

# Laboratory Procedure Manual

*Analyte:* Insulin

*Matrix:* Serum

*Method* Human Insulin Immunoassay

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**Public Release Data Set Information**

This document details the Lab Protocol for NHANES 2005-2006.

A tabular list of the released variables follows:

<b>Data File Name</b>	<b>Variable Name</b>	<b>SAS Label</b>
GLU_D	LBXIN	Insulin ( $\mu$ U/mL )
	LBDINSI	Insulin (pmol/L)

## 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Insulin is the primary hormone responsible for controlling glucose metabolism, and its secretion is governed by plasma glucose concentration. The insulin molecule is synthesized in the pancreas as pro-insulin and is later cleaved to form C-peptide and insulin. The principal function of insulin is to control the uptake and utilization of glucose in the peripheral tissues. Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions, while concentrations are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, and some endocrine dysfunctions.

The Merocodia Insulin ELISA is a two-site enzyme immunoassay utilizing the direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants of the insulin molecule. Specimen, control, or standard is pipetted into the sample well, then followed by the addition of peroxidase-conjugated anti-insulin antibodies. Insulin present in the sample will bind to anti-insulin antibodies bound to the sample well, while the peroxidase-conjugated anti-insulin antibodies will also bind to the insulin at the same time. After washing to remove unbound enzyme-labelled antibodies, TMB-labelled substrate is added and binds to the conjugated antibodies. Acid is added to the sample well to stop the reaction, and the colorimetric endpoint is read on a microplate spectrophotometer set to the appropriate light wavelength.

## 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

- A. Open the Study Patients Folder located in the "aren0085" server folder.
- B. Open the *Merocodia Insulin in duplicate* file with the matching date of the results.
- C. Click on the Read Data tab (at the bottom of the page).
- D. Put in your floppy disk with the OD readings on it.
- E. Open the floppy disk under My Computer, 3 ½ Floppy Disk, open the data file that you want.
- F. Highlight the data, right-click, and copy.
- G. Open the appropriate results file, click the Read Data tab, right-click in the top left cell, and paste the results.
- H. **If samples are run singly**, while the data is highlighted click on the Excel Sort button to order the results so that no spaces remain between lines. Do not do this step if samples are run in duplicate.

- I. Highlight and copy the well numbers to the appropriate positions in the Samples to Run page.
- J. Go back to the Read Data tab and highlight and copy the result values.
- K. Go to the Samples to Run page and paste the values in the correct positions corresponding to the well numbers.
- L. Check your control values to make sure that they are in the specified range.
- M. Plot the QC values in the appropriate QC file in the computer.
- N. Print the Samples to Run page.
- O. Check your results against your spectrophotometer raw data.
- P. Open the appropriate results file.
- Q. Find the Lab ID numbers that correspond to those you have data for. Place the cursor in the correct position in the list.
- R. Go back to the Insulin results file and highlight your results. Right-click, then select Copy.
- S. Go back to the appropriate results file, right-click, and paste the results.
- T. Double-check your spreadsheet (copy and paste procedure) against your results hard copy.
- U. Fill in the other information on the Result spreadsheet: date, volume used, kit lot number, and any other comments about the assay run.
- V. Save the information on the spreadsheet.
- W. Have a second Technologist review your work, sign off, and file the results.

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

Serum or EDTA- or heparin-plasma may be used to test for insulin concentration with this kit. Either type of sample should be stored at -20°C or lower if not assayed immediately (within 24 hours). Avoid repeated freeze-thaw cycles of the sample. The CSCL laboratory has determined that insulin is stable up to five freeze-thaw cycles. Twenty-five (25) uL of each sample of serum or plasma is required to test each specimen singly per assay. Grossly lipemic, icteric, or hemolyzed samples do not interfere with this assay. All patient samples should be handled as if capable of transmitting infections. No dilution is required for most samples. If a dilution is required, use Calibrator 0 as the diluent.

