Emit® 2000 Vancomycin Assay

June 2011

1 INTENDED USE

The Emit® 2000 Vancomycin Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of vancomycin in human serum or plasma. Measurements obtained by this assay are used in the diagnosis and treatment of vancomycin overdose and in monitoring the level of vancomycin to ensure appropriate therapy.

2 SUMMARY AND EXPLANATION OF THE TEST

Monitoring vancomycin concentrations in serum, along with careful clinical assessment, is the most effective means of ensuring adequate therapy for several reasons:

- Individual patients exhibit a high degree of variability in response to a given dose of vancomycin in terms of the volume of distribution and the rate of drug clearance from plasma.
- The risk of ototoxicity and nephrotoxicity from vancomycin is increased in patients with impaired renal function and in patients receiving concurrent aminoglycoside therapy.
- Patients with impaired renal or hepatic function, dialysis patients, morbidly obese patients, patients receiving concurrent aminoglycoside therapy, and pediatric or elderly patients should be monitored closely while on vancomycin therapy.

Methods historically used to monitor serum vancomycin concentrations are microbiological assays, immunoassays, and chromatographic assays.

3 PRINCIPLE

The Emit® 2000 Vancomycin Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of vancomycin in human serum or plasma. Serum or plasma is mixed with Reagent 1, which contains vancomycin labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Subsequently, Reagent 2, which contains antibodies to vancomycin and the coenzyme nicotinamide adenine dinucleotide (NAD), is added. Vancomycin in the sample and vancomycin-labeled G6PDH compete for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the vancomycin concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized 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 (Luesnocostoc mesenteroides) enzyme employed in the assay.

4 REAGENTS

Reagents contain the following substances:

- **Enzyme Reagent 1**: Vancomycin labeled with bacterial G6PDH (0.21 U/mL),* HEPES buffer, bovine serum albumin, preservatives, and stabilizers

- **Antibody/Substrate Reagent 2**: Mouse monoclonal antibodies to vancomycin (27 µg/mL),* bovine serum albumin, G6P (44 mM), NAD (36 mM), preservatives, and stabilizers

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

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

5 SPECIMEN COLLECTION AND PREPARATION

Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants EDTA, sodium heparin, lithium 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.

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.

- To obtain a vancomycin concentration that best represents the peak tissue level, draw the sample 0.5–2 hours after an infusion.
- To avoid in vitro degradation, store serum or plasma frozen at -20°C or at -70°C if not analyzed immediately.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious. It is recommended that human specimens be handled in accordance with government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known vancomycin concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

6 PROCEDURE

Materials Provided

- Emit® 2000 Vancomycin Assay
- Reagent 1
- Reagent 2

Materials Required But Not Provided

- 4W109UL  Emit® 2000 Vancomycin Calibrators 0*, 5, 10, 20, 30, 50 µg/mL

*Additional negative calibrator is provided.

Quality Control

- Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.
- Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known vancomycin concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
- Refer to the instrument operator’s manual for further instructions.

If a new set of reagents with the same lot number is used, validate the system by assaying controls. Recalibrate whenever a new lot of reagents is used or as indicated by control results using Emit® 2000 Vancomycin Calibrators (0–50 µg/mL). Refer to the instrument operator’s manual for on-instrument stability information.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

Precautions:

Reagents contain materials that may cause sensitivity on contact with skin. Wear suitable protective clothing and gloves.

Preparation of Reagents:

The Emit® 2000 Vancomycin Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent vials when not in use.

Storage of assay components:

- When not in use, store reagents at 2–8°C (36–46°F), upright, and with the screw caps tightly closed. When stored as directed, reagents are stable until the expiration date printed on the label. Refer to the analyzer-specific application sheets for on-instrument stability information.
- Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). Improper storage of reagents can affect assay performance.

For in vitro diagnostic use.

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**Table: Reagent Quantities**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Product Description</th>
<th>Quantity/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSR4W229</td>
<td>Emit® 2000 Vancomycin Assay</td>
<td></td>
</tr>
<tr>
<td>OSR4W518 R1</td>
<td>(Enzyme Reagent 1)</td>
<td>32 mL</td>
</tr>
<tr>
<td>OSR4W548 R2</td>
<td>(Antibody/Substrate Reagent 2)</td>
<td>16 mL</td>
</tr>
</tbody>
</table>
Diluting High-Concentration Samples
To estimate vancomycin concentrations above the assay range, patient samples containing more than 50 µg/mL (34 µmol/L) vancomycin may be diluted with one or two parts of distilled or deionized water or Emit® 2000 Vancomycin Calibrator 0. After diluting the sample, test and multiply by the results by the dilution factor.

