TOBR2

cobas®

Tobramycin

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04491033 190	ONLINE TDM Tobramycin 100 tests	System-ID 07 6985 1	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03375790 190	Preciset TDM I Calibrators CAL A-F (6 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	
04708725 190	Sample Cleaner 1 (59 mL)		

English

System information

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

TOBR2: ACN 607

For **cobas c** 502 analyzers:

TOBR2: ACN 8607

Intended use

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

Summary

Tobramycin is an aminoglycoside antibiotic used in the treatment of infections caused by *Pseudomonas aeruginosa, Proteus species, E. coli, Klebsiella, Serratia, Citrobacter, Staphylococcus aureus, Enterobacter* and other microorganisms. Tobramycin's toxic effect is produced by interfering with ribosomal protein synthesis.[1] Tobramycin undergoes very little, if any, metabolization and is, therefore, eliminated as the parent drug by glomerular filtration. The half-life of tobramycin in serum or plasma correlates closely with renal function and thus is quite variable between individuals and within one individual over time.[2,3] Serum or plasma tobramycin concentration is also impacted by mode of administration, the volume of extracellular fluid, the duration of the treatment and physiological changes during the illness and therapy. The therapeutic range of tobramycin should be measured at peak as well as trough concentrations. In patients with pre-existing renal damage or those to whom tobramycin has been administered for prolonged periods or in doses above the therapeutic range, hearing impairment and/or nephrotoxicity may develop. Therefore, monitoring of peak and trough tobramycin serum or plasma levels is critical in the prevention of these serious complications with the adjustment of dosage administration as indicated.[4]

Test principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of tobramycin in human serum or plasma.[5] 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

R2 Tobramycin labeled with bacterial G6PDH and bovine serum albumin in buffer

R3 Anti-tobramycin antibody (sheep polyclonal), G6P, NAD and bovine serum albumin in buffer

R2 is in position A and R3 is in position B.

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 hydrochloride.

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

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

Stability:[6]	3 days capped at 4-8 °C
	1 month 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.

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.

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Application for serum and plasma

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

cobas c 311 **test definition**

Assay type	Rate-A assay		
Reaction time /Assay points:	10 / 28-40		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R2	145 µL	–	
R3	73 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.4 µL	–	–
Decreased	2.4 µL	–	–
Increased	2.4 µL	–	–

cobas c 501/502 **test definition**

Assay type	Rate-A assay		
Reaction time /Assay points:	10 / 42-54		
Wavelength (sub/main)	415 /340 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R2	145 µL	–	
R3	73 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.4 µL	–	–
Decreased	2.4 µL	–	–
Increased	2.4 µL	–	–

Calibration

Calibrator	S1-6: Preciset TDM I Calibrators
Calibration mode	RCM
Calibration frequency	6-point calibration • after reagent lot change • every 6 weeks • 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 tobramycin in normal human serum.

ACTION REQUIRED

NOTE: Due to potential carryover from the last calibrator (Cal F) into the first quality control sample (Level 1) following calibration, assaying a non-reportable blank quality control sample is required prior to assaying the controls. The blank quality control sample should be programmed in the first position followed by quality control levels 1-3. Use Sample Cleaner 1 (Cat. no. 04708725 190) as the blank quality control sample.

The blank quality control sample is not required when assaying controls without calibration.

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.

Conversion factor:[7] µg/mL x 2.14 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at tobramycin levels of approximately 3.5 and 8 µg/mL (7.5 and 17 µmol/L).

Serum/Plasma

Icterus:[8] No significant interference up to an I index of 30 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 30 mg/dL or 513 µmol/L).

Lipemia (Intralipid):[8] No significant interference up to an L index of 750. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Hemolysis:[8] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 800 mg/dL or 497 µmol/L).

Criterion: Recovery within ± 10 % of initial value at a tobramycin level of approximately 3 µg/mL (6.4 µmol/L).

No significant interference from triglycerides up to 750 mg/dL (8.5 mmol/L).

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

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

Amikacin cross-reacts with this assay. Kanamycin cross-reacts significantly; however, the assay has not been optimized to quantitate this aminoglycoside. Aminoglycosides are not generally coadministered in clinical practice, although more than one aminoglycoside may be present when switching from treatment with one to another. Samples that contain tobramycin in combination with either amikacin or kanamycin cannot be reliably quantitated by this assay.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

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

0.33-10 µg/mL (0.71-21.4 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

0.33 µg/mL (0.71 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 lowest standard (standard 1 + 2 SD, repeatability, n = 21).

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Expected values

Investigator	Peak		Trough	
	µg/mL	µmol/L	µg/mL	µmol/L
Baselt and Cravey[10]	6-10	12.8-21.4	0.5-2.0	1.1-4.3
Sande and Mandell[4]	5-8	10.7-17.1	1-2	2.1-4.3
Dipersio[11]	4-8	8.6-17.1	1-2	2.1-4.3
Lew[12]	5-10	10.7-21.4	-	-

Equivalent diagnostic technologies have shown that in most adults, a peak therapeutic response is achieved with tobramycin concentrations in the 6-10 µg/mL (12.8-21.4 µmol/L) range and trough concentrations in the 0.5-2.0 µg/mL (1.1-4.3 µmol/L) range. A peak therapeutic range is suggested for optimal antimicrobial effectiveness. Concentrations above the therapeutic range for a prolonged period of time or in patients with pre-existing renal impairment can cause nephrotoxicity and/or hearing impairment. Elevated or increasing trough levels are an indication of drug accumulation due to renal impairment. Both peak and trough levels should be monitored to ensure prevention of serious complications associated with drug dosage.

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 in a modified NCCLS EP5-A 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	1.63	3.49	0.07	0.15	4.2
Control 2	3.64	7.79	0.10	0.21	2.8
Control 3	7.72	16.52	0.22	0.47	2.9
HS 1	4.02	8.60	0.11	0.24	2.7
HS 2	8.72	18.66	0.20	0.43	2.3

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	1.63	3.49	0.07	0.15	4.5
Control 2	3.64	7.79	0.11	0.24	3.1
Control 3	7.72	16.52	0.21	0.45	2.8
HS 1	4.02	8.60	0.12	0.26	2.9
HS 2	8.72	18.66	0.21	0.45	2.4

Method comparison

Serum/plasma

Tobramycin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 700 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 69
Passing/Bablok[13]	Linear regression
y = 0.960x + 0.020 µg/mL	y = 0.973x + 0.007 µg/mL
τ = 0.946	r = 0.998

The sample concentrations were between 0.370 and 9.92 µg/mL (0.792 and 21.2 µmol/L).

COBAS INTEGRA 700 analyzer	Sample size (n) = 68
Passing/Bablok[13]	Linear regression
y = 0.963x + 0.152 µg/mL	y = 0.966x + 0.193 µg/mL
τ = 0.916	r = 0.995

The sample concentrations were between 0.3 and 10.0 µg/mL (0.642 and 21.4 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-reactivity
Amikacin	100	1.6
Carbenicillin	1000	ND
Cephalothin	1000	ND
Chloramphenicol	1000	ND
Clindamycin	1000	ND
Erythromycin	1000	ND
Gentamicin	100	0.5
Gentamicin	25	ND
Kanamycin	100	5.3
Neomycin	100	ND
Netilmicin	100	ND
Penicillin G	1000	ND
Sisomicin	100	ND
Streptomycin	100	ND
Sulphamethoxazole	600	ND
Tetracycline	1000	ND
Trimethoprim	25	ND
Vancomycin	200	ND

ND = not detectable

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

Acetaminophen	Doxycycline (Tetracycline)
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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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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