Tobramycin Assay

**3 METHODOLOGY**

The Emit® 2000 Tobramycin Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of tobramycin in human serum or plasma. This assay is based on competition for antibody binding sites between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH). 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 G6PDH does not interfere because the coenzyme functions only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

**4 REAGENTS**

Reagents contain the following substances:

- Tobramycin labeled with bacterial G6PDH (0.35 U/mL), sheep antibodies reactive to tobramycin (15.5 µg/mL),* glucose-6-phosphate (56 mM), nicotinamide adenine dinucleotide (36 mM), Tris buffer, bovine serum albumin, preservatives, and stabilizers.

*The antibody titer and enzyme conjugate activity may vary from lot to lot.

For in vitro diagnostic use.

Precautions

- Reagent 1 contains nonsterile mouse monoclonal antibodies. Reagent 2 contains nonsterile sheep antibodies. Reagents and calibrators contain nonsterile bovine serum albumin.
- Reagents 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 the drain with a large volume of water to prevent azide buildup.
- Reagents and calibrators contain materials that may cause sensitivity on contact with skin.
- Do not use kit after the expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents

The Emit® 2000 Tobramycin 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 at 2–8°C (36–46°F), upright, and with the 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.

**5 SPECIMEN COLLECTION AND PREPARATION**

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants EDTA, heparin, citrate, and oxalate/fluoride 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 volume requirements.
- 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.1 2
- Concentrations of β-lactam antibiotics (penicillins and cephalosporins) at therapeutic levels may inactivate tobramycin in vivo and in vitro.2 3 Analyze tobramycin specimens containing β-lactam antibiotics immediately upon receipt, store them frozen to prevent in vitro inactivation and low quantitation, or treat them with β-lactamase.1 2
- For patients on a regimen of divided daily tobramycin doses, collect a peak sample 30–60 minutes after intravenous infusion or 60–90 minutes after intramuscular injection and collect a trough sample just before the next scheduled dose.1 3 When adjusting dosage, measure peak and trough levels during the same dosing interval.
- For patients on a single daily dosage regimen, either collect a trough sample 3–5 hours before the next scheduled dose or, if using a published nomogram to determine the proper dosing interval, collect a sample 7–14 hours after dosing.4
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.7 8
- Blood specimens should be separated and tested immediately after collection, or the separated specimens should be frozen. At the time of testing, thaw the frozen specimens and test them immediately.
6 PROCEDURE

Materials Provided
Emit® 2000 Tobramycin Assay
Reagent 1
Reagent 2

Materials Required But Not Provided
Emit® 2000 Tobramycin Calibrators
Multilevel 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 assaysing 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 ± 12% of the mean, whichever is greater.

Establish new permanent control limits whenever a new lot of controls or reagents is used.

Daily Quality Control. Assay at least 1 control every 8 hours, alternating the control levels tested. Ensure that a minimum of 2 controls is assayed in every 24-hour period. If controls are within their control limits, calibration is verified. If any control is not within its control limits, rerun that control. If the result is then within the control limits, calibration is verified. If the control is not within the control limits after repeat testing, recalibrate according to the instructions in the Calibration section and verify calibration using 2 or more controls.

If, after recalibration, any control is not within its control limits, check the handling of the control, calibrator, and reagent, and then retest. If a control is still not within its control limits, call the Technical Assistance Center in the USA or your local Siemens Healthcare Diagnostics representative.

Each laboratory must establish and follow its own quality control procedures. At a minimum, perform the quality control procedures recommended by Siemens as described above. Ensure that the quality control results meet the acceptance criteria before reporting patient results.

Diluting High Concentration Samples

To estimate tobramycin concentrations above the assay range, patient samples containing more than 10 µg/mL (21 µmol/L) tobramycin may be diluted with one or two parts of distilled or deionized water or Emit® 2000 Tobramycin Calibrator 0. Ensure that the sample is transferred using only PLASTIC pipettes and containers. 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 analyzer User’s Guide or appropriate Application Sheet for instructions.

Evaluation and Interpretation of Results

• The assay uses Math Model No. 1
• Results are automatically calculated; no additional manipulation of data is required.
• The factors that can influence the relationship between tobramycin serum or plasma concentrations and clinical response include the type and severity of infection, the susceptibility of the infecting organism to tobramycin, renal function, general state of health, and use of other drugs. 1,2,4
• The concentration of tobramycin 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. 1,2,4

7 LIMITATIONS OF THE PROCEDURE

• When diluting patient samples containing high tobramycin concentrations, the following factors can affect the accuracy of the result: diluting with the correct fluid (Emit® 2000 Tobramycin Calibrator 0 or distilled or deionized water), failure to use plastic pipettes and containers, and the accuracy of the dilution.
• Patient samples containing amikacin or kanamycin cannot be reliably quantitated by this assay (see Section 9, Specific Performance Characteristics).
• Concentrations of β-lactam antibiotics (penicillins and cephalosporins) at therapeutic levels may inactivate tobramycin in vivo and in vitro (see Section 5, Specimen Collection and Preparation).
• The cross-reactivity of tobramycin (4 µg/mL) with gentamicin (25 µg/mL and 100 µg/mL) was 108% and 118%, respectively. The levels of gentamicin tested were approximately two to eight times the upper limit of the toxic range for this drug.