Samples from individuals undergoing insulin therapy may produce incorrect results due to formation of anti-insulin antibodies that are capable of interfering with this assay.

**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**

A. Instrumentation

1. Microplate reader capable of measuring absorbance at 450 nm. (Molecular Devices, SpectraMax 250).

2. Beckman Coulter Biomek 2000 Workstation, Beckman Coulter Biomek P250 pipette tips (catalog #373689) as pictured in the "Biomek Workstation" figure below, Biomek 48-tube holders, Biomek quarter reservoirs

B. Materials and Reagents

**Reagents:**

All reagents must be brought to room temperature before use. \* **Follow appropriate reagent preparation guidelines according to either a single kit or a ten-pack of kits, depending upon what is used. The instructions below are for a ten-pack of kits.**

1. 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.

2. Mercodia Insulin ELISA Kit. Catalog number 10-1113-10 (10 x 96 determinations) or 10-1113-01 (96 determinations). Mercodia AB, Uppsala, Sweden. See outer label of kit for expiration date. The kit includes the following reagents:

a. Microplates:

Ten 96-well microplates coated with a murine monoclonal anti-insulin antibody. Store at 2-8°C. After opening package, any unused microplate wells should be returned to the foil pouch (containing the desiccant pack) and sealed; these may be stored for up to 2 months at 2-8°C. The expiration date is printed on the outer packaging of the plates; unopened plates that are stored at 2-8°C are stable until this date.

b. Enzyme Conjugate 11X:

Peroxidase conjugated mouse monoclonal anti-insulin, ~6ug/mL. Store at 2-8°C.

Dilute as instructed below. Diluted enzyme conjugate should be used within one day. The expiration date is printed on the outer packaging of the enzyme conjugate; enzyme conjugate that is stored at 2-8°C is stable until this date.

- c. Enzyme Conjugate Buffer:  
One hundred twenty (120) mL of solution, ready for use. Store at 2-8°C. The expiration date is printed on the outer packaging of the enzyme conjugate buffer; enzyme conjugate buffer that is stored at 2-8°C is stable until this date.
- d. Calibrators:  
Recombinant human insulin in concentrations of 2, 3, 10, 30, 100, and 200 mU/L, ready to use, each at a volume of 1mL, except calibrator 2.0 which contains 0.5mL.
- e. Calibrator 0:  
Five (5) mL of solution, ready to use. This calibrator is used as a plate blank in the calculation of results and for the dilution of any samples with an insulin concentration greater than 200mU/L. Store at 2-8°C.
- f. Wash Buffer 21X:  
Store at 2-8°C. Dilute as instructed below. Store diluted buffer at 2-8°C for up to 4 weeks. The expiration date is printed on the outer packaging of the wash buffer; wash buffer that is stored at 2-8°C is stable until this date.
- g. Substrate TMB:  
3, 3',5,5'-tetramethylbenzidine (TMB) colorless solution, ready to use. *Note: light sensitive.* Store at 2-8°C. The expiration date is printed on the outer packaging of the substrate TMB; substrate TMB that is stored at 2-8°C is stable until this date.
- h. Stop Solution:  
0.5M H<sub>2</sub>SO<sub>4</sub>, ready to use. Store at 2-8°C. The expiration date is printed on the outer packaging of the stop solution; stop solution that is stored at 2-8°C is stable until this date.

*Caution: This is an acid solution. Wear eye, hand, face, and clothing protection when handling this substance.*

**Equipment and Supplies Required:**

1. 20 µL, 200 µL, and 1000 µL pipets (for manual method; or See 10.)
2. Pipet tips appropriate for above pipets (1, for manual method; or See 10.)
3. 20-200µL 12-channel pipet
4. 1L graduated cylinder
5. Nunc-Immuno Wash 12 (catalog #470175) microplate washer
6. 0.5 mL and 2.0mL microcentrifuge tubes, graduated free-standing
7. 10mL disposable serological pipettes
8. Plastic reagent reservoirs
9. Test tube tipper

10. Plastic transfer pipettes

C. Reagent Preparation

All reagents must be brought to room temperature before use.

