0. Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2002 data.

Two laboratories performed this testing during 2001-2002. In order to maintain confidentiality of the participants the quality control summary statistics and graphs were combined to mask the individual analysis dates from the two laboratories. Methods for both labs are included in this release. Most methods for Lab18 analytes are in one combined file. Methods Lab40 are described in a separate file for each analyte tested.

A list of the released analytes follows:

Lab	Analytes	SAS Label	Description
l40_b	LBXLH	Luteinizing hormone (mIU/mL)	Luteinizing hormone
l40_b	LBDLHSI	Luteinizing hormone (IU/L)	

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The IMx LH assay is based on the Microparticle Enzyme Immunoassay (MEIA) technology. The IMx LH reagents and samples are added to the reaction cell in the following sequence:

The probe/electrode assembly delivers the sample and Anti-beta LH Coated Microparticles to the incubation well of the reaction cell. The LH binds to the Anti-beta FSH Coated Microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix. The matrix is washed with the Wash Buffer to remove unbound materials. The Anti-alpha LH subunit specific Alkaline Phosphatase conjugated is dispensed onto the matrix and binds with the antibody-antigen complex. The matrix is washed to remove unbound materials. The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

Human LH levels are used in investigations of menstrual, fertility, and pubertal development disorders, menopause, ovulatory disorders and pituitary failure. The ration of LH/FSH has been used to assist in the diagnosis of polycystic ovary disease.

Low levels of hLH and hFSH may indicate pituitary failure, while elevated hLH and hFSH levels along with decreased levels of gonadal steroids may indicate gonadal failure (menopause, ovariectomy), premature ovarian syndrome, Turner's syndrome). Low gonadotropin levels are usually seen in females taking oral steroid-based contraceptives. In the male, elevated hFSH and hLH with low levels of gonadal steroids may indicate testicular failure or anorchia.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact serum and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Sani-Cloth HB, Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Abbott IMX System. When ordered tests are completed for each sample, the results are printed out by IMX instrument.
b. A statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC. Completed sample data is entered into an Excel spreadsheet for evaluation. The Excel spreadsheet results file data are copied to the shipment file and saved as a comma delimited file (CSV) and e-mailed to Westat within 21 days of sample receipt.
c. The Excel files containing all raw data and results are backed up once a week using a CD writer or Zip drive for storage.

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

a. Interferences:
   1) No interference from <10 mg/dL bilirubin or <1800 mg/dL triglycerides.
   2) No interference from <500 mg/dL hemoglobin.
b. Separated serum or plasma should not remain at +15°C to +30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Manufacturer recommends frozen specimens can be stored up to six months before testing. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
c. Fasting is not required.
d. A minimum of 0.3 mL serum is needed for LH.
e. Sample volume for individual test is 55 µl.
f. Sample is run singly.

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: Abbott IMX System
b. Materials

1. One bottle (6.5) mL) Anti-LH (Mouse, Monoclonal) Coated Microparticles in buffer. Store 2 to 8°C until expiration date on the package.

2. One bottle (8.5 mL) Anti-LH (Goat): Alkaline Phosphatase Conjugated in buffer with protein stabilizers. Store 2 to 8°C until expiration date on the package.

3. One bottle (10 mL) 4-Methylumbelliferyl Phosphate, 1.2 mM in buffer. Store 2 to 8°C until expiration date on the package.

4. Three Bottles (4 mL) IMx LH Mode 1 Calibrator (D). Store 2 to 8°C until expiration date on the package.

5. Six bottles (4 mL each) of IMx LH Calibrators. Store 2 to 8°C until expiration date on the package.

6. The three bottles (8 mL each) of IMx LH Controls. Store 2 to 8°C until expiration date on the package.

c. The reagents are supplied ready to use.

d. Standards Preparation: No preparation required.

