GENTC

thermoscientific

CEDIA™ Gentamicin II

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08772525 190[a)]	CEDIA Gentamicin II (95 tests)	System-ID 07 7620 3	Roche/Hitachi **cobas c** 501/502
08777934 190[a)]	CEDIA Antibiotic TDM Multi-Cal Low, 2 x 7.5 mL High, 2 x 5 mL	 Code 618 Code 619	
04521536 190[a)]	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	
04908856 160[b)]	Open/Close tool (5 pieces)		

a) Not all products are available in all countries.

b) Catalog number is for USA only. Open/Close tool is available upon request in other countries.

English

Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.

System information

For **cobas c** 501 analyzer:

GENTC: ACN 606

For **cobas c** 502 analyzer:

GENTC: ACN 8606

Intended use

The CEDIA Gentamicin II Assay is an in vitro diagnostic medical device intended for the quantitation of gentamicin in human serum or plasma.

Summary

Gentamicin is an aminoglycoside antibiotic used in the treatment of infections caused by *E. coli, Klebsiella, Enterobacter, Proteus mirabilis, Pseudomonas aeruginosa, Serratia, Staphylococcus aureus, Staphylococcus epidermidis* and other microorganisms. Gentamicin's toxic effect is produced by interfering with ribosomal protein synthesis.[1,2] Gentamicin undergoes very little, if any, metabolization and is, therefore, eliminated as the parent drug by glomerular filtration. The therapeutic range should be measured at peak as well as trough concentrations. Peak serum or plasma concentrations of gentamicin are suggested to ensure that adequate antimicrobial activity is obtained. Trough gentamicin concentrations usually ensure that drug elimination is adequate and the drug concentration is above minimum inhibitory concentration. Serum or plasma gentamicin 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. Therefore, monitoring of peak and trough gentamicin serum or plasma levels is critical in the prevention of these serious complications with the adjustment of dosage administration as indicated.[3,4]

Test principle

The CEDIA Gentamicin II Assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system.[5] The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments: enzyme acceptor (EA) and enzyme donor (ED). These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically. In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment of β-galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragments free to form active enzyme. If analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.[6]

Reagents - working solutions

R1	EA Reconstitution Buffer: Contains 3-(N-morpholino)propanesulfonic acid, buffer salts, surfactant and preservative (sodium azide) (13 mL).
R1a	EA Reagent: Contains 0.14 g/L Enzyme acceptor, 9.3 mg/L monoclonal anti-gentamicin antibody, buffer salts, stabilizer and preservative (sodium azide).
R2	ED Reconstitution Buffer: Contains 3-(N-morpholino)propanesulfonic acid, buffer salts, surfactant and preservative (sodium azide) (11 mL).
R2a	ED Reagent: Contains 38 µg/L Enzyme donor conjugated to gentamicin, 2.36 g/L chlorophenol red-β-D-galactopyranoside, 1.7 g/L goat anti-mouse antibodies, buffer salts, stabilizer and preservative (sodium azide).

Precautions and warnings

Danger

• **R1 & R2 Reconstitution Buffers:** Contain sodium azide.

EUH032 – Contact with acids liberates very toxic gas.

• **R1a & R2a Reagents:** Contain goat serum, BSA, sodium phosphate (dibasic, anhydrous), sodium phosphate (monobasic), sodium azide, drug-specific monoclonal antibody (mouse).

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

EUH032 – Contact with acids liberates very toxic gas.

Avoid breathing mist or vapor. Wash hands thoroughly after handling. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation or rash occurs: Get medical advice/attention. If eye irritation persists: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Take off contaminated clothing and wash

before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10 % sodium hydroxide.

• Do not use the reagents beyond their expiration dates.

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Remove the kit from refrigerated storage immediately prior to preparation of the solutions. Prepare the solutions in the following order to minimize possible contamination.

R2 Enzyme donor solution: Connect Bottle R2a (ED Reagent) to Bottle R2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle R2a is transferred into Bottle R2. Avoid the formation of foam. Detach Bottle R2a and adapter from Bottle R2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at 15-25 °C. Mix again. Record the reconstitution date on the bottle label. Place the bottle into refrigerated storage and let stand 30 minutes before use.

R1 Enzyme acceptor solution: Connect Bottle R1a (EA Reagent) to Bottle R1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle R1a is transferred into Bottle R1. Avoid the formation of foam. Detach Bottle R1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at 15-25 °C. Mix again. Record the reconstitution date on the bottle label. Place the bottle into refrigerated storage and let stand 30 minutes before use.

