

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
06779336190	ONLINE TDM Vancomycin Gen.3 (100 tests)	System-ID 07 7571 1 COBAS INTEGRA 400 plus
06779344190	ONLINE TDM Vancomycin Gen.3 (200 tests)	System-ID 07 7571 1 COBAS INTEGRA 400 plus

Materials required (but not provided):

03375790190	Preciset TDM I Calibrators A-F (1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8
04521536190	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

English
System information

Test VANC3, test ID 0-459

Intended use

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

Summary^{1,2,3,4}

Vancomycin is a complex glycopeptide antibiotic, which is used for the treatment of infections caused by Gram-positive organisms, primarily methicillin resistant *Staphylococcus aureus* (MRSA), coagulase-negative *Staphylococci*, *Streptococci* or *Enterococci*, particularly in patients allergic to β -lactams.

Common side effects include, amongst others, the following: (a) red man syndrome, a histamine-mediated flushing during or immediately following infusion, (b) nephrotoxicity, and (c) ototoxicity; the latter two adverse events are dose/level dependent.

In former years the monitoring of peak and trough levels has been recommended. Meanwhile the relevance of monitoring peak concentrations is questioned by some clinicians due to limited clinical data. Monitoring of trough serum or plasma levels is necessary to ascertain clinical efficacy and to limit potentially dose-dependent serious side effects, e.g. ototoxicity and nephrotoxicity. The potential for the latter two serious adverse events has established therapeutic drug monitoring (TDM) of vancomycin as the standard of care. Trough levels are typically obtained before or after the 4th dose of the drug and then monitored at least once weekly.

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Vancomycin antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions, photometrically detected by turbidity measurements is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of vancomycin in the sample. A competitive reaction takes place between the drug conjugate and vancomycin in the serum sample for binding to the vancomycin antibody on the microparticles. The resulting turbidity is indirectly proportional to the amount of drug present in the sample.

Reagents - working solutions

- R1** Vancomycin conjugate; piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer, pH 7.2; preservative; stabilizer
- SR** Anti-vancomycin antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino)propane sulfonic acid (MOPS) buffer, pH 7.2; stabilizer

 100 tests/**cobas c** pack: R1 is in position A and SR is in position C.

 200 tests/**cobas c** pack: R1 is in position B and SR is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C

 See expiration date on
cobas c pack label

On-board in use at 10-15 °C

12 weeks

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

 Plasma: K₂- or K₃-EDTA and lithium heparin plasma

Sample collection tubes containing separating gel have not been verified for use.

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.

See the limitations and interferences section for details about possible sample interferences.

Stability: 48 hours capped at 15-25 °C
 14 days capped at 2-8 °C
 12 months capped at -20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Do not induce foaming of specimens. Specimens can be repeatedly frozen and thawed up to 5 times.

Invert thawed specimens several times prior to testing.

 Usual sampling time varies depending upon whether peak or trough values should be measured.⁵
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.

Application for serum and plasma**Test definition**

Measuring mode	Absorbance
Abs. calculation mode	2 Point End
Reaction mode	R1/S-SR
Reaction direction	Increase
Wavelength A	583 nm
Calc. first/last	19/69
Unit	µg/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	100 µL	-
Sample	2 µL	-
SR	70 µL	-
Total volume	172 µL	

Calibration

Calibrators	Preciset TDM I Calibrators A-F
Calibration mode	Spline
Calibration replicate	Duplicate recommended
Calibration interval	- after 2 weeks on-board the analyzer - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against USP reference standards.⁶ The calibrators are prepared to contain known quantities of vancomycin in normal human serum.

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.

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor:⁷ µg/mL × 0.690 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at vancomycin concentrations of approximately 7.5 and 30 µg/mL (5.18 and 20.7 µmol/L).

Icterus:⁸ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:⁸ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 622 µmol/L).

Lipemia (Intralipid):⁸ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Triglycerides: No significant interference from triglycerides up to a concentration of 1000 mg/dL (11.4 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

Total protein: No significant interference from total protein up to a concentration of 2 to 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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.⁹ In very rare cases (less than 1 reported case per 1000000 tests) certain immunoglobulins can unspecifically interfere with the agglutination reaction leading to unreliable results.

Note: A test result flagged with "HIGH ACT" indicates unusual reaction kinetics. There is a high possibility that the sample contains an interfering substance which accelerates the reaction kinetics. For such samples it is not possible to report a reliable analyte concentration with this assay.

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**

4.0-80 µg/mL (2.76-55.2 µmol/L)

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

Lower limits of measurement

Limit of Blank, Limit of Detection, and Limit of Quantitation

Limit of Blank = 1.0 µg/mL (0.69 µmol/L)

Limit of Detection = 1.5 µg/mL (1.04 µmol/L)

Limit of Quantitation = 4.0 µg/mL (2.76 µmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration vancomycin samples.

