CEDIA™ Tobramycin II Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100018 (13 mL, 11 mL Kit)

Intended Use
The CEDIA™ Tobramycin II Assay is an in vitro diagnostic medical device intended for the quantitation of tobramycin in human serum or plasma.

Summary and Explanation of the Test
Tobramycin is an aminoglycoside antibiotic used in the treatment of infections caused by Pseudomonas aeruginosa, Proteus species, E. coli, Klebsiella, Serratia, Citrobacter, Staphylococcus aureus, Enterobacter and other microorganisms. Tobramycin's toxic effect is produced by interfering with ribosomal protein synthesis.[1] Tobramycin 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 tobramycin are suggested to ensure that adequate antimicrobial activity is obtained. Trough tobramycin concentrations usually ensure that drug elimination is adequate and the drug concentration is above minimum inhibitory concentration. Serum or plasma tobramycin concentration is impacted by mode of administration, the volume of extracellular fluid, the duration of treatment and physiological changes during the illness and therapy. Therefore, monitoring of peak and trough tobramycin serum or plasma levels is critical in the prevention of these serious complications with the adjustment of dosage administration as indicated.[2]

The CEDIA Tobramycin II assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[3]

The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments i.e., 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.[5]

Reagents
1 **EA Reconstitution Buffer:** Contains 3-(N-morpholino) propanesulfonic acid, buffer salts, surfactant and preservative, (13 mL).
1a EA Reagent: Contains 0.22 g/L Enzyme acceptor, 25.5 mg/L monoclonal anti-tobramycin antibody, buffer salts, carrier protein, stabilizer and preservative.
2 **ED Reconstitution Buffer:** Contains 3-(N-morpholino) propanesulfonic acid, buffer salts, surfactant and preservative, (11 mL).
2a ED Reagent: Contains 23.4 µg/L Enzyme donor conjugated to tobramycin, 2.4 g/L chlorophenol red-β-D-galactopyranoside, 3.3 g/L goat anti-mouse antibodies, buffer salts, stabilizer and preservative.

Additonal Materials Required (sold separately):

REF	Kit Description
100017	CEDIA Antibiotic TDM Multi-Cal

Commercial Control(s) - Consult Customer Technical Support for recommendations

⚠ Precautions and Warnings
DANGER: The reagents contain ≤35% IgG Antisera (Goat), ≤21% Bovine serum albumin (BSA), ≤5% Sodium phosphate, monobasic, 5% Sodium phosphate, dibasic, anhydrous, ≤0.6% Sodium azide, and ≤0.2% Drug-specific antibody. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested. 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.

H315 - Causes skin irritation.
H317 - May cause 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. 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 skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Reagent Preparation and Storage
For preparation of the solutions for Hitachi analyzers, refer below. For all other analyzers, refer to the analyzer specific application sheet.

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 2a (ED Reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 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 directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 30 minutes before use.
R1 Enzyme acceptor solution: Connect Bottle 1a (EA Reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a 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 directly into the reagent compartment of the analyzer or into refrigerated storage and let stand 30 minutes before use.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.
NOTE 2: Avoid cross-contamination of reagents by matching reagent stoppers to the proper reagent bottle. The R2 Solution (Enzyme Donor) should be yellow-orange in color. A red or purple-red color indicates that the reagent has been contaminated and must be discarded.
NOTE 3: The R1 and R2 Solutions must be at the reagent compartment storage temperature of the analyzer before performing the assay. Refer to the analyzer specific application sheet for additional information.
NOTE 4: To ensure reconstituted EA solution stability, protect from prolonged, continuous exposure to bright light.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

R1 Solution: 30 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 30 days refrigerated on analyzer or at 2-8°C.

Specimen Collection and Handling
Serum or plasma (Na or Li heparin; Na EDTA) samples are suitable for use in the assay. Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the sample from the time it is collected until the time it is assayed. Centrifuge specimens containing particulate matter. 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. Handle all patient samples as if they were potentially infectious.

Assay Procedure
Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instrument parameters are available from Microgenics, a part of Thermo Fisher Scientific.

NOTE: If the bar code is not read by the analyzer, the numerical sequence on the bar code label can be entered manually via the keyboard.

Quality Control and Calibration[4]
2-Point calibration is recommended
- after reagent bottle change
- after reagent lot change
- as required following quality control procedures

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters. Contact Customer Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

CEDIA Tobramycin II Assay is designed to quantitate patient samples between 0.24 µg/mL and the value of the Antibiotic TDM High Calibrator (12 µg/mL). Specimen results below 0.24 µg/mL should be reported as < 0.24 µg/mL. Specimen results greater than 12 µg/mL can be reported as > 12 µg/mL or diluted one part sample with one part Antibiotic TDM Low Calibrator and reassayed. The value obtained on reassay should be derived as follows:

Actual value = (2 x diluted value) - conc. of Antibiotic TDM Low Calibrator

Use the following conversion factor to convert µg/mL to µmol/L:

µg/mL x 2.14 = µmol/L
µmol/L x 0.47 = µg/mL

The therapeutic efficacy and toxic effects are closely related to the serum drug concentration. In most adults, a peak therapeutic response is achieved with tobramycin concentrations between 6-10 µg/mL and trough concentrations between 0.5-2.0 µg/mL.[5] Different tobramycin therapeutic ranges have also been reported by other investigators.[6,7]

Limitations

1. The incidence of patients having antibodies to E. coli β-galactosidase is extremely low. However, some samples containing such antibodies can result in artificially high results that do not fit the clinical profile.
2. Kanamycin A (> 10%), kanamycin B (> 100%), and dideoxykanamycin (> 100%) show significant interference with the CEDIA Tobramycin II Assay.
3. 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.

