Gentamicin

Advia Centaur System

Principle of the Test

The ADVIA Centaur® Gentamicin assay is a competitive immunoassay using direct, chemiluminescent technology. Gentamicin in the patient sample competes with acridinium ester-labeled gentamicin derivative in the Lite Reagent for a limited amount of monoclonal mouse anti-gentamicin antibody, which is coupled to paramagnetic particles in the Solid Phase.

The system automatically performs the following steps:

• dispenses 15 µL of the sample into a cuvette
• washes the reagent probe twice with 450 µL of Probe Wash 2, if necessary
• dispenses 100 µL of Lite Reagent and 400 µL of Solid Phase and incubates for 7.5 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

An inverse relationship exists between the amount of gentamicin 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 gentamicin in serum or plasma 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. Heparinized plasma is also acceptable.
• This assay requires 15 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**
- 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 -70°C if the sample is not assayed within 48 hours. Frozen specimens can remain frozen for up to 1 month in non frost-free freezers.
- 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.
- Lite Reagent and Solid Phase stable until the expiration date on the pack label, or for 14 days onboard the system.
- Probe Wash 2 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. Discard the Probe Wash 2 reagent pack after 28 days onboard.
- Do not use reagents beyond the expiration date.

**Ingredients**

Reagent ingredients for the ADVIA Centaur Gentamicin 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>gentamicin derivative labeled with acridinium ester (~0.46 ng/ml) in phosphate buffer with BSA, sodium azide (&lt; 0.1%), and preservatives</td>
</tr>
</tbody>
</table>
Solid Phase 20.0 mL/ReadyPack  monoclonal mouse anti-gentamicin antibody (~20 µg/ml) covalently coupled to paramagnetic particles in phosphate buffered saline with BSA, sodium azide (< 0.1%), and preservatives

Probe Wash 2 22.5 mL/ReadyPack 0.2N hydrochloric acid

Multi-Diluent 7 5.0 mL/ReadyPack human serum with sodium azide (< 0.1%)

**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 Gentamicin Calibrator 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**

*Note:* A Calibrator Definition is required for new lots of calibrator only. Instrument status must be at “Ready” in order to perform a Calibrator Definition.

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. Review the values on the Calibrator Assigned Value card to ensure that they are correct. Scan again, if required.

6. 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. A Master Curve Definition must be performed when loading a new lot of primary reagent onto the Centaur. Once a Master Curve has been entered for a particular lot of primary reagent, it does not need to be re-entered when loading subsequent primary reagent packs for that lot #. A Master Curve Definition can be performed while the Centaur is “In Process”.

**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 Gentamicin Master Curve Card.
5. Select Save.

**Quality Control (QC)**

**QC Materials**

BioRad Immunoassay Plus Control 1 & 3

Each control is reconstituted with 5.0 mL of Clinical Laboratory Reagent Water (CLRW). The reconstituted controls are stable for 7 days when stored at 2-8° C.

See posted QC chart for acceptability limits.

**QC Frequency**

Analyze both levels of quality control material on each day that samples are analyzed.

Analyze both 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 Gentamicin 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
- 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 with the barcode facing you 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 Gentamicin ReadyPack before placing it on the reagent shelf for the first time.

2. Load the Gentamicin ReadyPack by matching the color on the pack to the color-coded reagent shelf.

3. Load one Probe Wash 2 ReadyPack by matching the color on the pack to the color-coded reagent shelf.

4. 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**

Four samples were assayed 6 times in 26 assays, on 7 systems (n = 156 for each sample), over a period of 3 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (mg/L)</th>
<th>Mean (µmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.52</td>
<td>3.2</td>
<td>5.95</td>
<td>1.99</td>
<td>6.27</td>
</tr>
<tr>
<td>4.59</td>
<td>9.6</td>
<td>4.13</td>
<td>2.85</td>
<td>5.01</td>
</tr>
<tr>
<td>9.20</td>
<td>19.2</td>
<td>6.85</td>
<td>3.75</td>
<td>7.81</td>
</tr>
<tr>
<td>11.78</td>
<td>24.6</td>
<td>5.99</td>
<td>4.96</td>
<td>7.78</td>
</tr>
</tbody>
</table>

**Standardization**

The ADVIA Centaur Gentamicin assay is traceable to an internal standard manufactured using U.S.P. (United States Pharmacopeia) material. Assigned values of calibrators and ranges of Ligand Plus controls are traceable to this standardization.

