GENT2

cobas®

Gentamicin

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04490843 190	ONLINE TDM Gentamicin 100 Tests	System-ID 07 6922 3	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
03375790 190	Preciset TDM I Calibrators CAL A-F (1 x 5 mL) Preciset TDM I Calibrators Diluent (1 x 10 mL)	Codes 691-696	
04521536 190	TDM Control Set Level I (2 x 5 mL) TDM Control Set Level II (2 x 5 mL) TDM Control Set Level III (2 x 5 mL)	Code 310 Code 311 Code 312	

English

System information

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

GENT2: ACN 416

For **cobas c** 502 analyzer:

GENT2: ACN 8416

Intended use

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

Summary

Gentamicin is an aminoglycoside antibiotic that displays broad spectrum, high potency, anti-bacterial action for most susceptible organisms.[1,2,3,4,5,6,7,8,9] At therapeutic serum concentrations ranging from 4 to 10 µg/mL (8.4 to 20.9 µmol/L), gentamicin is capable of inhibiting the growth of many gram positive cocci, especially penicillinase-producing staphylococci. At concentrations of 10 µg/mL (20.9 µmol/L), most strains of *E. coli, Proteus spp., Klebsiella, Aerobacter, Clostridium, Brucella spp., Salmonella, Serratia,* and *Shigella* are inhibited. At concentrations ranging from 4 to 10 µg/mL (8.4 to 20.9 µmol/L), gentamicin displays activity against most strains of *Pseudomonas aeruginosa* . Because of these characteristics, gentamicin has been most successfully used in the treatment of serious infections, especially those caused by gram-negative bacilli.[10,11]

The therapeutic range of gentamicin should be measured at peak as well as trough concentrations. In patients with pre-existing renal damage or those to whom gentamicin 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 gentamicin levels is critical in the prevention of these serious complications with the adjustment of dosage administration as indicated.[12,13]

Test principle

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

Reagents - working solutions

R1 Gentamicin conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.2; preservative

R2 Anti-gentamicin antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino) propane sulfonic acid (MOPS) buffer, pH 7.5; stabilizer; preservative

R1 is in position B and R2 is in position C.

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.

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 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, or sodium, lithium, or ammonium heparin 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.

Stability:[14]	1 week capped at 2-8 °C
	4 weeks capped at -20 °C

Centrifuge samples containing precipitates before performing the assay.

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 through values.[15]

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	2-Point End
Reaction time / Assay points	10 / 11-32
Wavelength (sub/main)	800/600 nm
Reaction direction	Increase
Unit	µg/mL (µmol/L)

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Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	95 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End
Reaction time / Assay points	10 / 18-49
Wavelength (sub/main)	800/600 nm
Reaction direction	Increase
Unit	µg/mL (µmol/L)

Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	95 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.0 µL	–	–
Decreased	2.0 µL	–	–
Increased	2.0 µL	–	–

Calibration

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

ACTION REQUIRED

After any calibration with Preciset TDM I Calibrators, the TDM Control Set have to be run in the order Level 3 to Level 1. Prior to running samples run a blank serum sample. The blank serum sample can be sheduled for any R1/R2 assay.

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

Conversion factor:[16] µg/mL x 2.09 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at gentamicin levels of approximately 2 and 6 µg/mL (4.2 and 12.5 µmol/L).

Serum/Plasma

Icterus:[17] No significant interference up to an I index of 50 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 855 µmol/L or 50 mg/dL).

Hemolysis:[17] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[17] No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides up to 1000 mg/dL (11.3 mmol/L).

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

Total protein: No interference from total protein up to 12 g/dL.

Note

A negative bias of up to approximately 20 % has been observed with this assay for some samples artificially spiked with Gentamicin sulfate. Patient samples have been verified to recover correctly.

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/Multiclean/SCCS or 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

Measuring range: 0.4-10.0 µg/mL (0.84-20.9 µmol/L)

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

Lower limits of measurement

Lower detection limit of the test

0.3 µg/mL (0.63 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 two standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 21).

Functional sensitivity

0.4 µg/mL (0.84 µmol/L)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with a coefficient of variation of ≤ 20 % (repeatability, n = 21).