1. Wash buffer: If catalog #10-1113-01 (the single is kit) is used: dilute the bottle of 21X wash buffer in 800mL of MilliQ water. If catalog #10-1113-10 (**the 10 pack of kits**) is used, prepare wash buffer according to the table below. Using 40mL of wash buffer and 800mL of water (1 plate instructions) will be enough for two plates. Discard any remaining wash after two plates and make fresh. If only one plate is done in a day, save the wash until the next plate is run.

Number of strips/plates	Wash buffer 21X	MilliQ water
6 strips	20mL	400mL
<b>1 plate</b>	<b>40mL</b>	<b>800mL</b>
2 plates	70mL	1400mL
3 plates	110mL	2200mL

2. Enzyme conjugate: Dilute the 11X enzyme conjugate according to the table below.

*if catalog #10-1113-01 (the single is kit) is used:*

Number of strips	Enzyme conjugate 11X	Enzyme conjugate buffer
4	400uL	4.0mL
6	600uL	6.0mL
8	700uL	7.0mL
10	900uL	9.0mL
12	1 vial (1.2mL)	1 vial (12mL)

*if catalog #10-1113-10 (the 10 pack of kits) is used:*

Number of strips/plates	Enzyme conjugate 11X	Enzyme conjugate buffer
6 strips	0.5mL	5mL
1 plate	1.0mL	10mL
2 plates	2.0mL	20mL
3 plates	3.0mL	30mL

**Note:** Prepare the conjugate directly in a Biomek quarter reservoir, using a disposable 10mL serological pipet.

D. Standards Preparation

1. Calibrators:

Upon receipt of the ten-pack of kits, aliquot each calibrator into 14, 0.5mL microcentrifuge vials, each containing 70uL, calibrator 2 will only have 7 vials due to its smaller volume. Each vial is adequate for 1 plate. Store at 2-8°C. The expiration date is printed on the outer packaging of the calibrators; calibrators that are stored at 2-8°C are stable until this date.

2. Calibrator 0:

Upon receipt of the ten-pack of kits, aliquot calibrator 0 into 14, 0.5mL microcentrifuge vials, each containing 70uL. Each vial is adequate for 1 plate. The remaining solution can be kept in the original vial to use in making sample dilutions. Store at 2-8°C. The expiration date is printed on the outer packaging of calibrator 0; calibrator 0 that is stored at 2-8°C is stable until this date.

E. Preparation of Quality Control Materials

Control Low (Catalog #10-1134-01)  
Control High (Catalog #10-1164-01)

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

Reconstitute each Mercodia Diabetes-antigen Control by adding 500uL deionized water to each vial. Replace the rubber stopper and allow standing for 5 minutes. Gently swirl until contents are thoroughly mixed. Avoid foaming.

Aliquot each control into 11, 0.5mL microcentrifuge vials, each containing 45uL.

Each vial is adequate for 1 plate. Store at -70°C. Avoid repeated freezing and thawing. Reconstituted Controls are stable for three months at -70°C.

7. **CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES**

A daily position calibration is performed on the Biomek, as well as a monthly cleaning procedure, and a calibration of the pipet tools every 6 months. \*See Biomek Maintenance Procedures.

8. **PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS**

A. PROCEDURE

Making the Worksheet

1. Open the Study Patients Folder located in the "aren0085" server or the "shortcut to study patients" folder on the desktop.
2. Open the appropriate Study Folder (Mesa, NHANES, etc...)
3. Open the *Mercodia Insulin in duplicate template* file.
4. Click on "Samples to Run" tab (at the bottom of page).
5. Place the cursor in the S1 box.
6. Open the file that contains the ID numbers of the appropriate study.
7. Find Lab ID numbers you will assay and "highlight" them by selecting with the cursor.



