

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
20767166 322	Amikacin (150 tests)	System-ID 07 6716 6 COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375781 190	Preciset TDM II Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6829 4
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 AMKMM, test ID 0-216

Intended use

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

Summary

Amikacin is a semi-synthetic aminoglycoside that exhibits bactericidal activity against a wide range of pathogens, including many organisms resistant to other aminoglycosides.^{1,2,3,4} Amikacin is active in vitro against gram-negative organisms, penicillinase and non-penicillinase producing staphylococci. The strength of this drug is due primarily to its high degree of resistance to aminoglycoside-inactivating enzymes.⁵

Determination of serum or plasma drug levels is required to achieve optimum therapeutic efficacy and minimize toxicity.⁶

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.⁷ Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum or plasma 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-amikacin monoclonal antibody (mouse) in buffer, pH 6.5, with stabilizer and preservative
- R2** Diluent
Buffer containing stabilizer and preservative.
- SR** Tracer reagent
Fluorescein-labeled amikacin derivative in buffer, pH 8.0, with stabilizer and preservative.

R1 is in position A, R2 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

18 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 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.

Centrifuge samples containing precipitates before performing the assay.

Usual sampling time varies dependent upon desired measurement of peak or trough values.⁸ Specimens should be tested within 8 hours of collection if kept at room temperature. If specimens must be stored for later testing, they may be kept at 2-8 °C for up to 48 hours or at -20 °C or below for longer periods. 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-R2-SDR2/S-SR
Wavelength	excitation 485 nm emission 515 nm
Reading cycle blank/test	45/61

Unit	µg/mL	
Pipetting parameters		
		Diluent (H ₂ O)
R1	90 µL	30 µL
R2	10 µL	10 µL
Sample	2.25 µL	4.75 µL
Special diluent (SDR II)	18 µL	
SR	9 µL	5 µL
Total volume	179 µL	

COBAS INTEGRA 800 test definition

Measuring mode	FP
Reaction mode	R1-R2-SDR2/S-SR
Wavelength	excitation 485 nm
	emission 515 nm
Reading cycle blank/test	40/60
Unit	µg/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	110 µL	10 µL
R2	10 µL	10 µL
Sample	2 µL	5 µL
Special diluent (SDR II)	18 µL	
SR	9 µL	5 µL
Total volume	179 µL	

Calibration

Calibrators	Preciset TDM II Calibrators A-F
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 2.5 µg/mL (≥ 4.3 µmol/L)
Calibration interval	
COBAS INTEGRA 400 plus analyzers:	Each lot, every 4 weeks, and as required following quality control procedures
COBAS INTEGRA 800 analyzers:	Each lot, every 18 weeks, and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM II 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 II calibrators are prepared to contain known quantities of amikacin 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 × 1.71 = 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 II Diluent (0 µg/mL). A 1:5 dilution of those samples is recommended. 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 an amikacin concentration of 4 µg/mL (6.7 µmol/L) and 25 µg/mL (42.7 µmol/L).

Icterus:⁹ No significant interference up to a bilirubin concentration of 301 µmol/L or 17.6 mg/dL.

Hemolysis:⁹ No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:⁹ No significant interference up to a triglycerides concentration of 1852 mg/dL.

Total protein: No significant interference up to a total protein concentration of 4-12 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

0.30-40 µg/mL (0.51-68.4 µmol/L)

Lower limits of measurement

Lower detection limit of the test:

0.30 µg/mL (0.51 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 of COBAS INTEGRA Amikacin reagent run on the COBAS INTEGRA systems.

Expected values

Although optimum values may vary, peak serum values of amikacin in the range of 20-25 µg/mL (34.2-42.8 µmol/L) and trough values in the range of 5-10 µg/mL (8.6-17.1 µmol/L) are generally accepted for therapeutic effectiveness. Toxicity is associated with peak levels greater than 35 µg/mL (59.9 µmol/L) and trough values greater than 10 µg/mL (17.1 µmol/L).⁶

The most serious toxic effect 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 amikacin is inherently stable,

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is not metabolized, and is excreted primarily by glomerular filtration, the presence of renal impairment drastically alters its pharmacokinetics. If dosage regimens are not adjusted, excess accumulation leading to ototoxicity and further renal impairment could be encountered.^{10,11,12,13}

While serum levels can be toxic, indiscriminately low dosages of amikacin will result in ineffective treatment for many strains of gram-negative bacteria. Organisms which are resistant to amikacin will often show increased resistance to all other available aminoglycosides. This observation¹⁴ points out the possibility that the indiscriminate use of low dosages of amikacin could engender the emergence of drug-resistant organisms and possibly render the drug ineffective in treating infectious disease.^{5,15}

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-T2¹⁶ requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on a COBAS INTEGRA 700 analyzer:

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	5.3 (9.1)	0.29 (0.50)	5.4
Level 2	14.3 (24.4)	0.25 (0.43)	1.8
Level 3	27.3 (46.7)	0.58 (0.99)	2.1

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	5.3 (9.1)	0.37 (0.63)	7.0
Level 2	14.3 (24.4)	0.36 (0.62)	2.5
Level 3	27.3 (46.7)	0.78 (1.33)	2.9

Method comparison

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

	FPIA
Number of samples	120
Range of values	min. 0.30 µg/mL max. 41.8 µg/mL
Slope	0.914
Intercept	0.511 µg/mL
Correlation coefficient	0.987

Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with amikacin at 20 µg/mL (34.2 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.¹⁷ 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 %
Erythromycin	200	ND

Drug	Level tested µg/mL	Cross-reactivity %
Gentamicin	100	ND
Kanamycin A	25	3.6
Kanamycin B	25	0.6
Neomycin	100	< 0.3
Netilmicin	80	ND
Streptomycin	200	< 0.3
Tobramycin	100	< 0.3
Vancomycin	400	ND

In a similar study, the following structurally related or potentially coadministered compounds were tested on the COBAS INTEGRA systems using normal human serum spiked with amikacin at 25 µg/mL (42.7 µmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
Amphotericin	20	1.1
Ampicillin	90	0.9
Carbenicillin	500	ND
Cephalexin	500	ND
Cephaloglycin	500	ND
Cephaloridine	160	< 0.3
Cephalosporin C	500	< 0.3
Cephalothin	60	ND
Chloramphenicol	300	< 0.3
Clindamycin	5	2.8
Ethacrynic acid	500	ND
5-Fluorocytosine	700	ND
Furosemide	100	ND
Fusidic acid	500	ND
Lincomycin	30	0.6
Methotrexate	23	ND
Methylprednisolone	500	ND
Oxytetracycline	40	ND
Penicillin V	50	ND
Prednisolone	500	ND
Rifampin	320	ND
Spectinomycin	200	ND
Sulfadiazine	1500	ND
Sulfamethoxazole	2000	ND
Tetracycline	40	ND
Trimethoprim	120	ND

ND = Not Detectable

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

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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.

	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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