Expected values

Although optimum values may vary, peak serum values in the range of 6 to 10 µg/mL (12.5 to 20.9 µmol/L) and trough values in the range of 0.5 to 2.0 µg/mL (1.0 to 4.2 µmol/L) are generally accepted for therapeutic effectiveness.[18] The achievement of non-toxic, but therapeutic, serum levels is often difficult, even in patients with normal renal function. Complications encountered with the use of gentamicin are ototoxicity and nephrotoxicity.[10, 19,20,21,22] However, these reactions are predictable, and close patient monitoring is essential for the successful use of this agent. The most serious toxic effect of gentamicin is permanent damage to the vestibular division of the eighth cranial nerve, which has been reported to occur most frequently in patients with renal failure. Since gentamicin is inherently unstable, is not metabolized and is excreted primarily by glomerular filtration, toxic concentrations of the drug may accumulate in the body when

the dosage is not adjusted for patients with impaired renal function. While high serum levels can be toxic, indiscriminately low dosages of gentamicin will result in ineffective treatment for many strains of gram-negative bacteria. The indiscriminate use of low dosages of gentamicin may not only engender the emergence of gentamicin-resistant organisms, but also the emergence of aminoglycoside-resistant organisms.[11,23,24] Current literature reflects increasing interest in once daily dosing versus the conventional administration of drug 2 to 4 times daily. Adoption of once daily dosing may require a revision of target peak and trough concentrations.[25,26,27]

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

Reference[14]

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-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	1.87	3.91	0.08	0.17	4.2
Control 2	4.37	9.13	0.08	0.17	1.8
Control 3	6.48	13.5	0.12	0.3	1.8
HS 1	1.90	3.97	0.07	0.15	3.6
HS 2	6.04	12.6	0.13	0.3	2.2

Intermediate precision	Mean		SD		CV
	µg/mL	µmol/L	µg/mL	µmol/L	%
Control 1	1.87	3.91	0.09	0.19	5.1
Control 2	4.37	9.13	0.09	0.19	2.0
Control 3	6.48	13.5	0.15	0.31	2.3
HS 1	1.90	3.97	0.08	0.17	4.1
HS 2	6.04	12.6	0.17	0.36	2.8

Method comparison

Serum/Plasma

Gentamicin values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined with the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and on a COBAS INTEGRA 800 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 64
Passing/Bablok[28]	Linear regression
y = 0.976x - 0.014 µg/mL	y = 0.976x - 0.016 µg/mL
τ = 0.962	r = 0.999

The sample concentrations were between 0.540 and 9.65 µg/mL (1.13 and 20.2 µmol/L).

COBAS INTEGRA 800 analyzer	Sample size (n) = 63
Passing/Bablok[28]	Linear regression
y = 0.983x - 0.214 µg/mL	y = 0.988x - 0.219 µg/mL
τ = 0.965	r = 0.997

The sample concentrations were between 0.530 and 9.14 µg/mL (1.11 and 19.1 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross-Reactivity
Netilmicin	70	9.13
Sisomicin	131	8.16
Methotrexate	23	< 1.0
Tetracycline	40	< 1.0
Amikacin	250	< 0.1
Cephalexin	500	< 0.1
Chloramphenicol	300	< 0.1
Clindamycin	500	< 0.1
Kanamycin	250	< 0.1
Neomycin	100	< 0.1
Spectinomycin	200	< 0.1
Streptomycin	200	< 0.1
Tobramycin	100	< 0.1
Vancomycin	400	< 0.1
Amphotericin B	50	< 0.01
Ampicillin	78	< 0.01
Carbenicillin	500	< 0.01
Cephalosporin C	432	< 0.01
Cephalothin	63	< 0.01
Erythromycin	200	< 0.01
5-Fluorocytosine	700	< 0.01
Furosemide	100	< 0.01
Methylprednisolone	500	< 0.01
Oxytetracycline	37	< 0.01
Prednisolone	500	< 0.01

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

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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

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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.

Significant additions or changes are indicated by a change bar in the margin.

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