8. Right-click on the selection, choose Copy, then go back to the *Mercodia Insulin in duplicate template* and Right-click on the S1 box and choose Paste.
9. Click on the “Sample Template” tab on the bottom of the page and check the plate layout and lot numbers of controls and reagents. Update if necessary.
10. Save the worksheet in the folder of the appropriate study, under Insulin Results Save as *Mercodia Insulin in duplicate results* “date of run”.
11. Print your Worksheet.

B. Quality Control Materials

1. Calibrators: Arrange the 70 µL calibrator aliquots in the Biomek 48-tube holder as shown below. The calibrator aliquots are sufficient for one plate.
2. Controls: Arrange the low and high Mercodia controls and lab (pooled) control in the Biomek 48-tube holder as shown below.

**Calibrators & Controls (tube holder #1), for samples in duplicate**

Tube holder #1	1	2	3	4	5	6	7	8
A	Calibrator 0	Cal 2.0	Cal 3.0	Cal 10.0	Cal 30.0	Cal 100.0	Cal 200.0	
B	Mercodia low ctrl	Mercodia high ctrl	Lab ctrl					
C								
D								
E								
F								

C. Operation

Thaw samples and controls, by placing on tube tipper if desired, mix well, and check each sample for fibrin clots with a wooden applicator stick. Pulse-spin the specimens, **if necessary**, to get the remaining sample out of the cap. Bring all the contents of the kit to room temperature before use.

**Note: Some positions are not filled because of the Biomek rack format.**

Samples: Arrange the samples as shown in the **Sample set 1 (tube holder #2) chart**. One insulin assay plate can test 39 samples in duplicate or 78 samples singly. Check the sample tube volume while placing samples in the tube holder. The Biomek requires a minimum volume of 70 µL. After all samples have been added to the assay plate, check the wells. If there are bubbles in the well, an incorrect amount of sample may have been added; note this on the worksheet. The sample will have to be repeated.

**Sample set 1 (tube holder #2), for samples in duplicate**

Tube holder #2	1	2	3	4	5	6	7	8
A	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6	sample 7	sample 8
B	sample 9	sample 10	sample 11	sample 12	sample 13	sample 14	sample 15	sample 16
C	sample 17	sample 18	sample 19	sample 20	sample 21	sample 22	sample 23	sample 24
D	sample	sample	sample	sample	sample	sample	sample	sample

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	25	26	27	28	29	30	31	32
E	sample 33	sample 34	sample 35	sample 36	sample 37	sample 38	sample 39	empty
F								

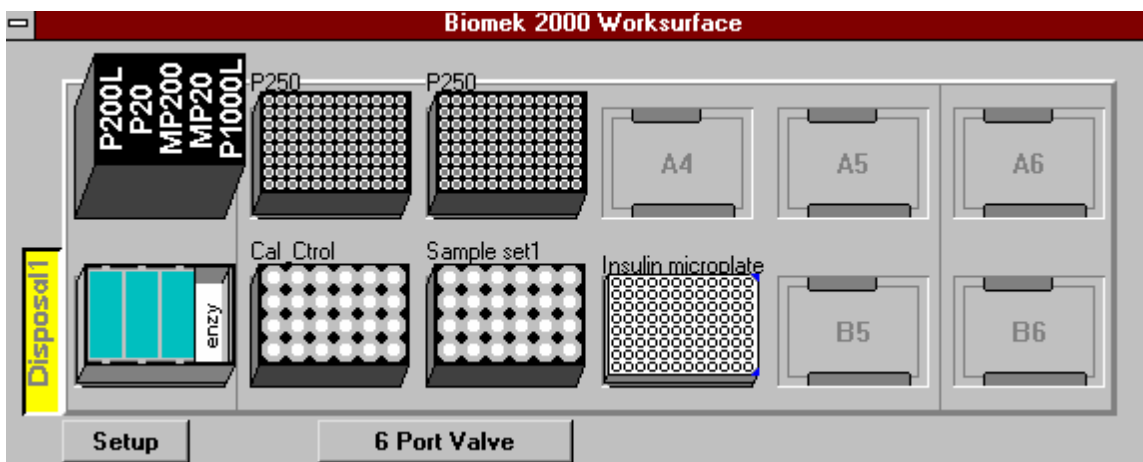
The samples in the microplate will then be arranged as shown in the table below.

