Luteinizing Hormone (LH) in Refrigerated Serum – NHANES 2001-2002 Collaborative Laboratory Services, L.L.C.

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

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1.   SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The Access LH assay is a paramagnetic particle, chemiluminescent, two-step enzyme immunoassay for the quantitative determination of hLH in human serum using the Access Immunoassay System. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-mouse: mouse anti-hLH complexes and Tris buffered saline with protein. The serum hLH binds to the immobilized mouse anti-hLH on the solid phase. Separation in a magnetic field and washing removes materials not bound to the solid phase. Alkaline phosphatase conjugated goat anti-hLH is then added and binds to the previously bound hLH on the particles. A second separation and wash step removes unbound conjugate. A chemiluminescent substrate, Lumi-Phos 530, is added to the reaction vessel and light generated by the reaction is measured with a luminometer. The photon production is proportional to the amount of hLH in the sample. The amount of analyte in the sample is determined by means of a stored, multi-point calibration curve.

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 plasma 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.

Reagents and solvents used in this study include those listed in Section 6. 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 Beckman Access2 Immunoassay System. When ordered tests are completed for each sample, the results are printed out by Beckman Access2 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: Beckman Access2 Immunoassay System

    b.   Materials

        1)   Access Immunoassay 2 mL Sample Cups (Cat. #81902)

        2)   Access Immunoassay Reaction Vessels (Cat. #81901)

        3)   S/P Plastic Transfer Pipette (Cat. #P5214-10)

    c.   Reagent Preparation:

        1)   Access hLH Reagent Pack (Cat. #33510), 100 determinations, 50 tests/pack. Contains the following components.

        R1a: Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-hLH complexes suspended in Tris buffered saline with bovine serum albumin, surfactant, <0.1% sodium azide and 0.1% sodium azide, and 0.1% ProClin 300.

        R1b: Tris buffered saline with BSA, protein, surfactant, <0.1% sodium azide, and 0.1% ProClin 300.

        R1c: Goat anti-hLH-alkaline phosphatase conjugate in Tris saline buffer with BSA, protein, surfactant, <0.1% sodium azide, and 0.1% ProClin 300.

        a)   Provided ready to use.

        b)   Store upright at 2-10°C.

        c)   Packs must be refrigerated at 2-10°C for two hours before loading on instrument.

        d)   Unopened packs are stable until expiration date when stored as directed.

        e)   After initial use, pack is stable for 28 days at 2-10°C.

        f)   CAUTION: Sodium azide may react with lead and copper plumbing. On disposal of liquid, flush drain with large volume of water. ProClin is a potential skin sensitizer; in case of contact with reagent, thoroughly flush with water.

        2)   Access Substrate (Cat. #81906)

            a)   Lumi-Phos 530 (buffered solution containing dioxetane Lumigen PPD, flourescer, and surfactant).

            b)   Allow substrate to equilibrate, unopened at room temperature for a minimum of 18 hours (maximum 14 days) prior to use.

            c)   Unopened substrate is stable until expiration date when stored at 2-10°C.

            d)   Opened substrate on board in external fluids tray is stable for 14 days.

            e)   Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

        3)   Access Wash Buffer (Cat. #81907)

            a)   Tris buffered saline, surfactant, 0.1% sodium azide and 0.1% ProClin 300.

            b)   Stable until expiration date when stored at room temperature.

    d.   Standards Preparation: No preparation required.

        1)   Beckman Access hLH Calibrators (Cat. #33515).

    e.   Control Material:

        1)   Bio-Rad Immunoassay Plus Controls (Levels 1, 2, and 3) (Cat. #371, 372, 373).

            a)   Reconstitute each vial with 5 mL deionized water using a volumetric pipette. Replace the stopper and let control stand for 15 minutes. Before using, invert vial several times to mix.

            b)   Reconstituted control is stable for 7 days when stored at 2-8°C.

            c)   At least three levels of control should be analyzed in a 24 hour time period.

            d)   Ensure that assay control values are within the concentration ranges stated in the package insert or calculated from cumulative data at CLS.

            e)   Refer to Quality Control Flow Chart for action decision guidelines.

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:

        1)   Calibration is required when a new lot of hLH reagent is loaded, when the calibration curve expires (curve stability is 28 days), or when controls are out of range.

        2)   Refer to Access2 Quick Reference Guide or Access2 "help" icon for detailed instructions on programming a calibration.

8.   PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

    a.   Preliminaries

        1)   Program requested tests on Access instrument using sample I.D. and slot I.D. (See Attachment G).

    b.   Sample Preparation

        1)   Thaw samples and vortex, mixing well.

    c.   Operation

        1)   For detailed instructions on operating the Access, refer to the Access2 Quick Reference Guide, or use the "help" icon on the instrument screen.

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)   55 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.

