

Luteinizing Hormone (LH)

ADVIA Centaur System

Principle of the Test

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

The system automatically performs the following steps:

- dispenses 50 µL of sample into a cuvette
- dispenses 100 µL of Lite Reagent and incubates for 5.0 minutes at 37°C
- dispenses 400 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 LH 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 luteinizing hormone (LH) in serum using the ADVIA Centaur System.

Luteinizing hormone (LH) is a glycoprotein hormone having two subunits. The alpha subunit is similar to those of follicle-stimulating hormone (FSH), 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

LH is secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus.¹ In males, LH is also called interstitial cell-stimulating hormone (ICSH).¹ In both males and females, LH 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 LH and the other pituitary gonadotropin, FSH, 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 50 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 28 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.

CAUTION:

- Discard the primary reagent packs at the end of the onboard stability interval.

- Do not use reagents beyond the expiration date.

Ingredients

Reagent ingredients for the ADVIA Centaur LH assay are as follows:

Reagent	Volume	Ingredients
Lite Reagent	6.0 mL/ReadyPack®	monoclonal mouse anti-LH antibody (~0.17 µg/mL) labeled with acridinium ester in buffer with sodium azide (< 0.1%) and preservatives
Solid Phase	24.0 mL/ReadyPack	monoclonal mouse anti-LH antibody (~0.05 mg/mL) covalently coupled to paramagnetic particles in buffer with sodium azide (0.1%) and preservatives
Multi-Diluent 1	25.0 mL/ReadyPack	equine serum with sodium azide (0.1%) and preservatives

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.



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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. Additionally, calibrate 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 LH 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 LH 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 sample 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



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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 LH ReadyPack before placing it on the reagent shelf for the first time.
2. Load the LH 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

Six 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:

Mean (mIU/mL) (IU/L)	Within-run % CV	Run-to-run % CV	Total % CV
4.2	2.3	1.5	2.7
7.4	2.9	2.4	3.8
16.4	2.3	2.9	3.7
21.7	2.9	2.4	3.8
58.4	3.0	2.5	3.8
118.2	2.6	2.3	3.5

Standardization

The ADVIA Centaur LH assay standardization is traceable to World Health Organization (WHO) 2nd International Standard for human LH (IS 80/552). A comparison over the full assay range gave the following correlation:

$$\text{ADVIA Centaur LH} = 1.05 (\text{WHO}) + 1.02 \text{ IU/mL}$$

$$r = 0.999$$

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

Specificity

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

Cross-reactant	LH value without cross-reactant (mIU/mL) (IU/L)	LH value with cross-reactant (mIU/mL) (IU/L)

TSH; 1000 μ IU/mL	13.3	13.1
	26.8	27.4
	46.3	47.2
β -LH; 5 ng/mL	8.4	9.6
	20.4	21.6
	39.5	41.4
	71.8	71.1
hCG; 200,000 mIU/mL	13.3	12.6
	26.8	24.5
	46.3	43.4
β -hCG; 10,000 mIU/mL	3.0	2.6
	8.6	7.4
	75.8	68.4
FSH; 200 mIU/mL	13.3	13.5
	26.8	27.4
	46.3	48.2

Interference testing was determined according to NCCLS Document EP7-P.

Analytical Sensitivity

The ADVIA Centaur LH assay has an analytical sensitivity of 0.1 mIU/mL (IU/L).

Analytical sensitivity is defined as the concentration of LH that corresponds to the RLUs that are two standard deviations greater than the mean RLUs of 20 replicate determinations of the LH zero standard.

Analytical Measuring Range (AMR)

The analytical measuring range of the ADVIA Centaur LH assay is 0.1 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 LH assay is 0.1 IU/L to 400.0 IU/L. Values below 0.1 IU/L are reported as <0.1.

Reference Interval for Females

Category	Range mIU/mL (IU/L)
Normally menstruating	
follicular phase	1.9–12.5
midcycle peak	8.7–76.3
luteal phase	0.5–16.9
Pregnant	< 0.1–1.5
Postmenopausal	15.9–54.0
Contraceptives	0.7–5.6

Reference Interval for Males (ages 20–70 years)

- 0.7–7.9 mIU/mL (IU/L)

Reference Interval for Males (> 70 years)

- 3.1–34 mIU/mL (IU/L)

Reportable Range

The reportable range of the ADVIA Centaur LH assay is 0.07 mIU/mL (IU/L) to 200 mIU/mL (IU/L).

Dilutions

- Serum samples with LH 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.
- **NOTE:** The sample volume required to perform onboard dilution (1:2) is 100 µL.
- 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

In ADVIA Centaur LH assay, patient samples spiked to LH levels up to 18,000 mIU/mL (IU/L) do not demonstrate a paradoxical decrease in the RLUs (high dose hook effect).

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

Administration of gonadotropin-releasing hormone (GnRH), clomiphene citrate, and human menopausal gonadotropin (hMG) to females can result in elevated endogenous LH values, while administration of estrogens or testosterone can result in decreased endogenous LH values. Administration of hMG to males can result in elevated endogenous LH values, while administration of testosterone decreases endogenous LH values.

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.

Serum specimens that are . . .	Have an insignificant effect on the assay up to . . .
hemolyzed	500 mg/dL of hemoglobin
lipemic	3000 mg/dL of triglycerides
icteric	20 mg/dL of bilirubin

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 LH 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% H₂O₂, 0.1N HNO₃)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

References

1. Bayer Diagnostics ADVIA Centaur LH product insert, Revision E.
2. Bayer Diagnostics ADVIA Centaur Reference Manual, Revision D.
3. Bayer Diagnostics ADVIA Centaur Assay Manual, Revision AT.
4. National Committee for Clinical Laboratory Standards (NCCLS). Clinical Laboratory Procedure Manuals—Third Edition (GP2-A3), 1996.

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