CEDIA™ Theophylline II Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100008 (17 mL, 17 mL Kit)

Intended Use

The CEDIA™ Theophylline II Assay is an in vitro diagnostic medical device intended for the quantitation of theophylline in human serum or plasma. Measurements are used in the diagnosis and treatment of theophylline overdose and in monitoring levels of theophylline to ensure proper therapy.

Summary and Explanation of the Test

Theophylline is a methylxanthine derivative which is widely used in the treatment of asthma, obstructive lung disease and neonatal apnea.[1]

The effect of theophylline is closely correlated with concentration of the drug in serum; the therapeutic range for theophylline is 10 to 20 µg/mL in adults[2] and 5 to 10 µg/mL in newborns for treatment of apnea.[3] Toxic effects of theophylline usually occur at concentrations above 20 µg/mL in adults,[4-7] although mild symptoms can occur above 15 µg/mL. These effects include anorexia, nausea, vomiting, headaches and nervousness. Severe side effects such as increased cardiac rate, arrhythmia, cerebral seizures, and respiratory or cardiac arrest usually occur at concentrations above 40 µg/mL, but may also occur at lower concentrations.

Monitoring of theophylline concentrations in serum is essential, since individuals can vary in their rates of theophylline clearance.[8,9] Theophylline elimination is slowed in obese patients, patients with hepatic disease, and in those on a high carbohydrate, low protein diet. Premature infants have very low rates of theophylline elimination.[4] Conversely, theophylline elimination is more rapid among cigarette smokers.[2] In combination with other clinical data, monitoring serum theophylline levels may provide the physician with useful information to aid in adjusting patient dosage to achieve optimal therapeutic effect while avoiding drug toxicity.

The CEDIA Theophylline II Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[10]

The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. 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 analyte present in the sample.[10]

Reagents

1 **EA Reconstitution Buffer:** Contains MOPS (3-(N-morpholino) propanesulfonic acid buffer), 150 mg/L monoclonal anti-theophylline antibody, stabilizer, preservative.
1a **EA Reagent:** Contains 0.171 g/L Enzyme acceptor, buffer salts, and preservative.
2 **ED Reconstitution Buffer:** Contains MES (2-(N-morpholino) ethanesulfonic acid buffer), stabilizers, preservative.
2a **ED Reagent:** Contains 0.06 mg/L Enzyme donor conjugated to theophylline, 1.637g/L chlorophenol red-β-D-galactopyranoside, preservative.

Additional Materials Required (sold separately):

REF	Kit Description
100007	CEDIA Core TDM Multi-Cal

Commercial Control(s) —
Consult Customer Technical Support for recommendations on suitable control material.

⚠ Precautions and Warnings

DANGER: Powder reagent contains ≤56% w/w bovine serum albumin (BSA), and ≤2% w/w sodium azide. Liquid reagent contains ≤1.0% bovine serum, ≤0.3% sodium azide and ≤0.1% Drug-specific antibody (Mouse).
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust/mist/vapors/spray. 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 room temperature (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 (2-8°C) and let stand 5 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 (2-8°C) and let stand 5 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 caps to the proper reagent bottle. The R2 Solution 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: 60 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 60 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, 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[11]

2-Point calibration is recommended
- after reagent bottle change
- after reagent lot change
- as required following quality control procedures

Calibration verification: Not necessary.

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 and recommendations on suitable control material. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

The CEDIA Theophylline II Assay is designed to quantitate patient samples between 0.8 µg/mL and 40 µg/mL.

Specimens quantitating greater than 40 µg/mL can be reported as greater than 40 µg/mL or diluted one part sample with one part Low Calibrator and reassayed. The value obtained on reassay should be derived as follows:

Actual Value = (2 x diluted value) – concentration of Low Calibrator

Specimens giving values below the minimum detectable concentration of the assay should be reported as < 0.8 µg/mL.

