Follicle-Stimulating Hormone (FSH)

ADVIA Centaur System

Principle of the Test

The ADVIA Centaur® FSH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two antibodies that have specificity for the intact FSH molecule. The first antibody, in the Lite Reagent, is a polyclonal sheep anti-FSH antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-FSH antibody, which is covalently coupled to paramagnetic particles.

The system automatically performs the following steps:

- dispenses 100 µL of sample into a cuvette
- dispenses 50 µL Lite Reagent and incubates for 5.0 minutes at 37°C
- dispenses 225 µL of Solid Phase and incubates for 2.5 minutes at 37°C
- separates, aspirates, and washes the cuvettes with reagent water
- dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
- reports results according to the selected option, as described in the system operating instructions or in the online help system

A direct relationship exists between the amount of FSH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Clinical Application and Usefulness

For in vitro diagnostic use in the quantitative determination of follicle-stimulating hormone (FSH) in serum using the ADVIA Centaur System.

Follicle-stimulating hormone (FSH) is a glycoprotein hormone with two subunits. The alpha subunit is similar to those of luteinizing hormone (LH), human chorionic gonadotropin (hCG), and thyroid-stimulating hormone (TSH).

The beta subunit is different from those of the other glycoprotein hormones and confers its biochemical specificity.¹

FSH is secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus.¹ In both males and females, FSH secretion is regulated by a balance of positive and negative feedback mechanisms involving the hypothalamic-pituitary axis, the reproductive organs, and the pituitary and sex steroid hormones.¹ FSH and LH play a critical role in maintaining the normal function of the male and female reproductive systems.
Specimen Collection and Handling

Specimen Collection

BIOHAZARD
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

• Serum is the recommended sample type for this assay.
• This assay requires 100 µL of sample for a single determination. Additional volume is required for onboard dilutions.
• Allow samples to clot adequately before centrifugation.
• Samples are free of fibrin or other particulate matter.
• Samples are free of bubbles.

Specimen Storage and Stability

• Keep tubes covered and upright at all times.
• Do not use samples that have been stored at room temperature for longer than 8 hours.
• Tightly cover and refrigerate specimens at 2 to 8°C if the assay is not completed within 8 hours.
• Freeze samples at or below -20°C if the sample is not assayed within 48 hours.
• Freeze samples only once and mix thoroughly after thawing.

Specimen Rejection Criteria

Unlabelled specimens are rejected
Grossly hemolyzed samples are rejected

Reagents

Storage and Stability

• Store the reagents upright at 2–8 °C.
• Primary reagents stable until the expiration date on the pack label, or for 14 days onboard the system.
• Diluent stable until the expiration date on the pack label, or for 28 consecutive days after accessing the ancillary reagent pack.

Ingredients

Reagent ingredients for the ADVIA Centaur FSH assay are as follows:
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lite Reagent</td>
<td>5.0 mL/ReadyPack®</td>
<td>polyclonal sheep anti-FSH antibody (~327.2 ng/mL) labeled with acridinium ester in buffer with sodium azide (0.1%) and preservatives</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>22.5 mL/ReadyPack</td>
<td>monoclonal mouse anti-FSH antibody (~0.01 mg/mL) covalently coupled to paramagnetic particles in buffer with protein stabilizers, sodium azide (0.1%), and preservatives</td>
</tr>
<tr>
<td>Multi-Diluent 1</td>
<td>25.0 mL/ReadyPack</td>
<td>equine serum with sodium azide (0.1%) and preservatives</td>
</tr>
</tbody>
</table>

**WARNING:** Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

**BIOHAZARD**

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**Reagents Special Preparation**

No special preparation of reagents is required.
**Calibration**

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

**Two-point Calibration Interval**

Use Calibrator B to perform two-point calibrations.

Perform a two-point calibration every 28 days when using the high volume usage option.

In addition, perform a two-point calibration when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

**Defining Calibrator Values for Two-point Calibration**

1. At the workspace, select **Calibration**.
2. Select **Calibrator Definition**.
3. Select **Scan Data**.
4. Scan the barcodes on the Calibrator Assigned Value Card.
5. Define the LIS field.
6. Review the values on the Calibrator Assigned Value card to ensure that they are correct. Scan again, if required.
7. Select **Save**.

**Master Curve**

Use the barcode reader to enter the Master Curve values from the Master Curve card onto the system. Ensure that the lot number on the Master Curve matches the lot number of the ReadyPack.

**Defining the Master Curve Using the Barcode Scanner**

1. At the workspace, select **Calibration**.
2. Select **Master Curve Definition**.
3. At the Calibration-Master Curve Definition window, select **Scan Data**.
4. Scan the barcodes on the FSH Master Curve Card.
5. Select **Save**.

**Quality Control (QC)**

BioRad Immunoassay Plus Control 1, 2 & 3

Each control is reconstituted with 5.0 mL of Type 1 de-ionized water. The reconstituted controls are stable for 7 days when stored at 2-8 °C.
See posted QC chart for acceptability limits.

**QC Frequency**
Analyze all three levels of quality control material on each day that samples are analyzed.
Analyze all three levels of quality control material each time a two-point calibration is performed.

