GENT

Gentamicin

cobas®

Therapeutic drug monitoring

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20737844 322	Gentamicin (200 tests)	System-ID 07 3784 4	COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8	
04521536 190	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	
20720720 322	COBAS FP Sample Dilution Reagent II (1 × 200 mL)	System-ID 07 2072 0	

English

System information

Test GENTM, test ID 0-284

Intended use

In vitro test for the quantitative determination of gentamicin in serum or plasma on COBAS INTEGRA systems.

Summary

Gentamicin is an aminoglycoside antibiotic which displays broad spectrum, high potency, and 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]

Test principle

Fluorescence polarization

COBAS INTEGRA therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.[12] Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum for the purpose of therapeutic drug monitoring.

Surface active agents are used to ensure dissociation of the drug from serum proteins and to prevent nonspecific binding of the tracer.

Reagents - working solutions

R1 Antibody reagent
Anti-gentamicin monoclonal antibody (mouse) in buffer, pH 7.5, with stabilizer and preservative.

SR Tracer reagent
Fluorescein-labeled gentamicin derivative in buffer, pH 8.5, with stabilizer and preservative.

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	26 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

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:
Unhemolyzed serum
Unhemolyzed heparinized 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.

Centrifuge samples containing precipitates before performing the assay.

Usual sampling time varies dependent upon desired measurement of peak or trough values.[13] If specimens must be stored for later testing, they may be kept capped at 2-8 °C for 1 week or capped at -20 °C for 4 weeks.[14] 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)

COBAS FP Sample Dilution Reagent (SDR II), Cat. No. 20720720 322
The SDR II is placed as special diluent in its predefined rack position and is stable for 7 days on-board COBAS INTEGRA 400 plus/800 analyzers.

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

COBAS INTEGRA 400 plus test definition

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm

Reading cycle blank/test		29/45
Unit		µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	140 µL	10 µL
Sample	2 µL	5 µL
Special diluent SDR II	18 µL	
SR	18 µL	10 µL
Total volume	203 µL	

COBAS INTEGRA 800 test definition

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm
Reading cycle blank/test		40/60
Unit		µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	140 µL	10 µL
Sample	2 µL	5 µL
Special diluent SDR II	18 µL	
SR	18 µL	10 µL
Total volume	203 µL	

Calibration

Calibrators	Preciset TDM I Calibrators A-F
Calibration mode	Logit/log 5
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 0.5 µg/mL (≥ 1.0 µmol/L)
Calibration interval	Each lot, every 12 weeks, and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of gentamicin in normal human serum and are traceable to USP reference standards.

Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

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.

Note

Controls should be assayed within 2 hours after placing on-board the instrument.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/mL × 2.09 = µmol/L

Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g. technical or procedural errors).

Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate manual dilution of the original sample with the Preciset TDM I Diluent (0 µg/mL). Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

Serum/plasma

Criterion: Recovery within ± 10 % of initial value at a gentamicin concentration of 1.1 µg/mL (2.3 µmol/L) and 5.5 µg/mL (11.5 µmol/L).

Icterus:[15] No significant interference up to a bilirubin concentration of 256.5 µmol/L or 15 mg/dL.

Hemolysis:[15] No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:[15] No significant interference up to a triglycerides concentration of 1602 mg/dL.

Total protein: No significant interference up to a total protein concentration of 14 g/dL.

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

COBAS INTEGRA 400 plus analyzer:
0.04-10 µg/mL (0.08-20.9 µmol/L)

COBAS INTEGRA 800 analyzer:
0.14-10 µg/mL (0.3-20.9 µmol/L)

Lower limits of measurement

Lower detection limit of the test:

COBAS INTEGRA 400 plus analyzer:
0.04 µg/mL (0.08 µmol/L)

COBAS INTEGRA 800 analyzer:
0.14 µg/mL (0.3 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

Expected values

Although optimum values may vary, peak serum values in the range of 6-10 µg/mL (12.5-20.9 µmol/L) and trough values in the range of 0.5-2.0 µg/mL (1.0-4.2 µmol/L) are generally accepted for therapeutic effectiveness.[16] 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, 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.[11,21,22]

Current literature reflects increasing interest in once-daily dosing versus the conventional administration of drug 2-4 times daily. Adoption of once-daily dosing may require a revision of target peak and trough concentrations.[23,24,25]

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 controls in accordance with the NCCLS EP5-T2[26] requirements with repeatability (n = 80) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on a COBAS INTEGRA 400 analyzer.

Repeatability	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Level 1	3.1 (6.5)	0.09 (0.19)	2.9
Level 2	5.7 (11.9)	0.07 (0.15)	1.3
Level 3	7.0 (14.6)	0.10 (0.21)	1.5

Intermediate precision	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Level 1	3.1 (6.5)	0.10 (0.21)	3.1
Level 2	5.7 (11.9)	0.11 (0.23)	2.0
Level 3	7.0 (14.6)	0.17 (0.36)	2.4

Method comparison

Gentamicin values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Gentamicin reagent (y) were compared with those determined using a commercially available FPIA method (x).

		FPIA
Number of samples		145
Range of values	min.	0.5 µg/mL
	max.	10.0 µg/mL
Slope		0.955
Intercept		-0.015 µg/mL
Correlation coefficient		0.992

Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with gentamicin at 5.4 µg/mL (11.3 µmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.[27] The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested µg/mL	Cross-reactivity %
Ampicillin	90	ND
Cephalosporin C	500	ND
Netilmicin	70	25.2
Oxytetracycline	40	0.7
Sisomicin	200	47.3

ND = Not Detectable

Netilmicin and sisomicin are structurally very similar to gentamicin and can be expected to cross-react to a significant degree. The concurrent administration of gentamicin with netilmicin or sisomicin to the same patient would be highly unlikely.

In a similar study, the following structurally related or potentially co-administered compounds were tested on the COBAS FARA II analyzer using normal human serum spiked with gentamicin at 5.5 µg/mL (11.5 µmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
Amikacin	250	ND
Amphotericin B	50	ND
Carbenicillin	500	ND
Cephalexin	500	ND
Cephaloglycin	500	ND
Cephalothin	63	ND
Chloramphenicol	300	ND
Clindamycin	500	ND
Erythromycin	200	ND
5-Fluorocytosine	700	ND
Furosemide	100	ND
Fusidic acid	500	ND
Kanamycin	250	0.04
Methotrexate	23	ND
Methylprednisolone	500	ND
Neomycin	100	ND
Prednisolone	500	ND
Spectinomycin	200	ND
Streptomycin	200	ND
Tetracycline	40	ND
Ticarcillin	500	ND
Tobramycin	100	ND
Vancomycin	400	ND

ND = Not Detectable

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

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

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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