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Emit® 2000 Vancomycin Assay

See shaded sections:
Updated information from 2018-03 version.

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Vancomycin Assay

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.[1]
- The risk of ototoxicity and nephrotoxicity from vancomycin is increased in patients with impaired renal function and in patients receiving concurrent aminoglycoside therapy.[1]
- 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.[1]

Methods historically used to monitor serum vancomycin concentrations are microbiological assays, immunoassays, and chromatographic assays.[1]

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.[2] 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 *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

REF	Product Description	Quantity/Volume
4W019UL	**Emit® 2000 Vancomycin Assay**	
	Enzyme Reagent 1 Vancomycin labeled with bacterial G6PDH (0.21 U/mL),* HEPES buffer, bovine serum albumin, preservatives, and stabilizers	28 mL
	Antibody/Substrate Reagent 2 Mouse monoclonal antibodies to vancomycin (27 µg/mL),* bovine serum albumin, G6P (44 mM), NAD (36 mM), preservatives, and stabilizers	14 mL

**** 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.

Risk and Safety

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

For *in vitro* diagnostic use.

Precautions

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

Preparation and Storage of Assay Components

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.

Note: Caps must always be replaced on the original containers.

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.**

5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants EDTA, sodium 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 analyzer-specific application sheet.
- 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]
- To obtain a vancomycin concentration that best represents the peak tissue level, draw the sample 0.5–2 hours after an infusion.[3]
- To avoid *in vitro* degradation, store serum or plasma frozen at -20°C[4] 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 the OSHA Standard on Bloodborne Pathogens or other appropriate local practices.[5,6]

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.***

Instruments

Siemens Healthcare Diagnostics provides instructions for using this assay on a number of chemistry analyzers.

Contact the Technical Assistance Center in the USA or your local Siemens representative for analyzer-specific application sheets.

Analyzers must be capable of maintaining a constant reaction temperature, pipetting specimens/reagents and measuring enzyme rates precisely, timing the reaction accurately, and mixing reagents thoroughly.

Calibration

Recalibrate whenever a new lot of reagents is used or as indicated by control results (see Quality Control, below), using Emit® 2000 Vancomycin Calibrators (0–50 µg/mL). 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.

Quality Control

1. 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.
2. 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.
3. Refer to the instrument operator's manual for appropriate instrument checks.

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 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

- 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.
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.
- Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.

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.[1,3,7,8] For example, after completion of a 60-minute infusion of vancomycin in adults, samples drawn at 2 hours had concentrations of 18–26 µg/mL (12–17 µmol/L),[3] samples drawn at 1 hour had concentrations of 26.5–40 µg/mL (18–27 µmol/L),[8] and samples drawn at 30 minutes had concentrations of 30–40 µg/mL (20–27 µmol/L).[1,3]

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.[1,7]

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 Specific Performance Characteristics

The data appearing in this section were collected on the SYVA®-30R Biochemical System.

The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents. Performance may vary depending on the instrumentation used.

Specificity

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

The compounds listed in Table 1 do not interfere with the Emit® 2000 Vancomycin Assay when tested in the presence of 20 µg/mL vancomycin. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 1 — Compounds That Do Not Interfere

Compound	Concentration Tested (µg/mL)
Acyclovir	25
Amikacin	100
Amphotericin B	20
Aztreonam	200
Caffeine	2
CDP-1	20
Cefazoline	500
Cefotaxine	1000
Chloramphenicol	100
Ciprofloxicin	10
Cisplatin	25
Clindamycin	10
Cyclosporine	50
Digoxin	0.006
Epinephrine	1
Erythromycin	5
Ethacrynic acid	50
Flucytosine	100
Furosemide	100
Fusidic Acid	500
Gentamicin	100
Imipenem	70
Methicillin	500
Metronidazole	50
Netilmicin	100
Nitroprusside	60
Penicillin G*	10
Pentamidine	0.7
Phenobarbital	40
Rifampin	500
Salicylate	60
Sulphamethoxazole	600
Theophylline	20
Trimethoprim	25
Tobramycin	100

Approximately equivalent to 16.7 units/mL penicillin G.

For additional information, contact your Siemens representative.

Sensitivity

The sensitivity level of the Emit® 2000 Vancomycin Assay is 2.0 µg/mL vancomycin. 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 800 mg/dL (8 g/L) hemoglobin or 30 mg/dL (0.3 g/L) free bilirubin were added to simulate hemolytic or icteric samples.

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.

Precision

Precision values were obtained using the SYVA®-30R Biochemical System. Precision was determined by measuring two replicates each of internal Siemens 3-level controls on 20 days with two runs per day. Precision was calculated according to National Committee for Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). Results of these studies are summarized in Table 2.

Table 2 — Precision

Control	Number of Replicates	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
Within-Run				
1	80	7.4	0.3	4.2
2	80	17.5	0.4	2.4
3	80	34.0	1.5	4.6
Total				
1	80	7.4	0.4	4.9
2	80	17.5	0.6	3.5
3	80	34.0	1.9	5.7

Method Comparison

Patient samples with vancomycin concentrations ranging from 5 µg/mL to 50 µg/mL were analyzed by the Emit® 2000 Vancomycin Assay and by the Emit® Vancomycin Assay on the SYVA®-30R Biochemical System, and the results were compared. Data are summarized in Table 3.

Table 3 — Method Comparison

	Emit® 2000 vs Emit®
Slope	0.97
Intercept (µg/mL)	-0.24
Correlation Coefficient	0.97
Number of Samples	100

11 Bibliography

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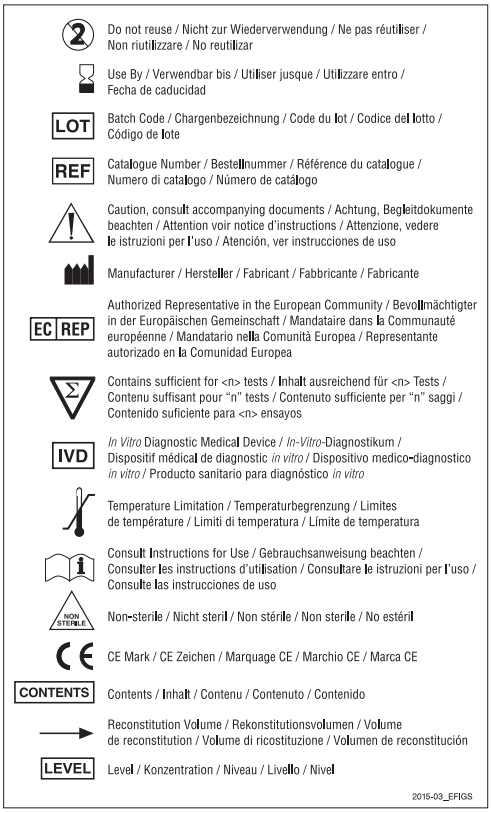
12 Symbols Key



For technical assistance, call Siemens Healthcare Diagnostics:
1-800-227-8994 in the USA
1-800-264-0083 in Canada
Outside the USA and Canada, call your local Siemens representative.

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