The Emit® 2000 Gentamicin Plus Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme enzymes only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

Reagents contain the following substances:
- Mouse monoclonal antibodies reactive to gentamicin (8.5 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), gentamicin labeled with glucose-6-phosphate dehydrogenase (0.46 U/mL), 0.1% sodium azide, Tris buffer, preservatives, and stabilizers.

Precautions
- For in vitro diagnostic use.
- Contains nonsterile mouse monoclonal antibodies.
- Assay components contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
- Do not use the kit after the expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents
The Emit® 2000 Gentamicin Plus Assay reagents are provided ready to use; no preparation is necessary.

Storage of Assay Components
- Improper storage of reagents can affect assay performance.
- When not in use, store reagents upright at 2–8°C and with screw caps tightly closed.
- Unopened reagents are stable until the expiration date printed on the label if stored upright at 2–8°C.
- Do not freeze reagents or expose them to temperatures above 32°C.

### 3 METHODOLOGY

#### 3.1 INTENDED USE

The Emit® 2000 Gentamicin Plus Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of gentamicin in human serum or plasma. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

#### 3.2 SUMMARY

Monitoring gentamicin concentrations in serum, along with careful clinical assessment, is the most effective means of ensuring adequate therapy for the following reasons:
- Gentamicin concentration in serum correlates better with antibacterial activity than does dosage.
- A standard dose of gentamicin does not always yield a predictable serum level because the drug's concentration also depends on the patient's volume of distribution and on drug elimination. These factors are influenced by the mode of administration, the volume of extracellular fluid, renal function, and physiological changes during therapy.
- Gentamicin has a narrow range of safe and effective serum concentrations.
- Exposure to high concentrations for a prolonged period may cause renal impairment or ototoxicity.
- Patients with impaired renal function should be monitored closely while on gentamicin therapy because nephrotoxicity caused by gentamicin may be difficult to distinguish from the symptoms of underlying renal disease. In addition, patients with compromised renal function eliminate gentamicin more slowly than patients with normal renal function.
- Methods historically used to monitor serum gentamicin concentrations are immunoassays, microbiological assays, and chromatographic assays.

#### 3.3 REAGENTS

- Mouse monoclonal antibodies reactive to gentamicin (8.5 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), gentamicin labeled with glucose-6-phosphate dehydrogenase (0.46 U/mL), 0.1% sodium azide, Tris buffer, preservatives, and stabilizers.

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- Do not freeze reagents or expose them to temperatures above 32°C.

### 5 SPECIMEN COLLECTION AND PREPARATION

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, citrate, oxalate, and EDTA have been tested and may be used with this assay. Some sample dilution may occur when samples are collected in tubes containing citrate anticoagulant. The amount of dilution and the possible need to correct for it should be considered when interpreting assay results for these samples.
- Sample volume is instrument-dependent. Refer to the appropriate Application Sheet for specific volumes.
- Store the serum or plasma refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C. Samples can be stored refrigerated at 2–8°C for up to six weeks or stored frozen for up to six months.
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.
- High concentrations of β-lactam antibiotics (penicillins and cephalosporins) inactivate gentamicin in vivo and in vitro. Analyze specimens containing a β-lactam antibiotic in addition to gentamicin immediately upon receipt, or store them frozen to prevent in vitro inactivation and low quantitation.
- Collect a trough sample just before the next scheduled dose. When adjusting dosage, measure peak and trough levels during the same dosing interval.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.
6 PROCEDURE

Materials Provided
Emit® 2000 Gentamicin Plus Assay
Reagent 1
Reagent 2

Materials Required But Not Provided
Emit® 2000 Gentamicin Plus Calibrators
Multi-level commercial controls

Calibration:
Recalibrate whenever a new lot of reagents is used or as indicated by control results (See Quality Control, below). If a new set of reagents with the same lot number is used, validate the system by assaying controls.

Quality Control
• Temporary Control Limits
  When establishing control limits for the first time, run 3 calibration curves and assay 3 replicates each of multi-level (2 or more) controls to determine a mean control concentration for each control level. Determine temporary control limits for each control level (± 20% of each mean control concentration). Use the temporary limits for at least 30 days—a minimum of 20 determinations for each level must be completed before establishing permanent control limits.

• Permanent Control Limits
  After 30 days (and a minimum of 20 determinations), recalculate the mean control concentrations, including all data that are within 3 standard deviations. Calculate the standard deviation for each control level and multiply it by 2.25. Permanent control ranges should be ± 2.25 SD of the mean or a 12% of the mean, whichever is greater. Establish new permanent control limits whenever a new lot of controls or reagents is used.

  • Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.

Diluting High Concentration Samples
To estimate gentamicin concentrations above the assay range, patient samples containing more than 10 µg/mL (22 µmol/L) gentamicin may be diluted with one or two parts distilled or deionized water or Emit® 2000 Gentamicin Plus Calibrator 0. Ensure that the sample is transferred using PLASTIC pipettes and containers only. After diluting the sample, repeat the entire assay sequence and multiply the results by the dilution factor. Some analyzers dilute and retest high concentration samples automatically. See the User’s Guide or appropriate Application Sheet for instructions.

