

Laboratory Procedure Manual

Analyte: **Follicle-Stimulating Hormone (FSH)**

Matrix: **Serum**

Method: **IMx FSH Assay**

Method No.:

Revised:

as performed by: **Coulston Foundation**
Alamogordo, New Mexico

Contact: **Ms. Julian Love**

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999–2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label (and SI units)
lab40	LBXFSH	Follicle-stimulating hormone (mIU/mL)
	LBDFSHSI	Follicle-stimulating hormone (IU/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The IMx follicle-stimulating hormone (FSH) assay is based on the Microparticle Enzyme Immunoassay (MEIA) technology. The IMx FSH reagents and sample are added to the reaction cell in the following sequence:

The probe/electrode assembly delivers the sample and adds anti- β FSH-coated microparticles to the incubation well of the reaction cell. The FSH binds to the anti- β 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- α FSH 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 FSH levels are used in investigations of menstrual, fertility, and pubertal development disorders, menopause, ovulatory disorders, and pituitary failure. The ratio of leuteinizing hormone (LH)/FSH has been used to assist in the diagnosis of polycystic ovary disease.

Low levels of hLH and hFSH may indicate pituitary failure, whereas 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 as 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 and 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.

Material safety data sheets (MSDS) 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 an IMX System. When ordered tests are completed for each sample, the results are printed out by 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 <1 g/dL hemoglobin.

B. Separated serum or plasma should not remain at 15–30°C longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at 2–8°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 FSH.

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-FSH-coated (mouse, monoclonal) microparticles in buffer with protein stabilizers. Preservative: 0.1% sodium azide. Store at 2–8°C until expiration date on the package.
- (2) One bottle (8.5 mL) anti-FSH (goat) alkaline phosphatase-conjugated in buffer with protein stabilizers. Minimum concentration: 0.1 µg/mL. Preservative: 0.1% sodium azide. Store at 2–8°C until expiration date on the package.
- (3) One bottle (10 mL) 4-methylumbelliferyl phosphate, 1.2 mM in buffer. Preservative: 0.1% sodium azide. Store at 2–8°C until expiration date on the package.
- (4) One bottle (13 mL) wash buffer containing surfactant. Store at 2–8°C until expiration date on the package.
- (5) One bottle (4 mL) IMx FSH Mode 1 Calibrator (C) Preservative: 0.2% sodium azide. Store at 2–8°C until expiration date on the package.
- (6) Six bottles (4 mL each) of IMx FSH calibrators; Preservative: 0.2% sodium azide. Store at 2–8°C until expiration date on the package.
- (7) Three bottles (8 mL each) of IMx FSH controls. Store at 2–8°C until expiration date on the package.

- C. All reagents are supplied ready for use.
- D. Standards Preparation: No preparation required.

The six bottles (4 mL each) of IMx FSH Calibrators are references against the WHO Second International Reference Preparation for human FSH (78/549). FSH is prepared in processed bovine serum at the following concentrations:

BOTTLE	FSH CONCENTRATION (mIU/mL)
A	0
B	1
C	10
D	50
E	100
F	150

Preservative: 0.2% sodium azide

- E. Control Material:

The three bottles (8 mL each) of IMx FSH controls are FSH prepared in calf serum to yield the following concentration ranges:

BOTTLE	FSH CONCENTRATION RANGE (mIU/mL)
L	3.5 to 6.5
M	18 to 32
H	53 to 97

Preservative: 0.2% sodium azide

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The IMx FSH assay utilizes a four-parameter logistic curve fit (4PLC) to 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)
± 20	≤ 0.5

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

- A. Preliminaries
Program requested tests on IMX instrument.
- B. Sample Preparation
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) Load the trays onto the instrument.
- (6) Press RUN.

D. The IMx FSH reagents and sample are added to the reaction cell in the following sequence:

- (1) The probe/electrode assembly delivers the sample and anti- β FSH-coated microparticles to the incubation well of the reaction cell.
- (2) The FSH binds to the anti- β FSH Coated Microparticles forming an antibody-antigen complex.
- (3) 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.
- (4) The matrix is washed with the wash buffer to remove unbound materials.
- (5) The anti- α FSH subunit-specific alkaline phosphatase-conjugated is dispensed onto the matrix and binds with the antibody-antigen complex.
- (6) The matrix is washed to remove unbound materials.
- (7) The substrate, 4-methylumbelliferyl phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

The list of required materials and the procedure to perform the IMx FSH assay can be found in the IMx System Operation Manual.

The IMx FSH assay requires a minimum volume of 300 mL of MEIA No. 2 diluent buffer in the buffer bottle to properly process an assay run. Before initiating the IMx FSH 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.