7 RESULTS
Results are calculated automatically by the analyzers. No additional manipulation of data is required unless samples have been manually diluted.

This assay uses Math Model No.1. Consult the appropriate instrument operating manual and analyzer-specific application sheet for complete instructions.

The factors that can influence the relationship between vancomycin serum or plasma concentrations and clinical response include the type and severity of infection, the susceptibility of the infecting organism to vancomycin, renal function, general state of health, and use of other drugs.1

The concentration of vancomycin 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

For purposes of diagnosis and treatment, results of this test should always be interpreted in conjunction with the patient’s medical history, clinical presentation and other findings.

8 LIMITATIONS OF THE PROCEDURE
When diluting patient samples containing high vancomycin concentrations, the following factors can affect the accuracy of the result: diluting with the correct fluid (Emit® 2000 Vancomycin Calibrator 0 or distilled or deionized water) and the accuracy of the dilution.

9 EXPECTED VALUES
The Emit® 2000 Vancomycin Assay accurately quantitates vancomycin concentrations in human serum or plasma containing 2.0–50 µg/mL (1.3–34 µmol/L) vancomycin.

Reported peak therapeutic ranges for vancomycin vary considerably. Both the dosage regimen and the timing of sample collection may affect the peak therapeutic range. For example, after completion of a 60-minute infusion of vancomycin in adults, samples drawn at 2 hours had concentrations of 18–28 µg/mL (12–17 µmol/L),1 samples drawn at 1 hour had concentrations of 26–40 µg/mL (18–27 µmol/L),1 and samples drawn at 30 minutes had concentrations of 30–40 µg/mL (20–27 µmol/L).1

Trough vancomycin serum concentrations of 5.0–10 µg/mL (3.4–6.7 µmol/L) usually ensure that the concentration is above the minimum inhibitory concentrations of most vancomycin-sensitive pathogens and that the drug elimination is adequate.1

For patients on concomitant vancomycin and aminoglycoside treatment, peak vancomycin concentrations exceeding 30 µg/mL (20 µmol/L) and trough concentrations above 10 µg/mL (6.7 µmol/L) are associated with nephrotoxicity.1 Serum concentrations above 80 µg/mL (54 µmol/L) are associated with ototoxicity.1

Note: To convert from µg/mL to µmol/L vancomycin, multiply by 0.67.

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.

10 PERFORMANCE
The data appearing in this section were collected on the AU400®/AU600® Clinical Chemistry System using the Emit® 2000 Vancomycin Assay. Results are current at the date of publication; however, results may vary due to analyzer-to-analyzer differences. Beckman Coulter customers and Siemens customers contact their respective technical assistance centers for additional information. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Method Comparison
Samples from patients were analyzed by the Emit® 2000 Vancomycin Assay on the SYVA®-30R and AU400 Clinical Chemistry System. A summary of the results is as follows:

Table 1 — Method Comparison
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (µg/mL)</td>
<td>7.3</td>
<td>18.7</td>
<td>30.2</td>
<td>39.3</td>
</tr>
<tr>
<td>SD</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>CV%</td>
<td>2.8%</td>
<td>1.6%</td>
<td>1.3%</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

Analytical Sensitivity
The sensitivity level of the Emit® 2000 Vancomycin Assay on the AU400 is 2.0 µg/mL. This level represents the lowest concentration of vancomycin that can be distinguished from 0 µg/mL with a confidence level of 95%.
Endogenous Substances

No interference has been found in samples to which 20 µg/mL vancomycin and either 400 mg/dL (4 g/L) hemoglobin or 30 mg/dL (0.3 g/L) free bilirubin were added to simulate hemolytic or icteric samples. Grossly hemolyzed samples should be recollected.

No interference has been found in lipemic patient samples containing 750 mg/dL (7.5 g/L) triglyceride to which 20 µg/mL vancomycin was added.

Calibration Stability

Studies have shown calibration stability of at least 14 days. The quality control limits used in these studies were established by following the instructions in Section 6, Procedure, Quality Control. 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.

11 BIBLIOGRAPHY


Symbols Key

- Do not reuse
- Use By
- Batch Code
- Catalogue Number
- Caution, consult accompanying documents
- Manufacturer
- Authorized Representative in the European Community
- Contains sufficient for <n> tests
- In Vitro Diagnostic Medical Device
- Temperature Limitation
- Consult Instructions for Use
- Non-sterile
- CE Mark
- Contents
- Reconstitution Volume
- Level

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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