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 patient 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.   Replacement and Periodic Maintenance of Key Components:
(See Attachment AC) for the Access Maintenance Schedule.

f.   Calculations:
1)   The Access Immunoassay System performs all calculations internally to produce the final reported result. Patient 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.

9.   REPORTABLE RANGE OF RESULTS

a.   Analytical Range:
1)   0.2 - 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 Access Sample Diluent A (Cat. #81908). After assaying the diluted sample, multiply the printed value by two to obtain the reportable answer.
3)   Limits of detection (LOD) are established by Beckman Coulter 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 0.2 mIU/mL.
5)   0 is not a reportable value. Report results below 0.2 as "<0.2".

10.   QUALITY CONTROL (QC) PROCEDURES

a.   Blind QC Specimens are included in the samples received from NHANES.
b.   Bio-Rad Immunoassay Plus Controls levels 1, 2, and 3 are assayed prior to running CDC-NHANES samples and after running CDC-NHANES samples.
c.   Acceptable Answer:
1)   Controls must be within ±2 S.D.
2)   Refer to Quality Control Flow Chart for action decisions guidelines (See Attachment I).

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)

LH

Group	Range (IU/L)
MALES	
prepubertal	<1*
adult	1.2-8.6
FEMALES	
prepubertal	<1*
follicular	2-11
mid-cycle	19-103
luteal	1-13
unspecified cycle	1-103
post-menopause	11-59

LH levels were measured in human serum samples from 50 adult males, 50 postmenopausal females, and 26 normal cycling females. The cycles were synchronized to the mid-cycle LH peak. The range of hLH levels generated at Beckman Instruments, Inc., is summarized above.

*MAYO clinic references

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 Access. 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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2001-2002 Leutinizing Hormone Quality Control
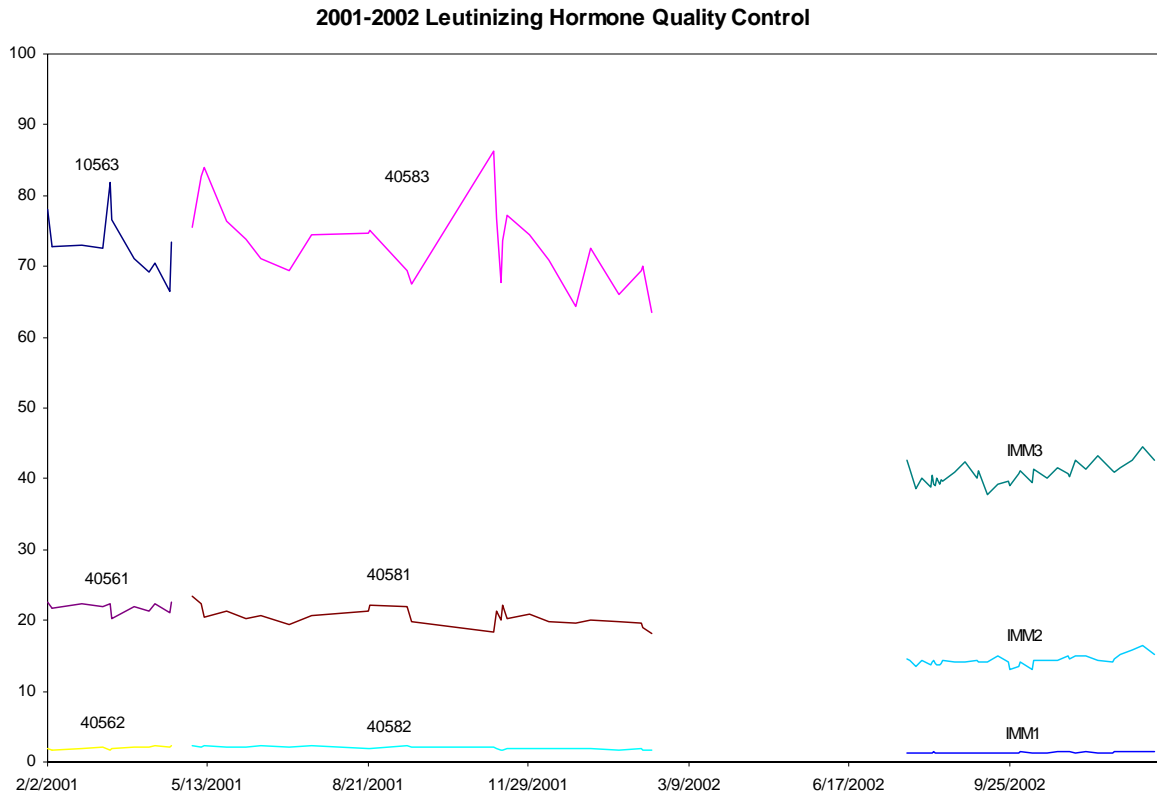
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