

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20737941 322	Vancomycin (200 tests)	System-ID 07 3794 1 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	System-ID 07 6900 2 System-ID 07 6901 0 System-ID 07 6902 9
20720720 322	COBAS FP Sample Dilution Reagent II (1 × 200 mL)	System-ID 07 2072 0

## English

### System information

Test VANCNM, test ID 0-394

### Intended use

In vitro diagnostic test for the quantitative determination of vancomycin in serum or heparinized plasma on COBAS INTEGRA systems.

### Summary

Vancomycin is a complex glycopeptide antibiotic, which has been used to treat penicillinase-producing staphylococci.<sup>1</sup> It is the drug of choice for the treatment of methicillin and related beta lactam antibiotic resistant *Staphylococcus aureus*<sup>2,3</sup> as well as for the treatment of serious gram-positive infections where allergies to penicillin or cephalosporin play a role.<sup>4,5</sup> Vancomycin is also used in the treatment of antibiotic-induced enterocolitis associated with *Clostridium difficile* and streptococcal or enterococcal endocarditis, the latter in conjunction with an aminoglycoside, when penicillin or ampicillin is not an option.<sup>4,6</sup>

Monitoring of peak and trough serum or plasma levels is necessary due to potentially serious side effects including ototoxicity, nephrotoxicity, phlebitis, and reversible neutropenia.<sup>7</sup>

### Test principle

Fluorescence polarization

COBAS INTEGRA therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.<sup>8</sup> Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum for the purpose of therapeutic drug monitoring.

Surface active agents are used to ensure dissociation of the drug from serum proteins and to prevent nonspecific binding of the tracer.

### Reagents - working solutions

**R1** Antibody reagent  
Anti-vancomycin monoclonal antibody (mouse) in buffer, pH 6.5, with stabilizer and preservative

**SR** Tracer reagent  
Fluorescein-labeled vancomycin derivative in buffer, pH 6.5, with stabilizer and preservative

R1 is in position B and SR is in position C.

### Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

### Reagent handling

Ready for use

### Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 26 weeks

### Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable:

Unhemolyzed serum

Unhemolyzed heparinized plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Usual sampling time varies dependent upon desired measurement of peak or trough values.<sup>9</sup> Specimens should be tested within 2 hours of collection if kept capped at 15-25 °C.<sup>10</sup> If specimens must be stored for later testing, they may be kept capped at 2-8 °C for up to 48 hours or at -20 °C for 4 weeks.<sup>11</sup> Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

### Materials provided

See "Reagents – working solutions" section for reagents.

### Materials required (but not provided)

COBAS FP Sample Dilution Reagent II (SDR II), Cat. No. 20720720 322  
The SDR II is placed as special diluent in its predefined rack position and is stable for 7 days on-board COBAS INTEGRA 400 plus/800 analyzers.

### Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

### Application for serum and plasma

#### COBAS INTEGRA 400 plus test definition

Measuring mode	FP
Reaction mode	R1-SDR2/S-SR
Wavelength	excitation 485 nm
	emission 515 nm

Reading cycle blank/test	29/45
Unit	µg/mL

## Pipetting parameters

		Diluent (H <sub>2</sub> O)
R1	85 µL	10 µL
Sample	2 µL	5 µL
Special diluent (SDR II)	24 µL	
SR	14 µL	10 µL
Total volume	150 µL	

## COBAS INTEGRA 800 test definition

Measuring mode	FP
Reaction mode	R1-SDR2/S-SR
Wavelength	excitation 485 nm
	emission 515 nm
Reading cycle blank/test	40/60
Unit	µg/mL

## Pipetting parameters

		Diluent (H <sub>2</sub> O)
R1	105 µL	10 µL
Sample	2 µL	5 µL
Special diluent (SDR II)	24 µL	
SR	14 µL	10 µL
Total volume	170 µL	

## Calibration

Calibrators	Preciset TDM I
	Calibrators A-F
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 5 µg/mL (≥ 3.5 µmol/L)
Calibration interval	

COBAS INTEGRA 400 plus analyzers Each lot, every 10 weeks and as required following quality control procedures

COBAS INTEGRA 800 analyzers Each lot, every 14 weeks and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of vancomycin in normal human serum and are traceable to USP reference standards.

## Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

## Quality control

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Note

Controls should be assayed within 2 hours after placing on-board the instrument.

## Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/mL × 0.690 = µmol/L

## Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate dilution of the original sample with the Preciset TDM I Diluent (0 µg/mL). Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

## Serum/plasma

Criterion: Recovery within ± 10 % of initial value at a vancomycin concentration of 29 µg/mL (20 µmol/L).

Icterus:<sup>12</sup> No significant interference up to a bilirubin concentration of 296 µmol/L or 17.3 mg/dL.

Hemolysis:<sup>12</sup> No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:<sup>12</sup> No significant interference up to a triglycerides concentration of 1261 mg/dL.

Total protein: No significant interference up to a total protein concentration of 3-12 g/dL.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

## Limits and ranges

### Measuring range

COBAS INTEGRA 400 plus analyzers:  
0.74-80 µg/mL (0.51-55.2 µmol/L)

COBAS INTEGRA 800 analyzers:  
1.39-80 µg/mL (0.96-55.2 µmol/L)

### Lower limits of measurement

Lower detection limit of the test:

COBAS INTEGRA 400 plus analyzers:  
0.74 µg/mL (0.51 µmol/L)

COBAS INTEGRA 800 analyzers:  
1.39 µg/mL (0.96 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

## Expected values

Although optimum values may vary, peak serum values in the range of 25-40 µg/mL (17.3-27.6 µmol/L) and trough values in the range of

5-10 µg/mL (3.5-6.9 µmol/L) are generally accepted for therapeutic effectiveness.<sup>4</sup>

Vancomycin is excreted primarily by the kidney in its unchanged active form although evidence of a nonrenal mechanism of elimination has been demonstrated.<sup>4,7</sup> Impaired renal function can cause accumulation of the drug. Vancomycin has several adverse reactions, the most severe being ototoxicity and nephrotoxicity, although the purity of recent vancomycin preparations appears to have lessened these effects as long as serum concentrations are monitored closely.<sup>4,9,13</sup> Nephrotoxicity is more likely to occur in patients receiving vancomycin in conjunction with an aminoglycoside.<sup>9</sup>

The measurement of vancomycin concentrations in serum is essential to optimize therapy and avoid dosage related toxicity. This is especially important in patients with renal insufficiency, where individualized patient therapy is the only method to ensure optimal therapeutic serum levels without serious side effects.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Precision was determined using controls in accordance with the NCCLS EP5-T2<sup>14</sup> requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	8.68 (5.99)	0.19 (0.13)	2.2
Level 2	26.3 (18.1)	0.4 (0.28)	1.6
Level 3	54.6 (37.7)	1.7 (1.17)	3.1

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	8.68 (5.99)	0.26 (0.18)	3.0
Level 2	26.3 (18.1)	0.6 (0.41)	2.2
Level 3	54.6 (37.7)	1.8 (1.24)	3.3

### Method comparison

Vancomycin values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Vancomycin reagent (y) were compared with those determined using a commercially available FPIA method (x).

	FPIA
Number of samples	193
Range of values	min. 1.41 µg/mL max. 68.1 µg/mL
Slope	0.958
Intercept	-0.386 µg/mL
Correlation coefficient	0.995

### Analytical specificity

Crystalline degradation product, CDP-1, has been described in the literature as being produced by the structural transformation of vancomycin in solution and is reported to have no antibiotic activity.<sup>15</sup> This product may occur in patients and can accumulate with renal failure.<sup>16</sup> The COBAS FP reagents for vancomycin has no detectable cross-reactivity with CDP-1.