Expected values

The practice of routine monitoring and adjustment of serum vancomycin concentrations has been the subject of intense debate for many years.³ Historically trough concentrations between 5 to 10 µg/mL and peak concentrations between 20 to 40 µg/mL were generally accepted for therapeutic effectiveness.^{3,4,7} The increased prevalence of resistant organisms, increasing vancomycin minimum inhibitory concentrations in target pathogens (particularly MRSA) and vancomycin failures have prompted more aggressive vancomycin dosing practices and recommendations.^{3,10} Therefore, current guidelines recommend higher trough concentrations in the range of 10-15 µg/mL for uncomplicated MRSA bacteremia and even 15-20 µg/mL in cases of sustained MRSA bacteremia or endocarditis and other severe invasive MRSA infections (i.e. prosthetic joint infections, hospital-acquired pneumonia or central nervous system infections).^{2,3} However, higher doses of vancomycin used have been associated with significantly higher vancomycin trough levels, acute renal failure and ototoxicity.^{10,11,12,13} The decision to target increased vancomycin trough concentrations should be based on an assessment of the severity of the infection and must consider the risk associated with increased vancomycin levels.

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 human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
TDMC1 ^{a)}	7.10 (4.90)	0.377 (0.260)	5.3
TDMC2 ^{b)}	21.2 (14.6)	0.394 (0.272)	1.9
TDMC3 ^{c)}	34.3 (23.7)	0.475 (0.328)	1.4
Human serum 1	7.37 (5.09)	0.332 (0.229)	4.5
Human serum 2	29.6 (20.4)	0.490 (0.338)	1.7
Human serum 3	5.36 (3.70)	0.384 (0.265)	7.2
Human serum 4	45.5 (31.4)	0.627 (0.433)	1.4
Human serum 5	75.7 (52.2)	1.64 (1.13)	2.2

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
TDMC1 ^{a)}	7.10 (4.90)	0.516 (0.356)	7.3
TDMC2 ^{b)}	21.2 (14.6)	0.518 (0.357)	2.4
TDMC3 ^{c)}	34.3 (23.7)	0.701 (0.484)	2.0
Human serum 1	7.77 (5.36)	0.520 (0.359)	6.7
Human serum 2	29.9 (20.6)	0.739 (0.510)	2.5
Human serum 3	5.84 (4.03)	0.480 (0.331)	8.2
Human serum 4	45.5 (31.4)	1.02 (0.704)	2.2
Human serum 5	75.7 (52.2)	2.36 (1.63)	3.1

a) TDM Control Set Level I

b) TDM Control Set Level II

c) TDM Control Set Level III

Method comparison

Vancomycin values for human serum samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Sample size (n) = 353

Passing/Bablok¹⁴

$$y = 1.030x + 0.0758 \text{ µg/mL}$$

$$\tau = 0.955$$

The sample concentrations were between 4.00 and 79.8 µg/mL (2.76 and 55.1 µmol/L).

Vancomycin values for human serum samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using LC-MS/MS¹⁶ (x).

Sample size (n) = 112

Passing/Bablok¹⁴

$$y = 0.973x + 1.00 \text{ µg/mL}$$

$$\tau = 0.945$$

The sample concentrations were between 4.10 and 79.6 µg/mL (2.83 and 54.9 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration tested µg/mL	Cross-reactivity %
Acyclovir	50	ND
Amikacin	100	ND
Amphotericin B	10	ND
Aztreonam	450	ND
Caffeine	60	ND
CDP-1	20	ND
Cefazoline	500	ND
Cefotaxime	300	ND
Chloramphenicol	60	ND
Ciprofloxacin	12	ND
Cisplatin	15	ND
Clindamycin	50	ND
Cyclosporine	3	ND
Digoxin	0.009	ND
Epinephrine	1	ND
Erythromycin	60	ND
Ethacrynic acid	1.5	ND
Flucytosine	300	ND
Furosemide	60	ND
Fusidic acid	600	ND
Gentamicin	30	ND
Imipenem	250	ND
Methicillin	250	ND
Methotrexate	455	ND
Metronidazole	150	ND
Netilmicin	30	ND
Nitroprusside	90	ND
Penicillin G	36	ND
Pentamidine	1.5	ND
Phenobarbital	150	ND
Rifampin	60	ND

Compound	Concentration tested µg/mL	Cross-reactivity %
Salicylate	750	ND
Sulphamethoxazole	400	ND
Theophylline	60	ND
Tobramycin	30	ND
Trimethoprim	40	ND

ND = not detectable

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

Acetaminophen	Heparin
Acetylcysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H ₂ O
Ascorbic acid	Metronidazole
Cefoxitin	Phenylbutazone
Cyclosporine	Rifampicin
Doxycycline (tetracycline)	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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: <https://ec.europa.eu/tools/eudamed>

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

	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.**

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0123



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