GENT2

cobas®

ONLINE TDM Gentamicin

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057770190	08057770500	ONLINE TDM Gentamicin (100 tests)	System-ID 2059 002	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	Codes 20691-20696	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	Code 20310 Code 20311 Code 20312	

English

System information

GENT2O: ACN 20591 (100 tests; System ID 2059 002)

Intended use

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

Summary

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

Gentamicin is an aminoglycoside antibiotic extracted from Micromonospora purpurea which is effective against a wide range of bacteria including most gram-negative bacteria and staphylococci. It is indicated in serious infections such as bacteraemia, urinary tract infections, chest infections, severe neonatal infections and other serious systemic infections due to susceptible organisms, in adults and children including neonates.[1,2] The bactericidal action of gentamicin is due to its ability to bind the 30S subunit proteins of the bacterial ribosomes, thereby inhibiting protein synthesis and acting in both the proliferation and resting phases of bacteria.[1,2,3]

The drug is mainly excreted by glomerular filtration and has a half life ranging from 2 to 3 hours.[3,4] Overexposure to gentamicin has been associated with nephrotoxicity and ototoxicity, especially in patients with pre-existing renal damage, in pediatric, elderly and critically ill patients.[4,5]

Therefore, serum concentration monitoring of gentamicin is recommended, especially in elderly, in newborns and in patients with impaired renal function in order to reduce the risk of serious complications and for adjustment of dosage administration as indicated.[1,4,5,6,7,8,9,10]

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

R3 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 R3 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.

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:	1 week capped at 2-8 °C
	4 weeks capped at -20 °C (± 5 °C)

Freeze only once.

Centrifuge samples containing precipitates before performing the assay.

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

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.[11]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	800/600 nm		
Reagent pipetting		Diluent (H_2O)	
R1	65 µL	–	
R3	62 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent* (H_2O)
Normal	1.3 µL	–	–
Decreased	1.3 µL	–	–
Increased	1.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1-6: Preciset TDM I calibrators
Calibration mode	Non-linear
Calibration frequency	Full calibration - after **cobas c** pack change - after reagent lot change - every 28 days on-board - 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. 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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks.

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 in the unit µg/mL (µmol/L, mg/L).

Conversion factors:[12]	µg/mL x 2.09 = µmol/L
	µg/mL x 1.0 = mg/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).

Icterus:[13] 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:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 150. 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 (11.3 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 up to a concentration of 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.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[14]

In very rare cases, patient samples may contain particle agglutinating proteins (e.g. heterophilic antibodies or antibodies due to abnormal immunoglobulin synthesis, such as gammopathies like MGUS[b)] or Waldenström's macroglobulinemia), which may lead to incorrect low or high results with this assay. Correct results cannot be obtained by sample dilution and these samples should be analyzed by an alternative method.

b) Monoclonal Gammopathy of Unknown Significance

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. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.3 µg/mL (0.63 µmol/L)
Limit of Detection	= 0.4 µg/mL (0.84 µmol/L)
Limit of Quantitation	= 0.8 µg/mL (1.67 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 gentamicin samples.

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.[15]

*calculated by unit conversion factor

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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.[16,17,18,19,20] 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.[21,22,23] 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.[24,25,26]

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

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). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean µg/mL*	*SD µg/mL*	*CV %*
TDMC1[a)]	1.81	0.0375	2.1
TDMC2[b)]	4.49	0.0558	1.2
TDMC3[c)]	6.48	0.0786	1.2
Human serum 1	0.663	0.0392	5.9
Human serum 2	1.94	0.0353	1.8
Human serum 3	4.60	0.0518	1.1
Human serum 4	7.76	0.108	1.4
Human serum 5	7.90	0.129	1.6

Intermediate precision	*Mean µg/mL*	*SD µg/mL*	*CV %*
TDMC1[a)]	1.81	0.0544	3.0
TDMC2[b)]	4.45	0.0720	1.6
TDMC3[c)]	6.48	0.106	1.6
Human serum 1	0.663	0.0525	7.9
Human serum 2	1.89	0.0581	3.1
Human serum 3	4.63	0.0876	1.9
Human serum 4	7.76	0.149	1.9
Human serum 5	7.90	0.167	2.1

a) TDM Control Set Level I

b) TDM Control Set Level II

c) TDM Control Set Level III

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Gentamicin values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 82

Passing/Bablok[27]	Linear regression
y = 1.000x - 0.0200 µg/mL	y = 1.002x - 0.0312 µg/mL
τ = 0.970	r = 0.999

The sample concentrations were between 0.41 and 9.81 µg/mL.

Gentamicin values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 77

Passing/Bablok[27]	Linear regression
y = 1.079x - 0.205 µg/mL	y = 1.091x - 0.231 µg/mL
τ = 0.954	r = 0.996

The sample concentrations were between 0.470 and 9.68 µg/mL.

Gentamicin values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 67

Passing/Bablok[27]	Linear regression
y = 1.015x - 0.0680 µg/mL	y = 1.026x - 0.106 µg/mL
τ = 0.954	r = 0.997

The sample concentrations were between 0.521 and 9.42 µg/mL.

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

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 navifyportal.roche.com for definition of symbols used):

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.

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CE 0123

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