The following structurally related or potentially co-administered compounds were tested in normal human serum spiked with vancomycin at 32 µg/mL (22.1 µmol/L). Each substance was tested at ten times the highest concentration of its therapeutic or normal range, as per the protocol described by NCCLS.<sup>17</sup> Cross-reactivity was determined to be not

detectable (less than the sensitivity of the assay). The imprecision of the assay was taken into account when determining cross-reactivity.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

All of these compounds were determined to be not detectable

Acetaminophen	Heparin
Amikacin	Hydrochlorothiazide
Amphotericin B	Isoniazide
Ampicillin	Kanamycin
Bendroflumethiazide	Lincomycin
Caffeine	Methicillin
Carbenicillin	Methotrexate
CDP-1	Methylprednisolone
Cefamandole Nafate	Nalidixic acid
Cefazoline	Neomycin
Cephalexin	Netilmicin
Cephaloglycin	Nitrofurantoin
Cephalexidine	Oxytetracycline
Cephalosporin C	Penicillin G
Cephalothin	Penicillin V
Chloramphenicol	Polythiazide
Chlorothiazide	Prednisolone
Clindamycin	Rifampin
Erythromycin	Spectinomycin
Ethacrynic acid	Streptomycin
Ethambutol	Sulfadiazine
5-Fluorocytosine	Sulfamethoxazole
Fortimicin A	Sulfisoxazole
Fortimicin B	Tetracycline
Furosemide	Ticarcillin
Fusidic acid	Tobramycin
Gentamicin	Trimethoprim

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

### References




- McCormick MH, Stark WM, Pittinger GE, et al. In: Antibiotics Annual. New York, NY: Medical Encyclopedia 1956;606-611.
- Crossley KB, Landesman B, Zaske D. An outbreak of infections caused by strains of Staphylococcus aureus resistant to methicillin and aminoglycosides. J Infect Dis 1979;139:273-279.
- Sorrell TC, Packham S, Shanker M, et al. Vancomycin therapy for methicillin-resistant Staphylococcus aureus. Ann Intern Med 1982;97:344-350.
- Cook FV. Vancomycin revisited. Ann Intern Med 1978;88:813-818.
- Geraci JE, Hermans PE. Vancomycin. Mayo Clin Proc 1983;58:88-91.
- Geraci JE, Wilson WR. Vancomycin therapy for infective endocarditis. Rev Infect Dis 1981;3(suppl):250-258.
- Moellering RC, Krogstad DJ, Greenblatt DJ. Vancomycin therapy in patients with impaired renal function: a nomogram for dosage. Ann Intern Med 1981;94:343-346.
- Dandliker WB, Feigen GA. Quantification of the antigen-antibody reaction by the polarization of fluorescence. Biochem Biophys Res Comm 1961;5:299-304.

- 9 Rotschafer JC, Crossley K, Zaske DE, et al. Pharmacokinetics of vancomycin: observations in 28 patients and dosage recommendations. *Antimicrob Agents Chemother* 1982;22:391-394.
- 10 Hammett-Stabler CA, Johns T. Laboratory guidelines for monitoring of antimicrobial drugs. *Clin Chem* 1998;44(5):1129-1140.
- 11 Data on file at Roche Diagnostics.
- 12 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. *Clin Chem* 1986;32:470-475.
- 13 Esposito AL, Gleckman RA. Vancomycin, a second look. *JAMA* 1977;238:1756-1757.
- 14 National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.
- 15 Harris CM, Kopecka H, Harris TM. Vancomycin: structure and transformation to CDP-1. *J AM Chem Soc* 1983;105:6915-6922.
- 16 Lakashmi A, Hu M, Chan K, et al. Potential problem with fluorescence polarization immunoassay cross-reactivity to vancomycin degradation product CDP-1: its detection in sera of renally impaired patients *Ther Drug Monit* 1989;11:585-591.
- 17 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

### FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

COBAS, COBAS C, COBAS INTEGRA and PRECISET are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

© 2015, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
www.roche.com

Distribution in USA by:  
Roche Diagnostics, Indianapolis, IN  
US Customer Technical Support 1-800-428-2336

