClean
Cat. No. 11706829, Elecsys 1010 Assay Cup, 12 x 32 reaction vessels or
Cat. No. 11706802, Elecsys 2010 Assay Cup, 60 x 60 reaction vessels
Cat. No. 11706799, Elecsys 2010 Assay Tip, 30 x 120 pipette tips

Accessories for all analyzers:
Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Materials and Reagents
1. Insulin Kit. Catalog number 12017547 122 (100 determinations) Roche Diagnostics Corporation, Indianapolis, IN 46250. See outer label of kit for expiration date. The kit includes the following reagents:

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	Anti-insulin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-insulin antibody (mouse) 1 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.
R2	Anti-insulin-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

Reagents must be brought to room temperature before use by allowing the reagent pack to sit at room temperature for a minimum of 45 minutes outside of the packaging before placing on the instrument.

2. MilliQ Water:
MilliQ is the trade name of the water system purchased from the Millipore Corporation (Continental Water System). MilliQ water is deionized water treated with activated carbon and deionization cartridges and filtered to remove microorganisms larger than 0.22 micrometers.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

Traceability: This method has been standardized using the 1st IRP WHO Reference Standard 66/304 (NIBSC). The American Diabetes Association Workgroup is continuing efforts on standardization of the assay.
Every Elecsys Insulin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Insulin CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

after 1 month (28 days) when using the same reagent lot
after 7 days (when using the same reagent kit on the analyzer)
as required: e.g. quality control findings outside the specified limits

Preparation of Calibration Material

Using a volumetric pipet, reconstitute Cal 1 and Cal 2 by adding 1.0 mL of milli-Q water. Allow the calibrators to stand at room temperature for 15 minutes before use. Before use, swirl gently until calibrator is well-mixed. Transfer reconstituted calibrator into the empty labeled snap-cap vials (CalSet vials). One calibrator bottle can be split into two CalSet vials by transferring 0.5 mL into each vial. The calibrator vials can be used only once on the instrument. The calibrator vials are stable for three months when frozen at -20° C.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Instrument Startup

The Elecsys 2010 is left overnight with the Operator's switch placed in the 'OFF' position. To "power on" the instrument, switch the Operator's switch to the 'ON' position. The instrument will go through a short initialization process when it is turned on. Check to ensure there is a disk in the disk drive. Open the lids of the ProCell/Clean Cell bottles.

The Elecsys 2010 has scheduled daily, weekly, every 2 weeks, monthly, and every 2 months maintenance. The maintenance is outlined on the check-off chart at the instrument. Details for the maintenance can be found in Section 4 of the User's Guide and Section 2 of the Tutorial Guide. Complete all necessary maintenance before beginning testing for the day.

Delete open requests from the previous day's run before beginning testing for the day.

- Touch the 'Status' tab to open the Status folder.
- Touch the 'disk number' field and type in the disk you wish to select (0-9).
- Touch the 'Open Requests' button to open the Open Requests pop-up window.
- Touch the 'Delete Open' button to delete open requests.
- Repeat for each disk used the previous day.

Delete documented results.

- Touch the 'Results' tab to open the Results folder.
- Touch the 'Delete documented results' button.
- A confirmation pop-up window will open asking 'Delete all documented results?'
- Touch the 'OK' button to confirm.

Inventory Checks

Touch the 'Inventory' tab on the screen to open the inventory screen. The amount of consumable materials can be seen from this screen.

1. Ensure there is adequate reagent on board to complete the anticipated number of tests for the day.
2. Remove any empty reagent packs from the reagent rotor.
3. Replace any empty assay tips and cups containers.
4. Empty the liquid waste and solid waste containers.
5. Fill the distilled water container and add 10 mL of SysWash per 1 L of water to the filled container.
6. When all consumables have been updated, touch the 'Reagent Scan' button in the inventory screen. The instrument will then perform a series of inventory checks to count the amount of consumables on board. The inventory screen will update at the end of the Reagent Scan.