IMX LH CALIBRATORS (No. 2239-02)
The six bottles (4 mL each) of IMx LH Calibrators are references against the WHO Second International Reference Standard 80/552 for LH. LH is prepared in calf serum at the following concentrations:

BOTTLE	LH CONCENTRATION (mIU/mL)
A	0
B	2
C	10
D	25
E	100
F	250

Preservative: Sodium Azide

e. The three bottles (8 mL each) of IMx LH Controls are LH prepared in processed bovine serum to yield the following concentration ranges:

BOTTLE	LH CONCENTRATION RANGE (mIU/mL)
L	3.5 to 6.5
M	30 to 50
H	57-103

Preservative: Sodium Azide

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibrators: Access hLH Calibrators (Cat. #33515).
   1) Six levels of calibrator.
   2) Provided ready to use.
   3) Mix contents by gently inverting prior to use.
   4) Stable until expiration date when stored at 2-10°C.
   5) Refer to calibration card enclosed with each set of calibrators for actual concentrations.

b. Calibration:

Calibration is required when a new lot of hLH reagent is loaded, when the calibration curve expires, or when controls are out of range.

IMX LH CALIBRATORS (No. 2239-02)
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BOTTLE	LH CONCENTRATION (mIU/mL)
A	0
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C	10
D	25
E	100
F	250

Preservative: Sodium Azide

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries
   1) Program requested tests on IMX instrument using sample I.D. and slot I.D.

b. Sample Preparation
   1) Thaw samples and vortex, mixing well.

c. Operation
   1) For detailed instructions on operating the IMX, refer to the manufacturer's instructions
   2) Check supplies.
   3) Program the requested tests.
   4) Prepare the primary sample tubes or sample cups and load them on the sample trays.
   5) 150 µL of sample is required for each determination in addition to the sample cup or sample tube dead volume.
   6) Load the trays onto the instrument.
   7) Press RUN.

The IMx LH assay parameters, illustrated in the package insert, have been factory set. These parameters can be printed, displayed, and edited according to the procedure in your IMx system Operation Manual, Section 6. Ensure that the assay parameters for IMx LH assay in the Assay Module match these parameters or edit accordingly. The assay parameters that cannot be edited are noted with an asterisk (*).

NOTE
Result Unit, assay parameter 42.12 or 47.12, can only be edited to "8" (mIU/mL) and Print Option, assay parameter 42.60 or 47.60, can only be edited to "0" or "1". Editing to another number will result in the displayed code "103 Bad Value in Assay File 12 or 60", respectively when the assay run is initiated. For further information on Changing Concentration Units and Print Options, refer to your IMx System Operation manual, Section 5.

IMX LH PROCEDURE
The list of required materials and the procedure to perform the IMx LH assay can be found in the IMx System Operation Manual, Section 5:
MEIA Assay Calibration Mode 1 Assay
Select Assay Calibration and Mode 1 Assay

The IMx LH assay requires a minimum volume of 200 mL of MEIA No. 2 Diluent Buffer in the buffer bottle in order to properly process an assay run. Before initiating the IMx LH assay, visually check that at least 300 mL of MEIA No. 2

Diluent Buffer is present. Do not add diluent buffer to the buffer bottle or switch buffer bottles during an assay run.

d. Recording of Data:
   1) Operator will review results.
   2) Operator will place printouts in file labeled for NHANES samples.
   3) Results and information about the run are entered into an Excel spreadsheet on a PC and copied into another Excel file to further evaluate the data.
   4) A printout of the Excel spreadsheet for each container ID results is made and comments noted.
   5) Project supervisor reviews the results. If problems noted with person results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.
   6) Daily log sheets are completed and any problems or issues noted.
   7) Repeat values are used when match the original results within 3 CSV's.

e. Calculations:
   1) The IMX System performs all calculations internally to produce the final reported result. Person test results are determined automatically by the system software using the smoothing "spline" math model. The amount of analyte in the sample is determined from the measured light production by means of a stored non-linear calibration curve.