Evaluation and Interpretation of Results
• This assay uses Math Model No. 1.
  • Results are automatically calculated by the analyzers; no additional manipulation of data is required.
  • The factors that can influence the relationship between gentamicin serum or plasma concentrations and clinical response include the type and severity of infection, the susceptibility of the infecting organism to gentamicin, renal function, general state of health, and the use of other drugs.
  • The concentration of gentamicin in serum or plasma depends on the time of the last drug dose; mode of administration; concomitant drug therapy; sample condition; time of sample collection; and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.1,2

7 LIMITATIONS OF THE PROCEDURE
• Carryover of the gentamicin preservative in some diagnostic reagents may affect the results of the Emit® 2000 Gentamicin Plus Assay. Complete details on how to eliminate any potential carryover are available from Siemens Healthcare Diagnostics.
• Samples containing gentamicin in combination with netilmicin or sisomicin cannot be reliably quantitated by this assay. (See section 9, Specific Performance Characteristics).
• High concentrations of β-lactam antibiotics (penicillins or cephalosporins) inactivate gentamicin in vivo and in vitro.

8 EXPECTED VALUES

The Emit® 2000 Gentamicin Plus Assay accurately quantitates gentamicin concentrations in human serum or plasma containing 0.25–10 µg/mL (0.5–22 µmol/L) gentamicin.

Although optimum concentrations vary according to the indication, peak gentamicin serum concentrations of 4.0–8.0 µg/mL (8.6–17 µmol/L) or 6.0–10 µg/mL (13–22 µmol/L) have been reported to effectively control serious infection by organisms susceptible to gentamicin.2,3 Reports show that trough gentamicin concentrations of 0.5–1.5 µg/mL (1.1–3.2 µmol/L) or 1.0–2.0 µg/mL (2.2–4.3 µmol/L) usually ensure that the concentration is above the minimum inhibitory concentrations of most gentamicin-sensitive pathogens and that the drug elimination is adequate.2,3 Further, trough concentrations above 2.0 µg/mL (4.5 µmol/L) and peak concentrations above 10 or 12 µg/mL (22 or 26 µmol/L) are often associated with renal impairment and ototoxicity.2,3

Note: To convert from µg/mL to µmol/L gentamicin, multiply by 2.16.

For effective treatment, some patients may require serum levels outside these ranges. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms. (See Section 6, Procedure, Evaluation and Interpretation of Results).

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 Gentamicin Assay studies performed on the AU4000®/AU600® Clinical Chemistry System. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Endogenous Substances
No clinically significant interference has been found in samples to which 800 mg/dL hemoglobin, 750 mg/dL triglycerides, or 30 mg/dL bilirubin were added to simulate hemolytic, lipemic, or icteric samples.

Precision
Within-run precision was determined by assaying 20 replicates of each level of a tri-level control. Table 1 summarizes the data.

Table 1 — Within-run Precision Summary
<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (µg/mL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.29</td>
<td>3.70</td>
</tr>
<tr>
<td>2</td>
<td>6.59</td>
<td>1.70</td>
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</tbody>
</table>

Total precision was calculated according to NCCLS guideline EP5-T2 using data collected from controls run in duplicate twice daily over twenty (20) days. Table 2 summarizes the data.

Table 2 — Total Precision Summary
<table>
<thead>
<tr>
<th>Level</th>
<th>Mean (µg/mL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.40</td>
<td>6.30</td>
</tr>
<tr>
<td>2</td>
<td>7.07</td>
<td>6.30</td>
</tr>
</tbody>
</table>

Comparative Analysis
In this study, patient samples were tested on the SYVA®-30R Biochemical System and on the AU600 Clinical Chemistry System. Table 3 summarizes the results.

Table 3 — Comparative Analysis Summary
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.938</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.381</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SYVA®-30R</td>
<td>4.132</td>
</tr>
<tr>
<td></td>
<td>AU600</td>
<td>4.258</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
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<td></td>
</tr>
<tr>
<td>Number</td>
<td>60</td>
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</tr>
</tbody>
</table>

Specificity
The Emit® 2000 Gentamicin Plus Assay measures the total (protein-bound plus unbound) gentamicin concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested.

Netilmicin and sisomicin—aminoglycosides structurally related to gentamicin—cross-react significantly with this assay; however, the assay has not been optimized to quantitate these aminoglycosides. Aminoglycosides are not generally co-administered in clinical practice, but more than one aminoglycoside may be present when switching from one treatment to another. Samples that contain gentamicin in combination with netilmicin or sisomicin cannot be reliably quantitated by this assay.
The compounds listed in Table 4 do not interfere with the Emit® 2000 Gentamicin Plus Assay when tested in the presence of 4.0 µg/mL gentamicin. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 4 — Compounds That Do Not Interfere

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>500</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>500</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>500</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>500</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>500</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>500</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>500*</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>600</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>100</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>50</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>25</td>
</tr>
</tbody>
</table>

*Approximately equivalent to 833 units/mL penicillin G.

Sensitivity

The sensitivity level of the Emit® 2000 Gentamicin Plus Assay is 0.25 µg/mL. This level represents the lowest measurable concentration of gentamicin that can be distinguished from 0 µg/mL with a confidence level of 95%.

Calibration Stability

Studies have shown calibration stability of at least 14 days. Calibration stability may vary from laboratory to laboratory depending on the following: handling of reagents, maintenance of instrument, adherence to operating procedures, establishment of control limits, and verification of calibration.

10 REFERENCES


For technical assistance:

Beckman Coulter customers contact their technical assistance center.
1-800-223-0130

Siemens Healthcare Diagnostics customers contact their technical assistance center.
1-800-227-8994 in the USA
1-800-264-0083 in Canada

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