Calibration

Determine if calibration is required by touching the button corresponding to each Insulin reagent pack in the Inventory folder. The pop up window will display information about the previous calibrations performed for that particular lot number and reagent pack. See calibration section for calibration frequency. If calibration is necessary, order the calibration by touching the 'Manual Calibration Selection' button. The button will toggle from 'OFF' to 'ON'. Touch the 'Close' button to close the pop up window

Quality Control

To order quality control samples:

- Touch 'Orders' tab to open the Orders folder
- Touch 'Sample, Control, Calibrator' button in the top right corner to toggle from 'Sample' to 'Control'
- Touch the 'Select Control' button
- Touch the appropriate control button to select the control to be run. Refer to current control ranges to determine the current lot and control name.
- Touch the disk/pos fields to change the disk/pos number to the correct location on the sample disk. Press Enter on the keypad to accept the changes
- Select the tests to run on the control by touching the button for the corresponding test. The button turns blue when the test is selected.
- Touch 'Register' to save the order.

Loading Samples

The Elecsys 2010 contains a sample barcode reader. Samples can be run on the Elecsys 2010 with or without a barcode. Specimens ordered in Misys can be run on the Misys barcode and downloaded to the computer. Results will be downloaded using the OEM function in Misys.

To order a specimen without a barcode:

- Write the ID number on the Insulin Results Worksheet corresponding to the position it will be placed on the sample disk.
- Place the specimen in the position on the sample disk. Use support tubes as needed. Ensure the tube is seated properly in the sample disk.
- Position the sample tube so that any barcode is facing away from the barcode reader.
- Touch the 'Orders' tab to open the Orders folder.
- Ensure the 'Sample, Control, Calibrator' button is toggled to 'Sample'.
- Touch the 'Sample ID' field to highlight.
- Enter a numeric sample ID number or allow the instrument to assign a sample ID.
- Touch the disk/pos fields to change the disk/pos to the appropriate position.
- Touch the 'Insulin' button to order the insulin test.
- Verify all fields are correct
- Touch 'Register' to save the information.
- Repeat until all samples are ordered.

To order a non-Misys barcoded sample:

- Write the ID number on the Insulin Results Worksheet corresponding to the position it will be placed on the sample disk.
- Place the specimen in the position on the sample disk. Use support tubes as needed.
- Position the sample tube so that any barcode is facing toward the barcode reader.
- After all samples are loaded, touch the 'Status' tab.
- Touch the disk field to change to the appropriate disk number.
- Touch the 'Sample Scan' button in the lower right corner. The instrument will then scan all position until it ends at position 30 or it reads the "STOP" barcode.
- When the Sample Scan completes, touch the button on the status screen corresponding to the first scanned sample. Note the sequence number in the pop up window for that sample.
- Touch the 'Orders' tab to open the Orders folder.
- Touch the Sequence number field to highlight the field. Enter the sequence number for the first scanned sample. Press 'Enter' on the keypad.
- Touch the 'Insulin' button to order the insulin test.
- Verify all fields are correct
- Touch 'Register' to save the information.
- Repeat until all samples are ordered.

To load a Misys barcoded sample:

- Place the specimen in the position on the sample disk. Use support tubes as needed.
- Position the sample tube so that any barcode is facing toward the barcode reader.
- When the Start button is pressed, the instrument will read the barcode and the ordered tests will download to the instrument.

When all samples are loaded and appropriately ordered, touch the 'Start' button on the keypad. A pop-up window will appear to confirm the correct disk to be run. If the disk number is correct touch the 'OK' button on the screen.

Instrument Shutdown

After the instrument finishes all tests ordered, it will go into a 'Sample Stop' status for 20 minutes. Then, it will automatically do Finalization Maintenance before going into 'Stand By' status. Finalization maintenance must occur at least once during the testing day. If Finalization Maintenance has not already occurred for the day, it must be manually requested at the end of the shift:

- Touch the 'Utility' tab to open the Utility folder.
- Touch the 'Maintenance' button
- Touch the 'Finalization Maintenance' button
- On the pop up window, touch the 'OK' button to confirm

Finalization Maintenance will take approximately 5 minutes to complete. Once the instrument status returns to Stand-by, close the caps on the ProCell/Clean Cell bottles. Turn off the instrument by switching the Operation switch to the 'OFF' position.