**Sample distribution chart, for samples in duplicate**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal 0	Cal 0	Lab Ctrl	sample 4	sample 8	sample 12	sample 16	sample 20	sample 24	sample 28	sample 32	sample 36
B	Cal 2.0	Cal 2.0	Lab Ctrl	sample 4	sample 8	sample 12	sample 16	sample 20	sample 24	sample 28	sample 32	sample 36
C	Cal 3.0	Cal 3.0	sample 1	sample 5	sample 9	sample 13	sample 17	sample 21	sample 25	sample 29	sample 33	sample 37
D	Cal 10.0	Cal 10.0	sample 1	sample 5	sample 9	sample 13	sample 17	sample 21	sample 25	sample 29	sample 33	sample 37
E	Cal 30.0	Cal 30.0	sample 2	sample 6	sample 10	sample 14	sample 18	sample 22	sample 26	sample 30	sample 34	sample 38
F	Cal 100.0	Cal 100.0	sample 2	sample 6	sample 10	sample 14	sample 18	sample 22	sample 26	sample 30	sample 34	sample 38
G	Cal 200.0	Cal 200.0	sample 3	sample 7	sample 11	sample 15	sample 19	sample 23	sample 27	sample 31	sample 35	sample 39
H	Low Ctrl	High ctrl	sample 3	sample 7	sample 11	sample 15	sample 19	sample 23	sample 27	sample 31	sample 35	sample 39

**\*Note: If calibrators, control types, sample types, tube type, dilution factor, position or number of samples are changed, make sure the Biomek method is changed accordingly.**

BIOMEK WORK SURFACE



PROTOCOL: Method name = **Insulin in dup (Other programs depending on Study)**  
Lab book = ELISA

DECK CONFIGURATION: set up the Biomek work surface as follows:  
**B1 – prepared Enzyme conjugate**

**B2 – Cal and control (tube holder #1)**  
**B3 – Samples (tube holder #2)**  
**B4 – Insulin microplate**  
**A2 – P250**  
**A3 – P250**

SEQUENCE:

	<b>Time</b>
- Add 25µL calibrators, controls, and samples(in duplicate)	20 min
- Add 100µL enzyme conjugate	10 min

**After the set up of the ELISA plate is complete, continue the assay manually, as described below:**

1. Cover the plate with an adhesive plate cover. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker set at 450 rpm (setting 45).
2. Aspirate each well and wash with a microplate washer, repeating the process 5 times for a total of 6 washes. Invert the plate and blot against clean paper towels between each wash. **Complete removal of the liquid at each step is essential to good performance.** After the last wash, remove remaining wash buffer, then invert the plate and blot it against clean paper towels, making sure all the liquid is removed.

**\*\*Avoid prolonged exposure of the wells to vacuum aspiration apparatus. Excessive drying of the wells can lead to poor assay performance. Subsequent reagents should be added immediately after washing the plate.** Do not let the plate dry out before adding the Substrate.

3. Add 200µL of Substrate TMB to each well using a 12-channel pipet. Cover plate with a new adhesive cover. Incubate for 15 minutes at room temperature. (Requires 20 mL per plate)
4. Add 50µL of Stop solution to each well using a 12-channel pipet. Pipet up and down a few times to mix solutions thoroughly; take care to avoid foaming or cross-contamination. (Need 6mL per plate)
5. Read plate on a microplate reader set to 450 nm immediately, if for some reason the plate can not be read immediately the assay plate is stable for 30 minutes after adding Stop solution.