The IMx FSH assay parameters, illustrated in the package insert, have been factory set. These parameters can be printed, displayed, and edited according to the procedure in the IMx system Operation Manual. Ensure that the assay parameters for IMx FSH 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 26.12 or 31.12, can be edited to "8" (mIU/mL) and Printer Option, assay parameter 26.60 or 31.60, 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

E. 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.

9. REPORTABLE RANGE OF RESULTS

Normal Menstruating Females

Follicular phase

n: 159 FSH value (mIU/mL)
 Mean: 7 FSH value (mIU/mL)
 Range: 3 to 2 FSH value (mIU/mL)

Mid-cycle peak

n: 23 FSH value (mIU/mL)
 Mean: 14 FSH value (mIU/mL)
 Range: 9 to 26 FSH value (mIU/mL)

Luteal phase

n: 161 FSH value (mIU/mL)
 Mean: 4 FSH value (mIU/mL)
 Range: 1 to 12 FSH value (mIU/mL)

Postmenopausal females

n: 57 FSH value (mIU/mL)
 Mean: 74 FSH value (mIU/mL)
 Range: 18 to 153 FSH value (mIU/mL)

10. QUALITY CONTROL (QC) PROCEDURES

For an IMx FSH or Select FSH calibration, all levels of FSH controls must be processed as a means of evaluating the calibration curve. WSRC minimum control requirement for an IMx FSH Mode 1 Assay is one control and one mode one calibrator on each carousel. All three levels of controls are processed at least one time during each 8-hour shift.

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

The three bottles (8 mL each) of IMx FSH controls are FSH-prepared in calf serum to yield the following concentration ranges:

BOTTLE	FSH CONCENTRATION RANGE (mIU/mL)
L	3.5 to 6.5
M	18 to 32
H	53 to 97

Preservative: 0.2% sodium azide

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 person means to look for confirmatory or disconfirmatory evidence. When the two 2s and/or one 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 hFSH 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. FSH results should be interpreted in conjunction with the person's clinical presentation and data from other tests.

13. REFERENCE RANGES (NORMAL VALUES)

Normal menstruating females

Follicular phase

n: 159 FSH value (mIU/mL)
Mean: 7 FSH value (mIU/mL)
Range: 3–2 FSH value (mIU/mL)

Mid-cycle peak

n: 23 FSH value (mIU/mL)
Mean: 14 FSH value (mIU/mL)
Range: 9–26 FSH value (mIU/mL)

Luteal phase

n: 161 FSH value (mIU/mL)
Mean: 4 FSH value (mIU/mL)
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Postmenopausal females

n: 57 FSH value (mIU/mL)
Mean: 74 FSH value (mIU/mL)
Range: 18–153 FSH 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 IMX.

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 a 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

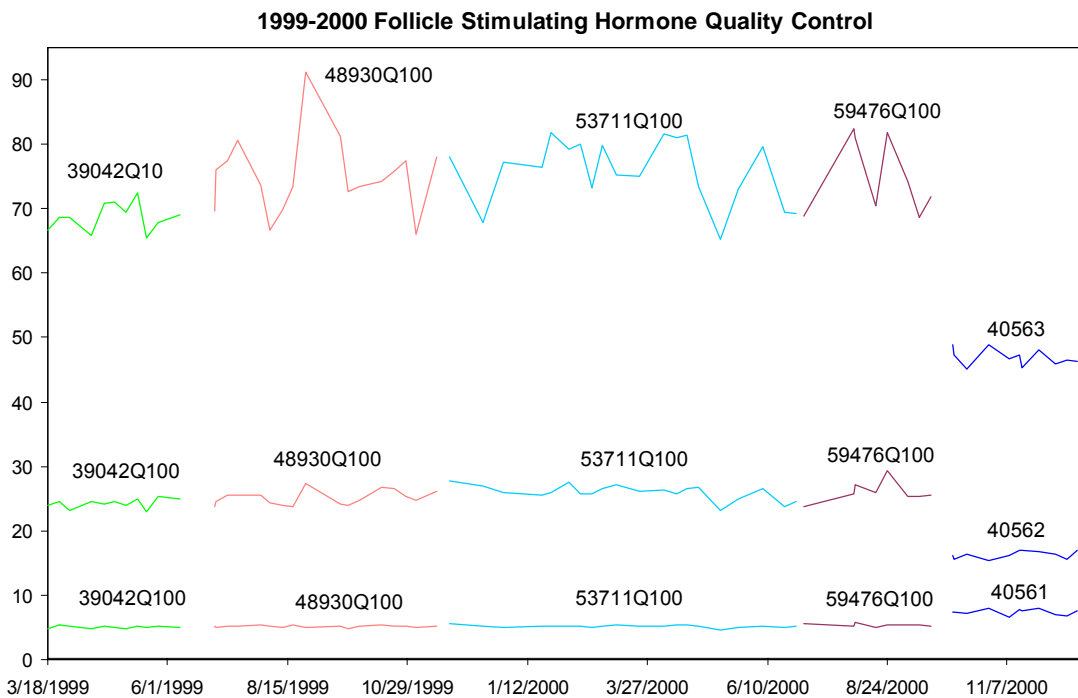
Microsoft Excel spreadsheets are used to keep records and track specimens analyzed on the IMX. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