**Troubleshooting Out-of-Range QC Values**
A QC run is acceptable when all values fall within the expected ranges.
If the FSH QC results do not fall within the defined ranges then the run is rejected, and you must take the following corrective action:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, recalibrate the system and then rerun the quality control samples or contact Bayer Diagnostics for more assistance

**Instrument Operation and System Description**
The ADVIA Centaur system is an automated, random access, direct chemiluminescent immunoassay analyzer that offers no-pause reloading of reagents, samples, and supplies. Comprehensive assay groups are available.

When the sample start button is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Paramagnetic particles are magnetically separated in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Acid and Base. The chemiluminescent reaction occurs in the luminometer. The PMT measures the chemical light reaction that takes place.

Refer to Section 4 in the ADVIA Centaur Reference Manual for detailed procedures that describe how to schedule samples and manage the worklist.

**A. System Start Button**
There is one (1) main system operation button on the ADVIA Centaur, the Start button. Pressing this button performs the following actions:

- Homes the subsystems.
- Starts specimen sampling.
- If the Start button is pressed while the system is processing samples, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

**B. Start-up**
1. Put the samples racks on the sample entry queue.
2. Press the Start button.
C. Verify Supplies:
While the system is running, you can manage the following supplies without interrupting the run.
- Reagent water supply
- Liquid waste container
- Acid and Base reagents
- Cuvette waste bin
- Sample tip waste bin and tip tray waste area
- Cuvette supply
- Sample tip supply
- Ancillary reagent packs
- Primary reagent packs

D. Scheduling a Run/Entering a Worklist
You can enter a worklist by different methods:

Automated Worklist
You can send the worklist to the ADVIA Centaur from a Lab Information System (LIS). This is done in a unidirectional manner (Dynamic Download) or in a bi-directional manner (Host Query).
Manual (Operator-initiated) Worklist

Scheduling Calibrators
1. At the workspace, select Worklist and then select Schedule.
2. Select the Calibrator box.
3. Select the test you want to schedule for calibration.
4. The reagent lot that is on the system and any defined calibrators are displayed. Select the appropriate reagent and calibrator lot combination.
5. Select Save.
6. Ensure that the lot numbers of reagent and calibrator are available for system use.

Scheduling Controls
1. At the workspace, select Worklist and then select Schedule.
2. Select the Control box.
3. Select each test you would like to schedule.
4. Select each control level and lot number you need to run.
5. Select Save.

Scheduling Patient Samples
1. At the workspace, select Worklist and then select Schedule.
2. Select the Patient box.
3. Enter a patient sample identification number (SID).
4. Press the Enter key.
5. Select each test, profile, dilution, and/or replicates needed.
6. Select Save.

For detailed operating procedural information, refer to the ADVIA Centaur Reference Manual or to the online help system.
E. Loading Sample Racks

**BIOHAZARD**
All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

**CAUTION:** Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Gently mix the calibrators and quality controls before dispensing into the sample cups.
2. Load the sample cups containing the calibrators, controls, or patient specimen into any Centaur rack.
3. Load racks onto the sample entry queue.
4. Press the Start button.

F. Loading Ancillary Reagents

Place the ancillary reagent pack on the ancillary entry queue until the green light comes on. The ADVIA Centaur moves the pack into the ancillary queue.

G. Loading Primary Reagents

**CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing reagents for use, refer to Appendix C, *Handling Reagents*, in your ADVIA Centaur Assay Manual.

1. Gently mix the FSH ReadyPack before placing it on the reagent shelf for the first time.
2. Load the FSH ReadyPack by matching the color on the pack to the color-coded reagent shelf.
3. Close the reagent compartment door.

H. Loading STAT Samples in the STAT Entry Queue

1. Press the Start button, if the green Start light is not lit.
2. Place the STAT sample tube or sample cup in the rack.
3. Load the rack in the STAT entry queue until the green STAT light comes on, indicating the system has accepted the rack.
Performance Characteristics

Precision

Five samples were assayed 6 times, in each of 12 runs, on 3 systems, (n = 72 for each sample), over a period of 2 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (mIU/mL) (IU/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>2.9</td>
<td>2.7</td>
<td>3.9</td>
</tr>
<tr>
<td>12.0</td>
<td>2.1</td>
<td>1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>23.4</td>
<td>2.0</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>53.1</td>
<td>2.2</td>
<td>0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>144.3</td>
<td>2.7</td>
<td>1.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Standardization

The ADVIA Centaur FSH assay standardization is traceable to World Health Organization (WHO) 2nd International Standard for human FSH (IS 94/632). A comparison over the full assay range gave the following correlation:

ADVIA Centaur FSH = 0.91 (WHO) – 0.18 IU/mL
\[ r = 0.999 \]

Assigned values for calibrators and ranges of Ligand Plus controls are traceable to this standardization.

Specificity

The cross-reactivity of the ADVIA Centaur FSH assay with TSH, LH, hCG, prolactin, and hGH was determined by adding these hormones to serum samples containing FSH. The level of FSH in the samples was then determined.

