TOBR2

cobas®

ONLINE TDM Tobramycin

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04491033190	04491033500	ONLINE TDM Tobramycin 100 tests	System-ID 07 6985 1	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	Codes 691-696	
04521536190	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	
04708725190	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 **cobas c** systems.

Summary

Tobramycin measurements performed with this assay, in human serum and plasma, are used for monitoring tobramycin treatment to ensure appropriate therapy.

Tobramycin belongs to the aminoglycoside antibiotics, with antibiotic activity against Gram-positive and Gram-negative bacteria and is indicated in the treatment of serious infections caused by susceptible pathogens.[1,2,3] Like all aminoglycoside antibiotics, tobramycin blocks the production of protein by inhibiting messenger RNA translation in the bacterial cells.[4] Tobramycin undergoes very little, if any, metabolization and is excreted primarily by glomerular filtration.[3] Serum or plasma tobramycin concentration is impacted by mode of administration, the volume of extracellular fluid, the duration of the treatment and physiological changes during the illness and therapy.[1,2] Tobramycin serum concentrations should be monitored when feasible to ensure the correct dosage is given.[1] In addition, an inverse relationship exists between serum half-life and renal function, therefore, doses and dosing interval should be adjusted for patients with reduced renal function and for critically ill patients.[1,3,5,6] Pre-existing renal damage, prolonged periods of administration and higher doses than recommended increase the risk of hearing impairment and nephrotoxicity.[1] Monitoring of tobramycin serum or plasma levels is used to ascertain clinical efficacy and to limit potentially dose-dependent serious side effects, such as nephro- and ototoxicity and is recommended by guidelines and clinical societies.[6,7,8,9,10]

Test principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of tobramycin in human serum or plasma.[11] 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 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.

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

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:[12]	3 days capped at 4-8 °C
	1 month capped at -20 °C (± 5 °C)

Freeze only once.

Do not induce foaming of specimens.

Invert thawed specimens several times prior to testing.

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.

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.

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

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

Traceability: This method has been standardized against USP reference standards.[13] The calibrators are prepared to contain known quantities of tobramycin 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

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor:[14] 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).

Icterus:[15] 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):[15] 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:[15] 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).

Triglycerides: No significant interference from triglycerides up to a concentration of 750 mg/dL (8.5 mmol/L).

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

Total protein: No significant interference from total protein in the concentration range of 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.[16]

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 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 required in certain cases.

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).

Expected values

Investigator	Peak		Trough	
	µg/mL	µmol/L*	µg/mL	µmol/L*
Baselt and Cravey[17]	6-10	12.8-21.4	0.5-2.0	1.1-4.3
Sande and Mandell[18]	5-8	10.7-17.1	1-2	2.1-4.3
Dipersio[19]	4-8	8.6-17.1	1-2	2.1-4.3

* calculated by unit conversion factor

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.

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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 the **cobas c** 501 analyzer.

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

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Tobramycin values for human serum and plasma samples obtained on a **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[20]	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[20]	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).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

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.

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:

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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