8 EXPECTED VALUES

The Emit® 2000 Tobramycin Assay accurately quantitates tobramycin concentrations in human serum or plasma containing 0.6–10 µg/mL (1.3–21 µmol/L) tobramycin.

Although optimum concentrations vary according to the indication, peak tobramycin serum concentrations of 4–8 µg/mL (8.6–17 µmol/L) have been reported to effectively control serious infection by organisms susceptible to tobramycin in patients on divided daily dosages. 1 Peak tobramycin serum concentrations of 5–10 µg/mL (11–21 µmol/L) have been reported to effectively control life-threatening infection by organisms susceptible to tobramycin. 1 Reports show that in patients on divided daily dosage regimens, trough tobramycin concentrations of 1–2 µg/mL (2.1–4.3 µmol/L) usually ensure that the concentration is above the minimum inhibitory concentrations of most tobramycin-sensitive pathogens and that the drug elimination is adequate. 4

Prolonged trough concentrations above 2 µg/mL (4.3 µmol/L) are often associated with renal impairment and ototoxicity. 1,3 Prolonged peak concentrations above 12 µg/mL (26 µmol/L) are often associated with ototoxicity when patients are on divided daily dosing regimens. 1

When patients are on a single daily dosage regimen, reports show that trough concentrations less than 1 µg/mL (2.1 µmol/L) allow adequate clearance of tobramycin before the next dose. 6 Results from samples collected 7–14 hours after dosing can be plotted on a published nomogram to determine the proper dosing interval. 6

Note: To convert from µg/mL to µmol/L tobramycin, multiply by 2.14.

For effective treatment, some patients may require serum or plasma levels outside these ranges. Therefore, the expected ranges are provided only as guidelines, and individual patient results should be interpreted in light of other clinical signs and symptoms.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 Tobramycin Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Refer to the Application Sheet 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 significant interference has been found in samples to which 800 mg/L hemoglobin, 30 mg/dL free bilirubin, or 750 mg/dL triglycerides were added to simulate hemolytic, icteric, or lipemic samples.

Precision

Precision was determined by assaying two replicates each of in-house tri-level controls on 20 days with 2 runs per day. Precision was calculated according to National Committee for Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Table 1 summarizes the results.

Table 1 — Precision

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean (µg/mL)</th>
<th>Standard Deviation (µg/mL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-Run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>0.06</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>0.12</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>0.18</td>
<td>2.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>0.10</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>0.15</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>0.22</td>
<td>3.3</td>
</tr>
</tbody>
</table>

In a separate study, within-run precision at 0.6 µg/mL was determined from the testing data used to determine assay sensitivity. One 0.6 µg/mL tobramycin-spiked serum sample was prepared and run 20 times. Table 2 summarizes the results.

Table 2 — Within-Run Precision at 0.6 µg/mL

<table>
<thead>
<tr>
<th>Mean (µg/mL)</th>
<th>Standard Deviation (µg/mL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.09</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Comparative Analysis

In this study, samples from patients with tobramycin concentrations ranging from 0.47 to 8.75 µg/mL were analyzed on the SYVA®-30R Biochemical System and the AU600 Clinical Chemistry System, and the results were compared. Table 3 summarizes the results.

Table 3 — Comparative Analysis Results

| Slope       | 1.039                      |
| Intercep (µg/mL) | -0.139             |
| Mean (µg/mL) | SYVA®-30R: 2.85            |
|             | AU600: 2.82               |
| Correlation Coefficient | 0.996                 |
| Number of Samples | 47                        |
Specificity
The Emit® 2000 Tobramycin Assay measures the total (protein-bound plus unbound) tobramycin concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested. Amikacin cross-reacts with this assay. Kanamycin cross-reacts significantly; however, the assay has not been optimized to quantitate this aminoglycoside. Aminoglycosides are not generally coadministered in clinical practice, although more than one aminoglycoside may be present when switching from treatment with one to another. Samples that contain tobramycin in combination with either amikacin or kanamycin cannot be reliably quantitated by this assay.

The compounds listed in Table 4 do not interfere with the Emit® 2000 Tobramycin Assay when tested in the presence of 4 µg/mL tobramycin. Levels tested were at or above maximum pharmacological concentrations.

Table 4 — Compounds That Do Not Significantly Interfere

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbenicillin*</td>
<td>1000</td>
</tr>
<tr>
<td>Cephalothin*</td>
<td>1000</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1000</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1000</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1000</td>
</tr>
<tr>
<td>Neomycin</td>
<td>100</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>100</td>
</tr>
<tr>
<td>Penicillin G*</td>
<td>1000¹</td>
</tr>
<tr>
<td>Sisomicin</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>100</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>60</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1000</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>25</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>200</td>
</tr>
</tbody>
</table>

*See Section 5, Specimen Collection and Preparation, for information about β-lactam antibiotics.

1 Approximate equivalent to 1666 units/mL penicillin G.

Sensitivity
The sensitivity level of the Emit® 2000 Tobramycin Assay is 0.45 µg/mL. This level represents the lowest concentration of tobramycin 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 handling of reagents, maintenance of instruments, adherence to operating procedures, establishment of control limits, and verification of calibration.

10 REFERENCES