D. Reading Plate and Calculation of Results:

Computerized data reduction of absorbance for the Calibrators (2-200 mU/l) versus the concentration using log/log regression should be performed to obtain the concentration of insulin.

1. To read plate, set the parameters for the plate reader for this assay:  
Endpoint reading  
L1=450nm  
L2=650nm  
Plate blanking ON  
Standard curve calculation: log/log curve fit

2. Turn on the plate reader (5 to 10 minutes before use) by pressing the switch at the rear of the instrument.
3. Double-click on the SoftMax Pro 2.6 icon, located on the desktop, and open the folder containing the *Mercodia Insulin in duplicate template* for the SoftMax Pro software. If any changes need to be made to the template, follow the directions below; otherwise, proceed to step 4.
  - a. To change the number of wells to be read: Click the "Setup" box. Click the "Strips" box. Highlight the number of columns of wells to be read (whole columns only). Click "OK."
  - b. To change information about the calibrators or their placement: Click the "Template" box. Choose the appropriate units for measurement. Highlight each standard box and name the standards and set the concentration of each by typing the value in the space at the top of the dialog box. Press return to enter each value. Select the boxes (representing each well) individually to set the unknown wells. Each unknown may be named, if desired. Select the boxes (representing each well) individually to set the control wells. Each control may be named, if desired. Click "OK."
  - c. To change the dilution factor used in the assay (if necessary): Click the triangle next to "Unknowns" to open the results list. Click on the column labeled "FinalResult." Click on the "f(x)" button for the Unknowns list. Change the value listed (i.e. \*100) to the correct dilution factor used in the assay. Click "OK." **Note: this will change the dilution for all specimens read.**
  - d. If necessary, save these changes to the template file.
4. Open the door of the plate reader by pressing the "Drawer" button the top of the machine. Insert the plate in the correct orientation. Close the door by pressing the "Drawer" button again.
5. Click the "Read" button at the top of the open template on the computer desktop.
6. Examine the optical density (OD) values generated to ensure they look appropriate. Also examine the standard line generated (by clicking on the triangle next to "Standards"). Finally, examine the calculated values for the unknowns.
7. Print the results to the Tsai Research printer.
8. Save the results on the computer in the appropriate folder as *Insulin <assay date>*.
9. Insert a 3 ½ floppy disk.
10. Highlight your results.
11. Under Edit, choose copy.
12. Open Excel, and open a blank worksheet.
13. Under Edit, choose paste.
14. Save the results to the 3½ floppy disk: File, Save As, My computer, 3½ floppy, **save as** *Insulin <assay date>*.

15. Close the SoftMax Pro program on the computer.
16. Remove the microplate from the plate reader, close the drawer, then switch the machine off.

## 9. REPORTABLE RANGE OF RESULTS

The CSCL laboratory has determined that the Mercodia Insulin Kit is linear up to 135mU/L. Specimens above 135mU/L should be diluted with the 0 calibrator and reassayed. Values less than 2mU/L are repeated. If the duplicate agrees, the value is reported as <2mU/L.

The detection limit is <1mU/L calculated as two standard deviations above the Calibrator 0.

## 10. QUALITY CONTROL (QC) PROCEDURES

Two commercial Mercodia controls, Low and High, as well as a pooled in-house control are run in each plate. The values of these controls need to be evaluated after each plate is run. The controls are plotted daily with each run, and if the controls fall out of the established range, the run needs to be repeated.

Mercodia Low Control (Lot #13734) Range: 4.2-9.1mU/L

Mercodia High Control (Lot #13736) Range: 52-71mU/L

In-house Control: Range established within laboratory

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

The values of these controls need to be evaluated after each plate is run. The controls are plotted daily with each run, and if the controls fall out of the established range, the run needs to be repeated.

## 12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A definitive clinical diagnosis should not be based on the results of a single test, but should be made after all clinical findings have been evaluated. Sample results from individuals already undergoing insulin therapy may be complicated by formation of anti-insulin antibodies that are capable of interfering in the assay. Grossly lipemic, icteric, or hemolyzed samples do not interfere in the assay.