9. REPORTABLE RANGE OF RESULTS

The Roche reported linear range is 1.20-6000 pmol/L (0.200-1000 mU/L) (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as <1.20 pmol/L (< 0.200 mU/L). Values above the measuring range are reported as >6000 pmol/L (> 1000 mU/L). The Collaborative Studies Lab has verified the assay to be linear up to 4368 pmol/L (728 mU/L). It will be the policy of the CSCL lab to duplicate any specimen with an insulin value of <12 pmol/L (<2 mU/L) and any fasting sample >300 pmol/L (>50 mU/L). It is not necessary to duplicate elevated specimens associated with an oral glucose tolerance test (OGTT).

The analytical measurement range (AMR) is 1.2 – 4368 pmol/L (0.2 - 728 mU/L). The clinical reportable range (CRR) is 12 – 1800 pmol/L (2-300 mU/L) for specimens not associated with an Oral Glucose Tolerance Test (OGTT).

10. QUALITY CONTROL (QC) PROCEDURES

One commercial control (high range) and a pooled serum control (normal range) are run at the start of the day and then throughout the testing day along with test samples. The range of these controls is established within our laboratory. The values of the controls need to be evaluated as they are run on the instrument. The controls are plotted daily in the spreadsheet 'Elecsys 2010' within the CSCL Q drive, 'Daily QC tally' folder. Intra-assay precision is monitored by running one specimen in duplicate within the day, and inter-assay precision is monitored by running one specimen in duplicate between days.

Preparation of Quality Control Material

*Pooled Serum control (normal)
*Bio-Rad Immunoassay 2 (high) Cat No. 372
Bio-Rad Laboratories 9500 Jeronimo Rd. Irvine, CA 92618

Using a volumetric pipet, reconstitute the Bio-Rad Immunoassay 2 control by adding 5.0 mL of milli-Q water. Allow the control to sit at room temperature for 15 minutes. Before sampling, allow the control to reach room temperature (18-25° C) and swirl gently until control is well mixed. Reconstituted control is stable for 7 days at 2-8° C.

The pooled serum control is ready for use. Allow the control to reach room temperature (18-25° C) and mix thoroughly by inversion or gentle vortex before sampling.

***The control materials are obtained from human blood components, therefore all controls should be handled as if capable of transmitting infections.**

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

Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

after 1 month (28 days) when using the same reagent lot
after 7 days (when using the same reagent kit on the analyzer)
as required: e.g. quality control findings outside the specified limits

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

Hemolysis is reported to interfere with the insulin assay. The assay is unaffected by icterus (bilirubin < 1539 µmol/L or < 90 mg/dL), lipemia (Intralipid < 1800 mg/dL), and biotin < 246 nmol/L or < 60 ng/mL. Criterion: Recovery within ± 10% of initial value. In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 18,900 IU/mL.

There is no high-dose hook effect at insulin concentrations up to 20,000 µU/mL or 138,900 pmol/L.

In vitro tests were performed on 20 commonly used pharmaceuticals. No interference with the assay was found.

Samples from patients treated with bovine, porcine or human insulin sometimes contain anti-insulin antibodies which can affect the test results.

As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes.

The test contains additives which minimize these effects.
In rare cases interference due to extremely high titers of antibodies to ruthenium can occur.

The test contains additives which minimize these effects.
Extremely high titers of antibodies to streptavidin can occur in isolated cases and cause interference.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings

13. REFERENCE RANGES (NORMAL VALUES)

Fasting levels for 137 tested, apparently healthy individuals, yielded a mean of 9.2mU/L, a median of 6.9mU/L and a range of 2-25mU/L.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no panic values for insulin.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are stored at -70°C until analyzed. On the day of testing, the specimens are thawed and kept in the refrigerator when not on the instrument. The specimens are refrozen within 1-2 days.

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

If testing cannot be performed, the specimens are stored at -70°C.

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

NHANES insulin results are entered onto a spreadsheet provided electronically by WESTAT, Inc for NHANES. The spreadsheet is found on the Q drive in the NHANES folder. Select the insulin (023) folder and choose the file named with the corresponding box number.

Enter the analysis date, run number, technologist's initials, insulin value, and result comment code.