The IMx LH assay utilizes a four parameter logistic curve fit (4PLC) generate a calibration curve. The following are assay-specific checks used to evaluate a calibration curve:

ASSAY PARAMETERS	CALIBRATOR EVALUATION (AVGR)
MIN SPAN F-A	Calibrator F - Calibrator A
MAX SAPN F-A	Calibrator F - Calibrator A
MAX CHECK 1	Calibrator A/Calibrator B
MAX CHECK 5	Calibrator E/Calibrator F

RERR (Rate Error)	RMSE (Root Mean Square Error)
$\pm$ 20	$\leq$ 0.5

For further information, refer to your IMX System Operation Manual, Section 5 for:
System Verification
MEIA Calibration and MEIA Mode 1 Assay Test Results Tape Explanation
Select Calibration and Mode 1 Assay Test Results Tape Explanation

9. REPORTABLE RANGE OF RESULTS

a. Analytical Range:
   1) 0.5 - the value of the highest calibrator (~250) mIU/mL
   2) A result over range high should be reported as ">250". To obtain a numerical answer, the specimen may be diluted with and equal volume of the hLH 0.0 Calibrator or ample Diluent A. After assaying the diluted sample, multiply the printed value by two to obtain the reportable answer.
   3) Limits of detection (LOD) are established by IMX and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

4) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the LH determination is 0.5 mIU/mL.
5) 0 is not a reportable value. Report results below 0.5 as "<0.5".

10. QUALITY CONTROL (QC) PROCEDURES

For an IMx LH calibration, all levels of LH controls must be processed as a means of evaluating the calibration curve. The control requirement for an IMx LH Mode 1 Assay is 2 controls on each carousel. All levels of controls should be processed at least 1 time during each 8-hour shift.

When a new lot of IMx LH Reagent Pack is used, run all levels of IMx LH controls. If any one of the controls is out of its specified range, assay recalibration is indicated.

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

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. Hemolyzed samples with up to 500 mg/dL hemoglobin have no significant interference.
b. <10 mg/dL bilirubin has no significant interference.
c. Lipemia has no significant interference in samples containing equivalent of 1800 mg/dL triglycerides.
d. Addition of 3 g/dL protein to sample did not affect hLH concentration.
e. This assay has been formulated to minimize the effect of human anti-mouse antibodies or heterophile antibodies which may be present in some patient samples.
f. LH results should be interpreted in conjunction with the patient's clinical presentation and data from other tests.
g. A variant molecular form of LH occurs frequently. Occurrence varies from 7% in US Hispanics to 42% in Laplanders. This variant may not react normally with the Access antibodies. We are working with Beckman to resolve this question. When a value occurs below the reference range this possibility must be considered. Values below the reference range will be given to the pathologist to consider what action may be necessary.

13. REFERENCE RANGES (NORMAL VALUES)

Normal Menstruating Females

Follicular Phase
n: 211 LH Value (mIU/mL)
Mean: 6 LH Value (mIU/mL)
Range: 2 to 15 LH Value (mIU/mL)

Mid-Cycle Peak
n: 26 LH Value (mIU/mL)
Mean: 549 LH Value (mIU/mL)
Range: 22 to 105 LH Value (mIU/mL)

Luteal Phase
n: 207 LH Value (mIU/mL)
Mean: 5 LH Value (mIU/mL)
Range: 0.6 to 19 LH Value (mIU/mL)

Postmenopausal Females
n: 45 LH Value (mIU/mL)
Mean: 31 LH Value (mIU/mL)
Range: 16 to 64 LH Value (mIU/mL)

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no critical call back values.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen with dry ice. Specimens are kept frozen at -70°C until ready to analyze. Sample is thawed, mixed well by vortexing, and then transferred to sample cup on the Access.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of Sample I.D. Specimen vial container is placed in -70°C Freezer after testing is complete.

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

Samples will remain in -70°C freezer until instrument is back in operation.

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

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an comma delimited file, either through electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfers of files or dial up modem transfer options are available.

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

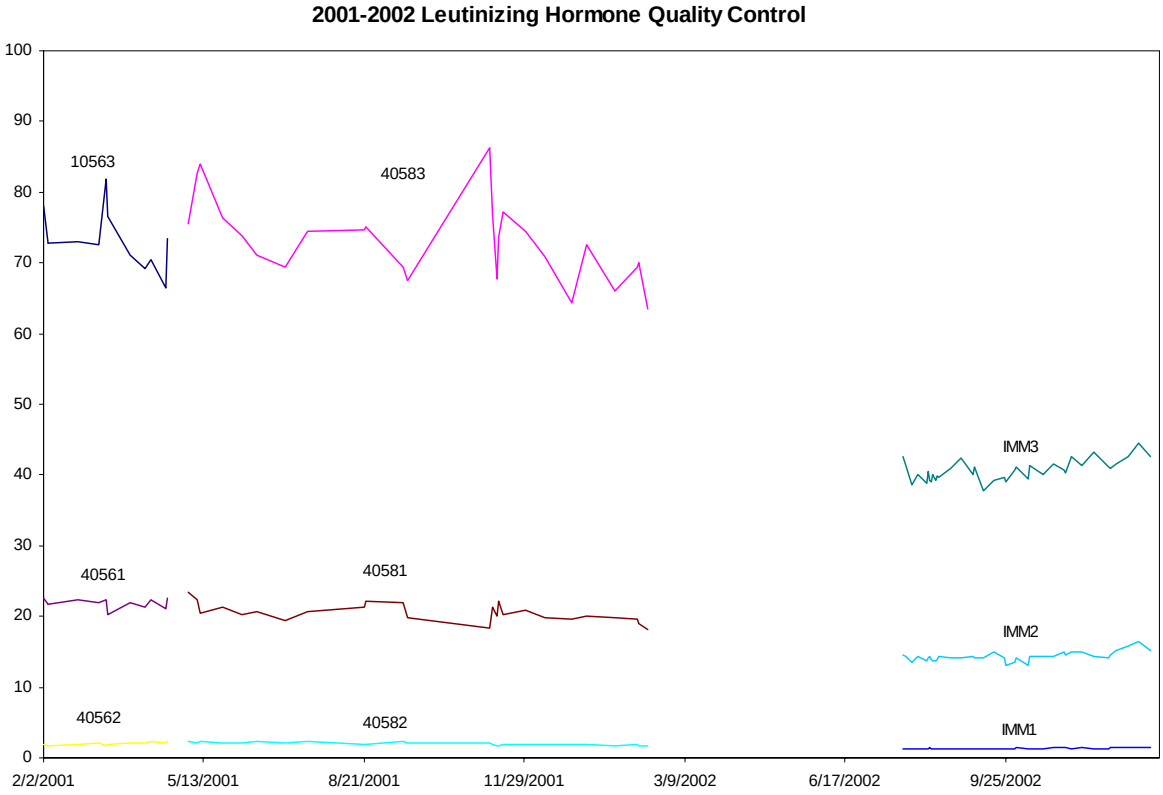
Microsoft Excel spreadsheets are used to keep records and track specimens analyzed on the IMX. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

Summary Statistics for Leutinizing Hormone by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
40561	11	2/2/2001	4/20/2001	1.991	0.183	9.2
40562	11	2/2/2001	4/20/2001	21.846	0.730	3.3
40563	11	2/2/2001	4/20/2001	73.230	4.294	5.9
40581	25	5/3/2001	2/14/2002	1.983	0.200	10.1
40582	25	5/3/2001	2/14/2002	20.510	1.268	6.2
40583	25	5/3/2001	2/14/2002	73.100	5.709	7.8
IMM1	37	7/23/2002	12/24/2002	1.33	0.07	5.4
IMM2	37	7/23/2002	12/24/2002	14.35	0.68	4.7
IMM3	37	7/23/2002	12/24/2002	40.69	1.47	3.6



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