The following cross-reactions have been found:

C-peptide	<0.01%	(by weight)
Proinsulin	<0.01%	(by weight)
Proinsulin des (31-32)	<0.5%	
Proinsulin split (32-33)	<0.5%	
Proinsulin des (64-65)	98%	
Proinsulin split (65-66)	56%	
Insulin lispro (Humalog®,Eli Lilly)	<0.006%	
Insulin aspart	<0.006%	
Rat Insulin	0.7%	

**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.

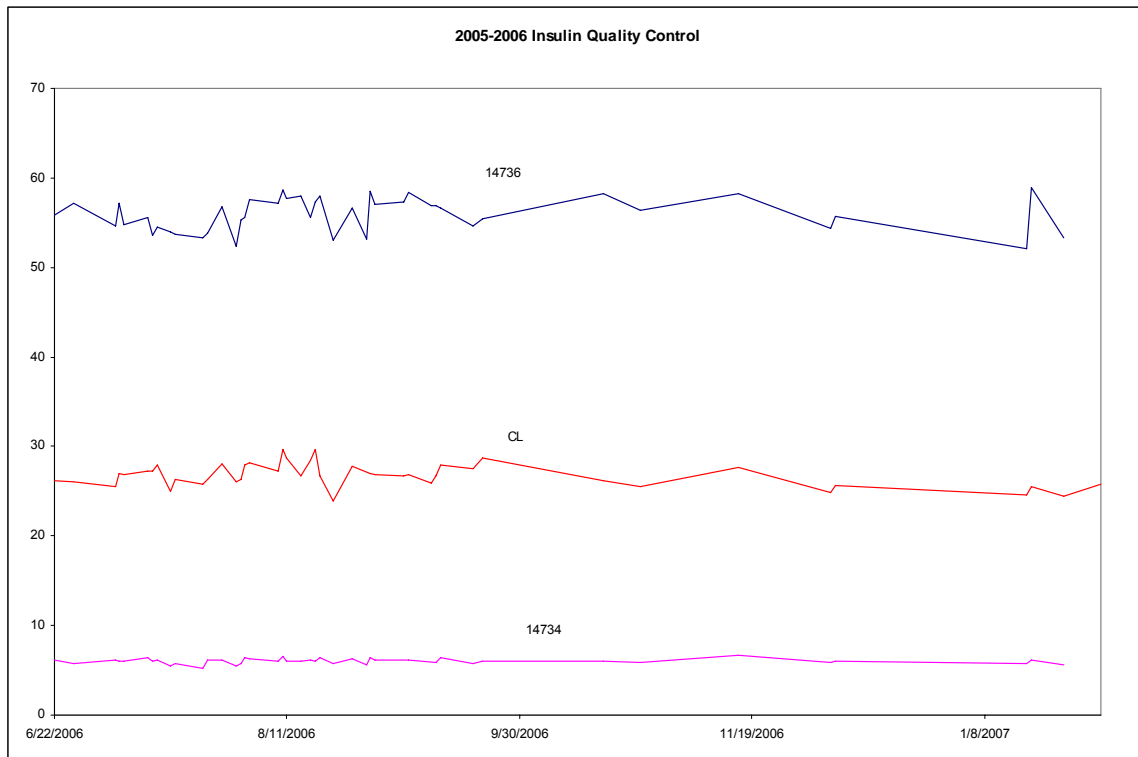
**18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING**

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

#### Summary Statistics for Insulin by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
14734	43	6/22/2006	1/25/2007	6.025	0.297	4.9
CL	44	6/22/2006	2/2/2007	26.749	1.313	4.9
14736	44	6/22/2006	1/25/2007	55.908	1.888	3.4



## **REFERENCES**

Mercodia AB. Mercodia Insulin ELISA kits insert. Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden.