CEDIA™ Carbamazepine II Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100006 (17 mL, 17 mL Kit)

Intended Use
The CEDIA™ Carbamazepine II Assay is an in-vitro diagnostic medical device intended for quantitation of carbamazepine in human serum or plasma.

Summary and Explanation of the Test
Carbamazepine is an anticonvulsant drug, used in particular for the treatment of trigeminal neuralgia,[1] all forms of partial epilepsy, generalized tonic-clonic seizures, and simple and complex partial seizures.[2-4] Effective serum concentration of carbamazepine is essential for seizure control. However, serum carbamazepine concentrations show only a moderate correlation to dose,[5,6] due to individual differences in absorption, metabolism and clearance. Moreover, co-administration of other antiepileptic agents can significantly increase serum carbamazepine levels.[6]

Toxicity of carbamazepine associated with therapy may or may not be dose related.[2-4] However, the central nervous system symptoms of vertigo, dizziness, and diplopia are dose-related with chronic therapy. In conjunction with other clinical information, monitoring carbamazepine levels will provide physicians with an effective tool to aid in adjusting dosage and achieving optimal therapeutic effect while avoiding both subtherapeutic and toxic drug levels.

The CEDIA Carbamazepine II Assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[7]

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 drug present in the sample.

Reagents
- **1 EA Reconstitution Buffer:** Contains 3-(N-morpholino) propane sulfonic acid, 49 mg/L monoclonal anti-carbamazepine antibody, stabilizer and preservative.
- **1a EA Reagent:** Contains 0.171 g/L Enzyme acceptor, stabilizer, buffer salts and preservative.
- **2 ED Reconstitution Buffer:** Contains 2-(N-morpholino) ethane sulfonic acid and preservative.
- **2a ED Reagents:** Contains 22.1 µg/L Enzyme donor conjugated to carbamazepine, 1.64 g/L chlorophenol red-β-D-galactopyranoside, buffer salts, stabilizer and preservative.

Additional Materials Required (sold separately):

REF	Kit Description
100007	CEDIA Core TDM Multi-Cal

Commercial Controls - Consult Customer Technical Support for recommendations

⚠ 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
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 caps 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: 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 CEDIA Carbamazepine II assay. Some gel separation tubes may not be suitable for use with therapeutic drug monitoring assays; refer to information provided by the tube manufacturer. Samples can be stored at 2-8°C for 1 week or 4 weeks at -20°C. Avoid repeated freezing and thawing. Centrifuge samples containing precipitate before performing the assay. Consistency of sample collection timing after administration of the last drug dose will improve the safety and efficacy of the anticonvulsant.

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.

Quality Control and Calibration[8]
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 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

CEDIA Carbamazepine II Assay is designed to quantitate patient samples between 0.5 µg/mL and the value of the Core TDM Multi-Cal High Calibrator (approximately 20 µg/mL). Specimens giving values below 0.5 µg/mL should be reported as < 0.5 µg/mL. Specimens quantitating greater than 20 µg/mL can be reported as > 20 µg/mL or diluted one part sample with one part Core TDM Multi-Cal Low Calibrator and reassayed. The value obtained on reassay should be derived as follows:

Actual Value = (2 x diluted value) - Concentration of Core TDM Multi-Cal Low Calibrator

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

µg/mL x 4.23 = µmol/L

µmol/L x 0.236 = µg/mL

The therapeutic efficacy and toxic effects are closely related to the serum drug concentration. The effective serum carbamazepine concentrations for seizure control have been reported as 8-12 µg/mL[4,9] for adults receiving carbamazepine as the sole antiepileptic agent. Other therapeutic ranges for carbamazepine have also been suggested.[5,10-13] Furthermore, lower concentrations may provide effective therapeutic response when other anticonvulsant agents are used in combination with carbamazepine. The therapeutic ranges are provided only as a guide for interpretation along with other clinical symptoms, and are not to be taken as the sole indicator for adjustment of dosage.

Limitations

1. The CEDIA Carbamazepine II Assay performance has not been established with body fluids other than human serum and plasma.
2. 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. If this occurs, contact Customer Technical Support for assistance.
3. This assay was validated on analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local representative.

Specific Performance Characteristics

Typical performance data obtained on the Hitachi 911 analyzer are shown below.[14] The results obtained in your laboratory may differ from these data.

Precision

Measured precision studies using pooled human serum and control sera yielded the following results using NCCLS modified replication experiment guidelines.

	Within-run precision			Total precision		
N	126	126	126	126	126	126
$\bar{x}$ (µg/mL)	4.2	10.6	16.8	4.2	10.6	16.8
SD (µg/mL)	0.06	0.08	0.12	0.15	0.21	0.29
CV%	1.5	0.8	0.7	3.5	2.0	1.7

Method Comparison

A comparison using the CEDIA Carbamazepine II Assay (y) with a commercially available fluorescence polarization immunoassay (x):

Linear regression

y = 1.01x + 0.17
r = 0.992

Number of samples measured: 100
The sample concentrations were between 1.9 and 19.3 µg/mL.

Linearity

A high sample was diluted with the Core TDM Multi-Cal 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	21.42	20.99	-
90	19.29	19.01	98.5
80	17.15	17.00	99.1
70	15.01	14.99	99.9
60	12.88	12.94	100.5
50	10.74	10.66	99.3
40	8.61	8.68	100.8
30	6.47	6.47	100.0
20	4.33	4.29	99.1
10	2.20	2.21	100.5
0	0.06	0.20	-

Recovery

Carbamazepine 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.

% Expected High Sample	Assayed Value (µg/mL)	% Value (µg/mL)	Recovery
100	21.74	21.03	-
90	19.58	19.21	98.1
80	17.41	17.13	98.4
70	15.25	14.92	97.8
60	13.09	13.34	101.9
50	10.93	11.03	100.9
40	8.77	8.91	101.6
30	6.61	6.57	99.4
20	4.44	4.46	100.5
10	2.28	2.15	94.3
0	0.12	0.13	-

Specificity

The following compounds were tested for cross-reactivity with the CEDIA Carbamazepine II Assay.

Compound	Concentration tested (µg/mL)	% Crossreactivity
Amitriptyline	100	18.6
Carbamazepine-10, 11-epoxide	250	7.4
Diazepam	250	4.8
Imipramine	200	5.6
Methsuximide	1000	1.0
Nortriptyline	50	17.2
Phenothiazine	200	8.6
Probenecid	500	2.0

Cross-reactivity is less than 1.0% for the following compounds:

Compound	Compound
2-Phenyl-2-ethylmalonamide	5-(p-Hydroxyphenyl)-phenylhydantoin
Amobarbital	p-Hydroxyphenobarbital
Chlorazepate	Mephenytoin
Chlordiazepoxide	Phenytoin
Ethosuximide	Primidone
Ethotoin	Promethazine
Glutethimide	Secobarbital
	Valproic Acid

No interference was found in the CEDIA Carbamazepine II Assay:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 60 mg/dL	Total protein	≤ 12 g/dL
Hemoglobin	≤ 1.0 g/dL	Triglyceride	≤ 1 g/dL
Rheumatoid factor	≤ 180 IU/mL		

Sensitivity

The minimum detectable concentration of the CEDIA Carbamazepine II Assay is 0.5 µg/mL (2.1 µmol/L). This value was determined by calculating the concentration of carbamazepine which would give a response equal to two standard deviations above that of the Core TDM Multi-Cal Low Calibrator.

References

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14. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

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

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