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

µg/mL x 5.55 = µmol/L
µmol/L x 0.18 = µg/mL

For therapeutic and toxic levels of theophylline, the following published data may be used as a reference:

Investigator	Therapeutic Range (µg/mL)	Toxic Range (µg/mL)
Mitenko and Ogilvie[12]	5-20	
Buelow et al.[13]	8-20	
Hendeles and Weinberger[2]	10-20	
Weinberger and Bronsky[14]	8-20	
Aranda et al.[3]	5-15	
Ogilvie[4]		> 20
Jacobs et al.[5]		> 20

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. Due to cross-reactivity with 1,3-Dimethyluric Acid, the CEDIA Theophylline II Assay should not be used to quantitate samples from uremic patients.[15-19]
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 911 analyzer are shown below.[20] The results obtained in your laboratory may differ from these data. For additional analyzer specific performance data, refer to the analyzer specific application protocol.

Precision

Measured precision studies using packaged reagents and control sera yielded the following results in µg/mL using NCCLS modified replication experiment guidelines.

	Within-run Precision			Total Precision		
n	120	120	120	120	120	120
$\bar{x}$ (µg/mL)	5.1	15.1	29.3	5.1	15.1	29.3
SD (µg/mL)	0.2	0.3	0.4	0.26	0.36	0.59
CV%	3.3	1.9	1.3	5.1	2.4	2.0

Method Comparison

A comparison using the CEDIA Theophylline II Assay (y) with a commercially available fluorescence polarization immunoassay (x) gave the following correlation (µg/mL):

Deming's regression	Linear regression
y = 1.01x – 0.41	y = 1.01x – 0.38
r = 0.997	r = 0.997
Sy.x = 0.47	Sy.x = 0.67

Number of samples measured: 125
The sample concentrations were between 0.9 and 37.4 µg/mL.

Linearity

A high sample was diluted with the 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
100.0	-	46.9	-
90.0	42.2	42.1	100
80.0	37.5	38.3	102
70.0	32.8	33.6	102
60.0	28.1	29.5	105
50.0	23.5	24.9	106
40.0	18.8	19.9	106
30.0	14.1	14.5	103
20.0	9.4	9.6	102
10.0	4.7	4.4	93.6
0.0	-	0.0	-

Recovery

A high theophylline sample (theophylline added to a low theophylline sample, spiked to within 10% of the assay range) was diluted with an analyte-free 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
100.0	-	46.3	-
90.0	41.7	42.3	101
80.0	37.1	38.2	103
70.0	32.5	34.3	106
60.0	27.9	29.8	107
50.0	23.2	25.1	108
40.0	18.6	20.0	108
30.0	14.0	14.8	106
20.0	9.4	10.0	106
10.0	4.8	4.7	100
0.0	-	0.1	-

Specificity

The following compounds were tested for cross-reactivity in the assay.

Compound	Concentration Tested (µg/mL)	% Cross-Reactivity
1,3,7-Trimethyluric Acid	1000	0.5
1,3-Dimethyluric Acid	200	9.8
1,7-Dimethyluric Acid	1000	0.1
1,7-Dimethylxanthine	1000	1.8
1-Methyluric Acid	1000	0.1
1-Methylxanthine	1000	1.3
3,7-Dimethyluric Acid	1000	0.5
3-Methyluric Acid	1000	0.2
3-Methylxanthine	1430	1.4
7-(2-hydroxyethyl) theophylline	1430	1.4
7-(b-hydroxypropyl) theophylline	1818	1.1
7-Methyluric Acid	1000	< 0.08
7-Methylxanthine	1000	0.2
8-Chlorotheophylline	360	5.6
Allopurinol	1000	0.1
Ampicillin	2000	< 0.08
Caffeine	645	3.1
Clindamycin	2000	< 0.08
Diprophylline	2000	0.7
Heparin	2000	< 0.08
Hypoxanthine	1000	< 0.08
Phenobarbital	2000	< 0.08
Prednisone	2000	< 0.08
Pseudoephedrine	2000	< 0.08
Sulthiame	1000	< 0.08
Terbutaline	2000	< 0.08
Theobromine	800	2.5
Urea	2000	< 0.08
Uric Acid	1000	< 0.08
Xanthine	1000	< 0.08
Xanthosine	1000	< 0.8

No interference was found in CEDIA Theophylline II Assay with:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 66 mg/dL	Triglycerides	≤ 1.0 g/dL
Hemoglobin	≤ 1000 mg/dL	Total protein	≤ 12.9 g/dL

Sensitivity
The minimum detectable concentration of the CEDIA Theophylline II Assay is 0.8 µg/mL (4.4 µmol/L). This value was determined by calculating the concentration of theophylline which would give a response equal to two standard deviations above that of the Low Calibrator.