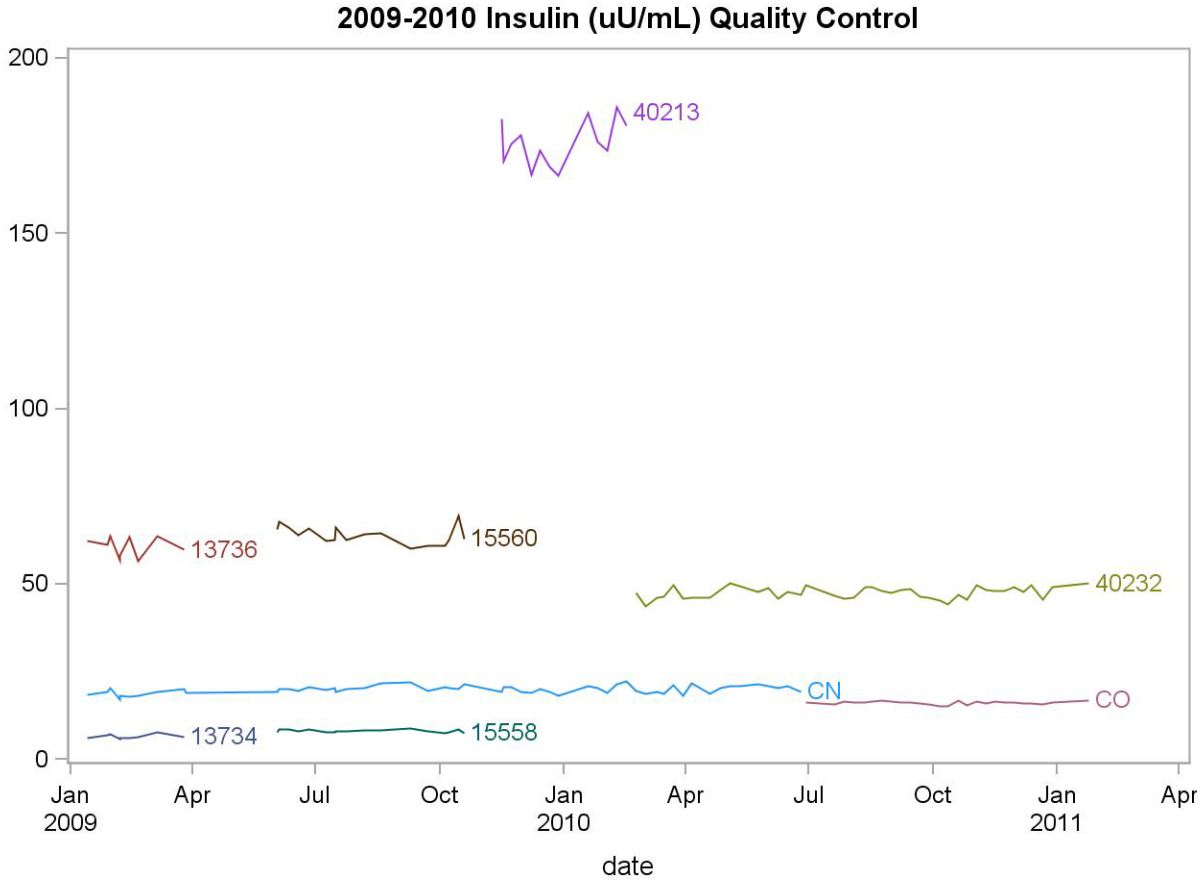
The spreadsheet will be sent electronically by the contact person.

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.

19 SUMMARY STATISTICS AND QC GRAPHS

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
13736	9	13JAN09	26MAR09	60.637	2.901	4.8
13734	9	13JAN09	26MAR09	6.488	0.591	9.1
CN	27	13JAN09	19OCT09	19.697	1.147	5.8
15560	17	03JUN09	19OCT09	64.000	2.519	3.9
15558	17	03JUN09	19OCT09	8.084	0.403	5.0
40213	13	16NOV09	16FEB10	175.662	6.491	3.7
CN	29	16NOV09	25JUN10	20.030	1.151	5.7
40232	42	23FEB10	24JAN11	47.516	1.649	3.5
CO	26	29JUN10	24JAN11	16.109	0.462	2.9



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