Cortisol

ADVIA Centaur System

Principle of the Test

The ADVIA Centaur® Cortisol assay is a competitive immunoassay using direct chemiluminescent technology. Cortisol in the patient sample competes with acridinium ester-labeled cortisol in the Lite Reagent for binding to polyclonal rabbit anti-cortisol antibody in the Solid Phase. The polyclonal rabbit anti-cortisol antibody is bound to monoclonal mouse anti-rabbit antibody, which is covalently coupled to paramagnetic particles in the Solid Phase.

The system automatically performs the following steps:

- dispenses 20 µL of sample into a cuvette
- dispenses 50 µL of Lite Reagent and 250 µL of Solid Phase and incubates for 5.0 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

An inverse relationship exists between the amount of cortisol 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 cortisol in serum using the ADVIA Centaur System.

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 20 µL of sample for a single determination. Additional volume is required for onboard dilutions.
- Allow serum samples to clot adequately before centrifugation.
Samples are free of fibrin or other particulate matter.
Samples are free of bubbles.

Specimen Storage and Stability
- 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.

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 10 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 Cortisol 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>2.5 mL/ReadyPack</td>
<td>cortisol (~1.7 ng/mL) labeled with acridinium ester in buffered saline with sodium salicylate (~50 mg/mL), sodium azide (0.1%) and preservatives</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>12.5 mL/ReadyPack</td>
<td>rabbit anti-cortisol antibody (~1.1 µg/mL) bound to monoclonal mouse anti-rabbit IgG antibody (~56 µg/mL) covalently coupled to paramagnetic particles in buffered saline with sodium azide (0.1%) and preservatives</td>
</tr>
<tr>
<td>Multi-Diluent 3</td>
<td>5.0 mL/ReadyPack</td>
<td>human plasma with sodium azide (0.1%)</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 E 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 Cortisol Master Curve Card.
5. Select Save.

Quality Control (QC)

BioRad Immunoassay Plus controls levels 1 and 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 at least two levels of quality control material on each day that samples are analyzed.
Analyze all 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 Cortisol 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 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 Cortisol ReadyPack before placing it on the reagent shelf for the first time.
2. Load the Cortisol 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 24 runs, on 6 systems, (n = 144 for each sample), over a period of 2 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (µg/dL)</th>
<th>Mean (nmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.88</td>
<td>107.05</td>
<td>3.69</td>
<td>5.45</td>
<td>6.58</td>
</tr>
<tr>
<td>5.63</td>
<td>155.33</td>
<td>3.09</td>
<td>3.83</td>
<td>4.92</td>
</tr>
<tr>
<td>14.17</td>
<td>390.95</td>
<td>2.89</td>
<td>3.07</td>
<td>4.22</td>
</tr>
<tr>
<td>27.53</td>
<td>759.55</td>
<td>3.82</td>
<td>1.86</td>
<td>4.25</td>
</tr>
<tr>
<td>37.15</td>
<td>1024.97</td>
<td>2.98</td>
<td>3.99</td>
<td>4.98</td>
</tr>
</tbody>
</table>

**Standardization**

The ADVIA Centaur Cortisol assay is standardized using internal standards manufactured analytically which are traceable to gas chromatography-mass spectroscopy (GCMS). The following equation describes the relationship between the cortisol standards and GCMS analysis throughout the range of the assay.

\[
\text{ADVIA Centaur Cortisol} = 0.99 \times \text{(GCMS)} + 0.75 \, \mu\text{g/dL}, \ r = 0.99
\]

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

**Specificity**

The Centaur Cortisol assay is highly specific for cortisol.

Cross-reactivity by structurally related compounds and pharmaceuticals was determined by spiking each compound into separate human serum samples to a final level of 1000 µg/dL (27,590 nmol/L), unless otherwise noted.

\[
\% \text{ cross-reactivity} = \left( \frac{\text{cortisol in spiked sample, } \mu\text{g/dL} - \text{cortisol in unspiked sample, } \mu\text{g/dL}}{\text{concentration of compound added, } \mu\text{g/dL}} \right) \times 100
\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of Compound tested</th>
<th>Updated % Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>1000 µg/dL</td>
<td>0.3</td>
</tr>
<tr>
<td>Allotetrahydrocortisol</td>
<td>100 µg/dL</td>
<td>6.5</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>1000 µg/dL</td>
<td>ND</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1000 µg/dL</td>
<td>5.3</td>
</tr>
</tbody>
</table>
### Analytical Sensitivity

The ADVIA Centaur Cortisol assay has an analytical sensitivity of 1 µg/dL.

Analytical sensitivity is defined as the concentration of cortisol that corresponds to the RLUs that are two standard deviations less than the mean RLUs of 20 replicate determinations of the Cortisol zero standard.
Analytical Measuring range (AMR)

The analytical measuring range of the ADVIA Centaur Cortisol assay is 1 µg/dL to 75 µg/dL.

Reportable Range

The reportable range of the ADVIA Centaur Cortisol assay is 1 µg/dL to dilute until an answer is achieved. Values below 1 µg/dL are reported as <1 µg/dL.

Dilutions

- NOTE: serum samples with cortisol levels greater than 75 µg/dL (2069 nmol/L) are programmed to rerun on a 1:2 instrument performed autodilution.
- The sample volume required to perform onboard dilutions (1:2) is 100 µL.
- For automatic dilutions, ensure that ADVIA Centaur Multi-Diluent 3 is on the Centaur.
- Ensure that results are mathematically corrected for dilution.
- If upon a 1:2 autodilution the result is >150, then perform a manual dilution until an answer is achieved. See dilution instructions for details.

Reference Interval

- 7–9 a.m. serum: 4–22 µg/dL
- 3–5 p.m. serum: 3–17 µg/dL

Units for Reporting Results

The system reports serum cortisol results in µg/dL (mass units) or nmol/L (SI units). The conversion formula is 1 µg/dL = 27.59 nmol/L. Units are user-defined in the system software.

The system reports direct urine results in µg/dL.

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.

**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**

Circulating cortisol results from patients receiving Prednisolone or Prednisone (which is converted to Prednisolone in vivo) therapy may be falsely elevated. Caution must be exercised with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids.

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 . . .

<table>
<thead>
<tr>
<th>Condition</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolyzed</td>
<td>500 mg/dL of hemoglobin</td>
</tr>
<tr>
<td>lipemic</td>
<td>1170 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 Cortisol ReadyPack
- ADVIA Centaur Calibrator E
- ADVIA Centaur Multi-Diluent 3
- 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 Cortisol product insert, Revision E.
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