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 Follicle-Stimulating Hormone by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
39042Q100	11	3/18/1999	6/9/1999	5.04	0.21	4.11
39042Q100	11	3/18/1999	6/9/1999	24.28	0.78	3.20
39042Q100	11	3/18/1999	6/9/1999	68.74	2.20	3.20
48930Q100	17	6/30/1999	11/16/1999	5.13	0.17	3.33
48930Q100	17	6/30/1999	11/16/1999	25.07	1.10	4.39
48930Q100	17	6/30/1999	11/16/1999	75.15	5.98	7.95
53711Q100	20	11/24/1999	6/28/2000	5.16	0.20	3.95
53711Q100	20	11/24/1999	6/28/2000	25.94	1.18	4.55
53711Q100	20	11/24/1999	6/28/2000	75.90	5.00	6.59
59476Q100	8	7/3/2000	9/20/2000	5.34	0.24	4.42
59476Q100	8	7/3/2000	9/20/2000	26.01	1.61	6.19
59476Q100	8	7/3/2000	9/20/2000	74.94	5.94	7.93
40561	11	10/4/2000	12/21/2000	7.40	0.48	6.48
40562	11	10/4/2000	12/21/2000	16.31	0.58	3.54
40563	11	10/4/2000	12/21/2000	46.95	1.28	2.73



REFERENCES

1. Beastall GH and others. Assays for follicle stimulating hormone and luteinizing hormone: Guidelines for the Provision of a Clinical Biochemistry Service. *Ann Clin Biochem.* 24:246-262.
2. Bonnar J. 1973. The hypothalamus and reproductive function. In: Scott RB and Walker RM, editors. *The Medical Annual.* Bristol (England): J Wright and Sons, pp. 251-258.
3. Catt KJ, Pierce JG. 1978. Gonadotropin hormones of the adenohypophysis. In: Yen SSC and Jaffe RB editors. *Reproductive Endocrinology.* Philadelphia: Saunders, pp. 75-113.
4. Crowley WF Jr, Filicori M, Santoro NF. 1987. GnRH section across the normal menstrual cycle. In: Crowley WF Jr, Hofler JG (eds). *The Episodic Section of Hormones.* New York: John Wiley and Sons, pp. 219-231.
5. Daughadny WH. 1985. The adenohypophysis. In: Wilson JD, Foster DW, editors. *Williams Textbook for Endocrinology.* Philadelphia: Saunders. pp. 80-83.
6. Franchimont P. 1973. Human gonadosecretion in male subjects. In: James VHT, Serio M, Martini L, editors. *The Endocrine Function of the Human Testis.* New York: Academic Press. pp. 439-458.
7. Griffin JE, Wilson JD. 1985. Disorders of the testes and male reproductive tract. In: Wilson JD, Foster DW, editors. *Williams Textbook of Endocrinology.* Philadelphia: Saunders. pp. 259-311.
8. Jeffcoate SL. 1975. The control of testicular function in the adult. *Clinics in Endocrinology and Metabolism.* 4:521-543.
9. Judd HL. Hormonal dynamics associated with the menopause. *Clin Obstet Gynecol* 19:775-788.
10. Pierce JG, Parsons TF. 1981. Glycoprotein Hormones: Structure and Function. *Annu Rev Biochem* 50:465-495.
11. National Committee for Clinical Laboratory Standards. Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue: Tentative Guideline. NCCLS Document M29-T2. Villanova, PA:NCCLS, 1991:1-43.
12. Primus FJ, Kelly EA, Hansen HJ, Goldenberg DM. 1988. "Sandwich" - Type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem.* 34:261-264.
13. Ross GT. 1985. Disorders of the ovary and female reproductive tract. In: Wilson JD, Foster DW, editors. *Williams Textbook of Endocrinology.* Philadelphia: Saunders. pp. 206-258.
14. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC Jr. 1985. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 45:879-885.
15. US Department of Labor, Occupational Safety and Health Administration, 29CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens; Final Rule. *Federal Register* 1991;56(235): 64175-182.
16. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication No. (CDC) 93-8395. Washington, DC: US Government Printing Office, May 1993.