Filling the cobas c pack:

1. Turn the **cobas c** pack toward you.

2. Position A of the **cobas c** pack is now in the center, position B on the left side, position C on the right side of the **cobas c** pack.

3. Unscrew the screw cap of the bottle in position B on the left side of the **cobas c** pack using the Open/Close tool.

4. Use one of the enclosed funnels to pour the content of the R1 bottle (13.0 mL) into the opened bottle of the **cobas c** pack (position B). Discard the funnel.

5. Close the bottle tightly using the Open/Close tool.

6. Unscrew the screw cap of the bottle in position C on the right side of the **cobas c** pack using the Open/Close tool.

7. Use one of the enclosed funnels to pour the content of the R2 bottle (11.0 mL) into the opened bottle of the **cobas c** pack (position C). Discard the funnel.

8. Close the bottle tightly using the Open/Close tool.

9. Leave position A empty.

The CEDIA Gentamicin II **cobas c** pack is now ready for use.

NOTE: Solutions must be at the reagent compartment storage temperature of the analyzer before performing assays.

Always use a new **cobas c** pack when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the **cobas c** pack bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.

Storage and stability

Shelf life at 2-8 °C:	For stability of the unopened components, refer to the box or bottle labels for the expiration date.

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.
Plasma: Na- or Li-heparin, Na-EDTA

Serum or plasma (Na- or Li-heparin; Na-EDTA) samples are suitable for use in the assay. Care should be taken to preserve the chemical integrity of the serum or plasma sample from the time it is collected until the time it is assayed. Cap samples, store at 2-8 °C and assay within 1 week after collection. If the assay cannot be performed within 1 week, or if the sample is to be shipped, cap the sample and keep it frozen. Store samples at -20 °C and assay within 4 weeks.

Invert thawed specimens several times prior to testing.

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. Centrifuge specimens containing particulate matter. Handle all patient samples as if they were potentially infectious.

Centrifuge samples containing precipitates before performing the assay.

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See “Reagents – working solutions” section for reagents.

cobas c pack, funnels

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.

Applications for serum/plasma

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 501/502 **test definition**

Assay type	Rate A
Reaction time / Assay points	10 / 60-70
Wavelength (sub/main)	660/570 nm
Reaction direction	Increase
Unit	µg/mL
Reagent pipetting	
R1	100 µL
R3	75 µL
Sample volumes	*Sample*
Normal	2 µL
Decreased	2 µL
Increased	2 µL

Calibration

Calibrators	*2-point calibration is recommended*

	S1: CEDIA Antibiotic TDM Multi-Cal Low calibrator
	S2: CEDIA Antibiotic TDM Multi-Cal High calibrator
Calibration mode	Linear
Calibration frequency	Recalibration is recommended • as 2-point after reagent bottle and/or lot change • 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 CEDIA Antibiotic TDM Multi-Cal calibrators are prepared to contain known quantities of gentamicin in bovine serum albumin.

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: µg/mL x 2.09 = µmol/L.

Limitations - interference

Samples containing gentamicin and the following maximal concentrations of potential interfering substances are quantitated accurately by the assay:

600 mg hemoglobin/dL

1000 mg triglyceride/dL

30 mg bilirubin/dL

Increasing total serum protein levels to 12.5 g/dL by addition of gamma globulin up to 5.0 g/dL; or increasing total serum protein levels to 12.5 g/dL by addition of human serum albumin up to 5.5 g/dL had no effect on patient sample quantitation by the assay.

Patient samples containing sisomicin will elevate gentamicin results.

The incidence of patients with antibodies to *E. coli* β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high gentamicin results that do not fit the clinical profile. If this occurs, contact Customer Technical Support.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely elevated results.

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-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

0.24-12.0 µg/mL (0.5-25.1 µmol/L)

Specimen dilution

Manually dilute samples above the measuring range 1 + 1 with the CEDIA Antibiotic TDM Multi-Cal Low calibrator diluent (0 ng/mL) and reassay. Multiply the result by 2 and subtract the concentration of the low calibrator to obtain the specimen value.

Lower limits of measurement

Analytical sensitivity (lower detection limit): 0.24 µg/mL (0.5 µmol/L).

Specimens results below 0.24 µg/mL should be reported as <0.24 µg/mL. Specimens results greater than 12 µg/mL should be reported as >12 µg/mL or dilute 1 part sample with 1 part CEDIA Antibiotic TDM Multi-Cal Low calibrator diluent (0 ng/mL) and reassay.

