VANC2

cobas®

Vancomycin

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04491050 190	ONLINE TDM Vancomycin 100 tests	System-ID 07 6914 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
05108420 190	ONLINE TDM Vancomycin 200 tests	System-ID 07 6914 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03375790 190	Preciset TDM I calibrators CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	Codes 691-696	
04521536 190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 310 Code 311 Code 312	

English

System information

For **cobas c** 311/501 analyzers:

VANC2: ACN 624

For **cobas c** 502 analyzers:

VANC2: ACN 8624

Intended use

In vitro test for the quantitative determination of vancomycin in serum and plasma on Roche/Hitachi **cobas c** systems.

Summary

Vancomycin is a complex glycopeptide antibiotic, which has been used to treat penicillinase-producing staphylococci.[1] It is the drug of choice for the treatment of methicillin and related beta lactam antibiotic resistant *Staphylococcus aureus*[2,3] as well as for the treatment of serious gram-positive infections where allergies to penicillin or cephalosporin play a role.[4,5] 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.[4,6]

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

Test principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of vancomycin in human serum or plasma.[8] 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 functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

Reagents - working solutions

R1 Vancomycin labeled with bacterial G6PDH in buffer

R2 Anti-vancomycin antibody (mouse monoclonal), G6P and NAD in buffer

R1 is in position A and R2 is in position C. Position B contains H_2O for technical reasons.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: For prescription use only.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one.

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

Reagent handling

Ready for use

Mix reagents by gentle inversion numerous times before placing on-board the analyzer.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	60 days

Do not freeze.

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.
Serum: Collect serum using standard sampling tubes.
Plasma: K_2- or K_3-EDTA, sodium citrate, fluoride oxalate plasma.

Stability:	2 hours capped at 15-25 °C[9]
	48 hours capped at 2-8 °C
	4 weeks capped at -20 °C

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.

Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

Usual sampling time varies dependent upon desired measurement of peak or trough values.[10]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

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Vancomycin

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 15-22		
Wavelength (sub/main)	415/340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	55 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–
Instrument factor	Set instrument factor a = 0.75 on the Calibration/Status/Instrument Factor display		

cobas c 501/502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 22-32		
Wavelength (sub/main)	415/340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	90 µL	–	
R2	55 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–
Instrument factor	Set instrument factor a = 0.75 on the Calibration/Status/Instrument Factor display		

The technical limit in the instrument settings is defined as 2.27-106.7 µg/mL (1.57-73.6 µmol/L) due to the instrument factor. See Calculation section.

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	RCM
Calibration frequency	6-point calibration • after **cobas c** pack change • every 3 days • as required following quality control procedures

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of vancomycin in normal human serum.

Quality control

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.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

ACTION REQUIRED

Instrument factor: An instrument factor, a = 0.75, is required for this vancomycin procedure. Set instrument factor on the Calibration/Status/Instrument Factor display.

NOTE: An adjustment to the analyzer's technical limit is necessary to reflect the use of an instrument factor.

Conversion factor:[11] µg/mL x 0.690 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at vancomycin levels of approximately 20 and 50 µg/mL (13.8 and 34.5 µmol/L).

Serum/Plasma

Icterus:[12] No significant interference up to an I index of 30 (approximate conjugated and unconjugated bilirubin concentration: 30 mg/dL or 513 µmol/L).

Hemolysis:[12] No significant interference up to an H index of 650 (approximate hemoglobin concentration: 650 mg/dL or 404 µmol/L).

Lipemia (Intralipid):[12] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at a vancomycin level of approximately 20 µg/mL (13.8 µmol/L).

No significant interference from triglycerides up to 500 mg/dL (5.7 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to 100 IU/mL.

Total protein: No significant interference from protein from 2-12 g/dL.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely lowered results.

Unspecific binding of heterophilic antibodies from the sample to Glucose-6-Phosphate Dehydrogenase of the reagent may lead to falsely lower test results in very rare cases (< 10^{-6}).

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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

Limits and ranges

Measuring range

1.7-80.0 µg/mL (1.2-55.2 µmol/L)

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value.

Lower limits of measurement

Lower detection limit of the test

1.7 µg/mL (1.2 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying

2 standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 21).

Expected values

Although optimum values may vary, peak serum values in the range of 25 to 40 µg/mL (17.3 to 27.6 µmol/L) and trough values in the range of 5 to 10 µg/mL (3.5 to 6.9 µmol/L) are generally accepted for therapeutic effectiveness.[4]

Vancomycin is excreted primarily by the kidney in its unchanged active form although evidence of a nonrenal mechanism of elimination has been demonstrated.[4,7] 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.[4,10,14] Nephrotoxicity is more likely to occur in patients receiving vancomycin in conjunction with an aminoglycoside.[10]

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 analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls and in a modified NCCLS EP5-T2 protocol (repeatability n = 63, intermediate precision n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum/Plasma

Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	4.7	0.1	0.1	1.8
Control 2	21.5	14.8	0.4	0.3	1.7
Control 3	40.9	28.2	1.1	0.7	2.6
HS 1	16.7	11.5	0.4	0.3	2.5
HS 2	61.0	42.1	2.3	1.6	3.7

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	6.8	4.7	0.2	0.2	3.5
Control 2	21.5	14.8	0.5	0.4	2.4
Control 3	40.9	28.2	1.3	0.9	3.1
HS 1	16.7	11.5	0.5	0.4	3.0
HS 2	61.0	42.1	2.7	1.9	4.4

Method comparison

Serum/plasma

Vancomycin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (Instrument Factor 0.75) (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi MOD P analyzer (Instrument Factor 0.79) (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi MOD P analyzer	Sample size (n) = 125
Passing/Bablok[15]	Linear regression
y = 1.00x - 0.100 µg/mL	y = 1.01x - 0.443 µg/mL
τ = 0.950	r = 0.994

The sample concentrations were between 4.10 and 78.6 µg/mL (2.83 and 54.2 µmol/L).

COBAS INTEGRA 800 analyzer	Sample size (n) = 122
Passing/Bablok[15]	Linear regression
y = 0.986x - 0.986 µg/mL	y = 0.988x - 0.926 µg/mL
τ = 0.964	r = 0.996

The sample concentrations were between 4.60 and 76.4 µg/mL (3.17 and 52.7 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

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

ND = Not Detected

Tests were performed on 16 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (Tetracycline)

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Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H_2O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline

References

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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.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	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.

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