Specific Performance Characteristics

Typical performance data obtained on the Hitachi 704 analyzer are shown below.[8] The results obtained in your laboratory may differ from these data.

Precision

Control sera and pooled human serum were analyzed for precision on a Hitachi analyzer using modified NCCLS replication experiment guidelines. The following results were obtained:

	Within-run Precision			Total Precision		
n	20	20	20	53	53	53
$\bar{x}$ (µg/mL)	1.80	5.06	9.12	1.95	5.32	9.54
SD (µg/mL)	0.09	0.10	0.12	0.12	0.19	0.30
CV %	5.0	2.0	1.3	6.2	3.6	3.1

Method Comparison

A comparison using the CEDIA Tobramycin II Assay (y) with a commercial fluorescence polarization immunoassay assay (x) gave the following correlation (µg/mL):

Linear regression
y = -0.01 + 0.989x
r = 0.993

Number of samples measured: 102

Linearity

A high sample was diluted with the Antibiotic TDM Low Calibrator. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/mL)	Assayed Value (µg/mL)	% Recovery
100	-	11.7	-
75	8.8	9.1	104
50	5.9	6.2	106
25	2.9	3.0	103

Recovery

Tobramycin in the form of a high (spiked) patient sample was added to a low patient sample. The percent recovery was then determined by dividing the assayed value by the expected value.

% High Sample	Expected Value (µg/mL)	Assayed Value (µg/mL)	% Recovery
100	-	10.1	-
75	7.6	7.7	101
50	5.1	5.1	101
25	2.6	2.5	96

Specificity

The CEDIA Tobramycin II Assay is very specific, having very low cross-reactivity to substances of similar structure, or co-administered drugs. Cross-reactivity is clinically insignificant (< 0.3%) for the following compounds.

Compound	Compound	Compound
5-Flurocytosine	Ethacrynic Acid	Rifampin
Amikacin	Furosemide	Sisomicin
Amphotericin	Fusidic Acid	Spectinomycin
Ampicillin	Gentamicin	Streptomycin
Carbenicillin	Lincomycin	Sulfadiazine
Cefamandole Nafate	Methicillin	Sulfamethoxazole
Cephalexin	Methotrexate	Sulfanilamide
Cephaloglycin	Methylprednisolone	Sulthiame
Cephalosporin C	Neomycin	Tetracycline
Cephaloridine	Netilmicin	Ticarcillin
Cephalothin	Oxytetracycline	Trimethoprim
Chloramphenicol	Penicillin G	Vancomycin
Clindamycin	Penicillin V	
Erythromycin	Prednisolone	

No interference was found in CEDIA Tobramycin Assay with:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 60 mg/dL	IgM	≤ 900 mg/dL
Hemoglobin	≤ 1000 mg/dL	Rheumatoid factor	≤ 1200 IU/mL
IgA	≤ 2700 mg/dL	Total Protein	≤ 13.2 g/dL
IgG	≤ 4900 mg/dL	Triglyceride	≤ 1000 mg/dL

Sensitivity

The minimum detectable concentration of the CEDIA Tobramycin II Assay is 0.24 µg/mL. (0.51 µmol/L)

References

1. Le Goffic F, Capmav ML, Tangy F, Baillarge M. Mechanism of Action of Aminoglycoside Antibiotics, Binding Studies of Tobramycin and Its 6′-N-acetyl derivatives to the Bacterial Ribosome and its Subunits. Eur. J. Biochem. 1979;102, 73-81.
2. Sande MA, Mandell GL. Antimicrobial Agents, The Aminoglycosides. In: Gilman, A.G. Goodman, L.S. and Gilman, A. eds. The Pharmacological Basis of Therapeutics. New York: MacMillan Publishing Company, 1980:1162-1180.
3. Henderson DR, Friedman SF, Harris JD, Manning WB, Zoccoli MA. CEDIA, A New Homogeneous Immunoassay System. Clin. Chem. 1986; 32(9): 1637-1641.
4. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
5. Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals in Man. 1990, 3rd edition, pp. 805-807.
6. Dipersio JR. Gentamicin and Other Aminoglycosides. In: Pesce AJ, Kaplan LA, eds. Methods in Clinical Chemistry, C.V. Mosby Co., 1987
7. Lew M. Interpretation of Aminoglycoside Serum Levels. Hosp. Pharm. 1979; 14: 465-472.
8. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Glossary:

http://www.thermofisher.com/symbols-glossary

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