**Specificity**

Pooled serum samples at therapeutic gentamicin levels were spiked with the compounds listed at the levels indicated. ADVIA Centaur Gentamicin assay results from the spiked samples were compared with those of unspiked control samples. These compounds did not have a significant effect on the ADVIA Centaur Gentamicin measurement (p > 0.05, t-test).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount Added (mg/L)</th>
<th>Compound</th>
<th>Amount Added (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>150</td>
<td>Kanamycin B</td>
<td>160</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>20</td>
<td>Lincomycin</td>
<td>200</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>50</td>
<td>Methicillin</td>
<td>200</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>2000</td>
<td>Methotrexate</td>
<td>4500</td>
</tr>
<tr>
<td>Cefamandole Nafate</td>
<td>500</td>
<td>Methylprednisolone</td>
<td>200</td>
</tr>
<tr>
<td>Cephalaxin</td>
<td>180</td>
<td>Neomycin</td>
<td>100</td>
</tr>
<tr>
<td>Cephaloglycin</td>
<td>160</td>
<td>Oxytetracycline</td>
<td>40</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>650</td>
<td>Penicillin V</td>
<td>15 U/mL</td>
</tr>
<tr>
<td>Cephalosporin C</td>
<td>160</td>
<td>Prednisolone</td>
<td>200</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>1000</td>
<td>Rifampin</td>
<td>50</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>250</td>
<td>Spectinomycin</td>
<td>300</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>200</td>
<td>Streptomycin</td>
<td>200</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>150</td>
<td>Sulfadiazine</td>
<td>500</td>
</tr>
</tbody>
</table>
In addition, the following compounds were spiked into patient serum samples without gentamicin and the following results were obtained. Percent change is calculated as:

% cross-reactivity = \( \frac{\text{concentration of spiked sample} - \text{concentration of unspiked sample}}{\text{amount of compound spiked}} \) \times 100

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount Added (µg/mL)</th>
<th>% cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netilmicin</td>
<td>10</td>
<td>24.1</td>
</tr>
<tr>
<td>Sisomicin</td>
<td>10</td>
<td>24.8</td>
</tr>
</tbody>
</table>

**Analytical Sensitivity**

The ADVIA Centaur Gentamicin assay has an analytical sensitivity of 0.17 µg/mL (0.36 µmol/L).

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

**Analytical Measuring Range (AMR)**

The analytical measuring range of the ADVIA Centaur Gentamicin assay is 0.2 to 12.0 mg/L.

**Reporting Results**

**Reportable Range**

The reportable range of the ADVIA Centaur Gentamicin assay is 0.2 mg/L to 24 mg/L. Results >24 are reported out as >24 mg/L.

**Dilutions**

- **NOTE:** samples with Gentamicin levels greater than 12 mg/L are programmed to rerun on a 1:2 instrument performed autodilution.
- 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 7 is loaded on the Centaur.
  For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.
- Ensure that results are mathematically corrected for dilution. **If a dilution factor is entered when scheduling the test, the system automatically calculates the result.**
Reference Interval

- Peak: 5 to 8 mg/L
- Trough for standard dosing: less than 2 mg/L
- Trough for once daily high dosing: less than 1 mg/L

Critical Values

Not applicable

Reporting Protocol for Critical Values

Not applicable

Units for Reporting Results

The system reports gentamicin results in µg/mL (mass units) or µmol/L (SI units). The conversion formula is 1 mg/L = 2.09 µmol/L. Units are user-defined in the system software.

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

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>Demonstrate ≤ 5% change in results up to . . .</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemolyzed</td>
<td>250 mg/dL of hemoglobin</td>
</tr>
<tr>
<td>lipemic</td>
<td>1000 mg/dL of triglycerides</td>
</tr>
<tr>
<td>icteric</td>
<td>10 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 Gentamicin ReadyPack
- ADVIA Centaur Probe Wash 2 ReadyPack
- ADVIA Centaur Gentamicin Calibrator
- ADVIA Centaur Multi-Diluent 7
- 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 Gentamicin product insert, Revision A.
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