References

1. Rall TW, The xanthines. In: Gilman, A.G., Goodman, L.S. and Gilman, A. eds: The Pharmacological Basis of Therapeutics. New York: MacMillan Publishing Company, 1980: 592-607.
2. Hendeles L, Weinberger MM: Theophylline therapeutic use and serum concentration monitoring. In: Taylor WJ, Finn AL, eds: Individualizing Drug Therapy: Practical Applications of Drug Monitoring. New York: Gross Townsend Frank, Inc., 1981: vol 1, pp 31-66.
3. Aranda JV,Sitar DS, Parsons WD, Loughnan PM, Neims AH: Pharmacokinetic aspects of theophylline in premature newborns. New England Journal of Medicine 1976; 295: 413-416.
4. Ogilvie RI: Clinical pharmacokinetics of theophylline. Clinical Pharmacokinetics 1978; 3:267-293.
5. Jacobs MH, Senior RM, Kessler G: Clinical experience with theophylline. Relationships between dosage, serum concentration, and toxicity. Journal of the American Medical Association 1976;235: 1983-1986.
6. Weinberger MW, Matthay RA, Ginchansky EJ, Chidsey CA, Petty TL: Intravenous aminophylline dosage. Use of serum theophylline measurement for guidance. Journal of the American Medical Association 1976;235: 2110-2113.
7. Zwillich CW, Sutton FD, Neff TA, Cohn WM, Matthay RA, Weinberger MM: Theophylline-induced seizures in adults. Correlation with serum concentrations. Annals of Internal Medicine 1975;82: 784-787.
8. Piafsky KM, Ogilvie RI: Drug therapy. Dosage of theophylline in bronchial asthma. New England Journal of Medicine 1975; 292:1218-1222.
9. Leung P, Kalisker A, Bell TD: Variation in theophylline clearance rate with time in chronic childhood asthma. Journal of Allergy and Clinical Immunology 1977;59: 440-444.
10. Henderson DR, Friedman SB, Harris JD, Manning WB, Zoccoli MA: CEDIA, a New Homogeneous Immunoassay System. Clin Chem 1986;32(9): 1637-1641.
11. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
12. Mitenko, P.A.and Ogilvie, R.I.: Rational intravenous doses of theophylline. New England Journal of Medicine 1973b;289: 600-603.
13. Buelow KB, Larsson H, Leideman T: Plasma theophylline level and ventilatory function in chronic obstructive pulmonary disease during prolonged oral treatment with choline theophyllinate. European Journal of Clinical Pharmacology 1975;8: 119-123.
14. Weinberger MM, Bronsky EA: Evaluation of oral bronchodilator therapy in asthmatic children. Journal of Pediatrics 1974; 84:421-427.
15. Breiner R, McComb Lewis S, Wong, SHY, Marzouk N, Kapke GF: Positive interference with immunoassay of theophylline in serum of uremics, letters to the editor. Clin. Chem. 1985;31:1575-1577.
16. Nelson KM, Mathews SE, Bowers LD: Theophylline concentrations may be falsely high in serum or uremic patients (letter to the editors). Clin Chem 1983;29: 2125-2126.
17. Nicot G, Charmes JP, Lachatre G, Sautereau D, Valette JP, Eichler E, Leroux-Robert C: Theophylline toxicity risks and chronic renal failure. Int J Clin Pharmacol 1989; 27:398-401.
18. Opein KE, Ainardi V, Raisys VA, Smith CM, Messenfer LJ: Increase in apparent theophylline concentration in the serum of two uremic patients as measured by some immunoassay methods (caused by 1,3-dimethyluric acid?) (letters to the ediitors). Clin Chem 1983;29:1698-1699.
19. Patel JA, Clayton LT, LeBel CP, McClatchey KD: Abnormal theophylline levels in plasma by fluorescence polarization immunoassay in patients with renal disease. Ther. Drug Monit. 1984;6:458-460.
20. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

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

Microgenics Corporation
46500 Kato Road
Fremont, CA 94538 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
B·R·A·H·M·S GmbH
Neuendorfstrasse 25
16761 Hennigsdorf, Germany

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