Expected values

The therapeutic efficacy and toxic effects are closely related to the serum drug concentration. In most adults a peak therapeutic response is achieved with gentamicin concentrations between 5-8 µg/mL. Trough concentrations between 1-2 µg/mL usually ensure that drug elimination is adequate.[1,4] Different gentamicin therapeutic ranges have also been reported by other investigators.[2,3,6,7,8]

Specific performance data

Representative performance data on the Roche/Hitachi 704 analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Intra-assay precision was determined by assaying 20 replicates of each of 3 control pools while inter-assay precision was determined by single point quantitation on 64 runs. The following results were obtained.

Intra-assay				**Inter-assay**		
N	20	20	20	64	64	64
x (µg/mL)	1.62	5.37	9.31	1.68	5.29	9.24
SD (µg/mL)	0.07	0.12	0.15	0.11	0.25	0.40
CV %	4.3	2.2	1.6	6.55	4.73	4.33

Method comparison

A comparison using the CEDIA Gentamicin Assay (y) with a commercially available FPIA (x) gave the following correlation (µg/mL).

Linear regression

y = 0.98x + 0.17

r = 0.992

Sy.x = 0.347

Linearity

To assess the linearity of the assay, a high sample was diluted with the CEDIA Antibiotic TDM Multi Cal Low calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

Dilution (%)	Expected value (µg/mL)	Measured value (µg/mL)	% Recovery
100	7.97	7.97	100
80	6.41	6.54	102
60	4.85	5.08	105
40	3.28	3.40	104
20	1.72	1.86	108
0	0.16	0.16	100

Recovery

To assess the recovery of the assay, gentamicin in the form of a high patient sample was added to a normal patient sample. The percent recovery was then determined by dividing the assayed value by the expected value.

Dilution (%)	Expected value (µg/mL)	Measured value (µg/mL)	% Recovery
100	8.85	8.85	100
80	7.08	7.27	103
60	5.31	5.52	104

40	3.54	3.70	105
20	1.77	1.74	98
0	0.00	0.00	100

Analytical specificity

The following compounds were tested for cross-reactivity with the assay, and the results are as follows:

Compound	Cross-reactivity (%)
3,4-Dideoxykanamycin	< 0.1
5-Fluorocytosine	< 1.0
Amikacin	< 0.1
Amphotericin	< 0.1
Ampicillin	< 0.1
Carbenicillin	< 0.1
Cefamandole nafate	< 0.1
Cephalexin	< 0.1
Cephalosporin C	< 0.1
Cephaloglycin	< 0.1
Cephaloridine	< 0.1
Cephalothin	< 0.1
Chloramphenicol	< 0.1
Clindamycin	< 0.1
Erythromycin	< 0.1
Ethacrynic acid	< 1.0
Furosemide	< 0.1
Fusidic acid	< 0.1
Kanamycin A	< 0.1
Kanamycin B	< 0.1
Lincomycin	< 0.1
Methicillin	< 0.1
Methotrexate	< 0.1
Methylprednisolone	< 0.1
Neomycin	< 0.1
Netilmycin	1.15
Oxytetracycline	< 0.1
Penicillin G	< 0.1
Penicillin V	< 0.1
Prednisolone	< 0.1
Rifampin	< 0.1
Sisomicin	62.1
Spectinomycin	< 0.1
Streptomycin	< 0.1
Sulfadiazine	< 0.1
Sulfamethoxazole	< 0.1
Sulfanilamide	< 0.1
Sulthiame	< 0.1
Tetracycline	< 0.1
Ticarcillin	< 0.1
Tobramycin	< 0.1
Trimethoprim	< 1.0
Vancomycin	< 0.1

References

1 Barza M, Scheife R. Drug therapy reviews: Antimicrobial spectrum, pharmacology and therapeutic use of antibiotics-part 4: aminoglycosides. Am J Hosp Pharm 1977;34:723-737.

2 Sande MA, Mandell GL. Antimicrobial agents, the aminoglycosides. In: Gilman AG, Goodman LS, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, NY: MacMillan 1980;1162-1180.

3 Kahlmeter G. Gentamicin and tobramycin. Clinical pharmacokinetics and nephrotoxicity. Aspects on assay techniques. Scand J Infect Dis 1979;132(Suppl 18):1-40.

4 Barza M, Lauermann M. Why monitor serum levels of gentamicin? Clin Pharm 1978;3:202-215.

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6 Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals in Man. 3rd ed 1990:805-807.

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8 Dipersio JR. Gentamicin and other aminoglycosides. In: Pesce AJ and Kaplan LA eds. Methods in Clinical Chemistry. St.Louis, Mo: CV Mosby Co 1987.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

As a distributor, 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.

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