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>FSH value without cross-reactant (mIU/mL) (IU/L)</th>
<th>FSH value with cross-reactant (mIU/mL) (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH; 1000 µIU/mL</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>29.7</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td>62.0</td>
<td>63.0</td>
</tr>
<tr>
<td>LH; 300 mIU/mL</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>30.3</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>64.3</td>
</tr>
<tr>
<td>hCG; 200,000 mIU/mL</td>
<td>2.7</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>29.5</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>62.2</td>
<td>65.5</td>
</tr>
<tr>
<td>Prolactin; 400 ng/mL</td>
<td>2.8</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>30.2</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>61.9</td>
<td>62.3</td>
</tr>
</tbody>
</table>
Analytical Sensitivity

The ADVIA Centaur FSH assay has an analytical sensitivity of 0.3 mIU/mL (IU/L).

Analytical sensitivity is defined as the concentration of FSH that corresponds to the RLUs that are two standard deviations greater than the mean RLUs of 9 replicate determinations of the FSH zero standard.

Analytical Measuring Range (AMR)

The analytical measuring range of the ADVIA Centaur LH assay is 0.3 to 200.0 IU/L.

Reporting Results

Units for Reporting Results

The system reports serum LH results in mIU/mL (mass units) or IU/L (SI units). The conversion formula is 1 mIU/mL = 1 IU/L. Units are user-defined in the system software.

Reportable Range

The reportable range of the ADVIA Centaur FSH assay is 0.3 mIU/mL (IU/L) to 400 mIU/mL (IU/L).

Reference Interval for Females

<table>
<thead>
<tr>
<th>Sample Category</th>
<th>Range (mIU/mL) (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normally menstruating</td>
<td></td>
</tr>
<tr>
<td>Follicular Phase</td>
<td>2.5–10.2</td>
</tr>
<tr>
<td>Midcycle Peak</td>
<td>3.4–33.4</td>
</tr>
<tr>
<td>Luteal Phase</td>
<td>1.5–9.1</td>
</tr>
<tr>
<td>Pregnant</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>23.0–116.3</td>
</tr>
</tbody>
</table>

Reference Interval for Males (ages 13-70)

- 0.6–14.7 mIU/mL (IU/L)
**Units for Reporting Results**

The system reports serum FSH results in mIU/mL (mass units) or IU/L (SI units). The conversion formula is 1 mIU/mL = 1 IU/L. Units are user-defined in the system software.

**Reportable Range**

The reportable range of the ADVIA Centaur FSH assay is 0.3 mIU/mL (IU/L) to 200 mIU/mL (IU/L).

**Dilutions**

- Serum samples with FSH levels greater than 200 mIU/mL (IU/L) must be diluted and retested to obtain accurate results.
- Patient samples can be automatically diluted by the system or prepared manually.
- For automatic dilutions, ensure that ADVIA Centaur Multi-Diluent 1 is loaded and set the system parameters as follows:
  - Dilution point: ≤ 200 mIU/mL (IU/L)
  - Dilution factor: 2
  
  For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.
- Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
- Use Multi-Diluent 1 to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

**Procedure Notes**

**Calculations**

For detailed information about how the system calculates results, refer to the ADVIA Centaur Reference Manual or to the online help system.

**High Dose Hook Effect**

Patient samples with high FSH levels can cause a paradoxical decrease in the RLUs (high dose hook effect). In this assay, patient samples with FSH levels as high as 1000 mIU/mL (IU/L) will assay greater than 200 mIU/mL (IU/L).

**Disposal**

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.
**Method Limitations**

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

<table>
<thead>
<tr>
<th>Serum specimens that are . . .</th>
<th>Have an insignificant effect on the assay up to . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolyzed</td>
<td>150 mg/dL of hemoglobin</td>
</tr>
<tr>
<td>lipemic</td>
<td>1000 mg/dL of triglycerides</td>
</tr>
<tr>
<td>icteric</td>
<td>20 mg/dL of bilirubin</td>
</tr>
</tbody>
</table>

If there is an interferant (hemolysis, lipemia and icterus) higher than the values listed in the above table, run the sample undiluted and send the corresponding ETC Code:

- **TUR** “Specimen turbid, result may be invalid”
- **ICTRQ** “Specimen icteric, result may be invalid”
- **HEMRQ** “Specimen hemolyzed, result may be invalid”

For additional information on performance characteristics including cross reactivity and dilution recovery, see the product information in the ADVIA Centaur Assay Manual.
**Equipment and Supplies**

- ADVIA Centaur FSH ReadyPack
- ADVIA Centaur Calibrator B
- ADVIA Centaur Multi-Diluent 1
- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- ADVIA Centaur Cleaning Solution Concentrate
- ADVIA Centaur Acid Reagent (0.5% H$_2$O$_2$, 0.1N HNO$_3$)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

**References**

1. Bayer Diagnostics ADVIA Centaur FSH product insert, Revision C.
2. Bayer Diagnostics ADVIA Centaur Reference Manual, Revision D.

**Technical Assistance**

Bayer Diagnostics Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number: IRL 80600727

Customer Account Number: 619504