CEDIA™ Valproic Acid II Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 100013 (13 mL, 11 mL Kit)

Intended Use
The CEDIA™ Valproic Acid II Assay is an in-vitro diagnostic medical device intended for the quantitation of valproic acid in human serum or plasma.

Summary and Explanation of the Test
Valproic acid (VPA; 2-propylpentanoic acid; Depakene®) is an anticonvulsant medication that is chiefly used for the treatment of primary and secondary generalized seizures, but is also effective against absence seizures.[1-5]

At therapeutic concentrations, over 90% of VPA in the circulation is bound to plasma proteins; primarily albumin.[6]

VPA has the fewest adverse effects of all the widely-used anti-epileptic agents.[7,8] The most common side effects are gastrointestinal disturbances such as nausea and vomiting.

Pharmacokinetics of VPA are highly variable, depending on the form of the drug and route of administration, as well as individual variations in volume of distribution, metabolism and clearance.[9,10] Monitoring VPA concentrations during therapy is essential in order to provide the physician with an indicator for adjusting dosage.

The CEDIA Valproic Acid II assay uses recombinant DNA technology (US Patent No, 4708929) to produce a unique homogeneous enzyme immunoassay system.[11]

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.

Reagents
- **1 EA Reconstitution Buffer:** Contains N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], stabilizer and preservative, (13 mL).
- **1a EA Reagent:** Contains 0.25 g/L Enzyme acceptor, 24 mg/L monoclonal anti-VPA antibody, BSA, Sodium Salicylate, buffer salts, and preservative.
- **2 ED Reconstitution Buffer:** Contains N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], stabilizers and preservative, (11 mL).
- **2a ED Reagent:** Contains 40 µg/L Enzyme donor conjugated to VPA, 2.4 g/L chlorophenol red-β-D-galactopyranoside, 27 ml/L goat anti-mouse antibodies, buffer salts, stabilizer, and preservative.

Additional Materials Required (sold separately):

REF	Kit Description
100007	CEDIA Core TDM Multi

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

⚠ Precautions and Warnings
DANGER: Powder reagent contains ≤42% w/w bovine serum albumin (BSA), ≤37% w/w potassium phosphate, dibasic, ≤3% w/w salicylic acid, ≤0.9% w/w sodium azide and ≤0.6% w/w antibody (Mouse). Liquid reagent contains ≤0.13% w/w sodium azide.

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 dust/mist/vapors/spray. 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 was before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

The reagents contain sodium azide. 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.

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 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: 45 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 45 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 enzymtic 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[12]
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 Valproic Acid assay is designed to quantitate patient samples between 3 µg/mL and the value of the Core TDM Multi-Cal High Calibrator (approximately 150 µg/mL). Specimen results below 3 µg/mL should be reported as < 3 µg/mL. Specimen results greater than 150 µg/mL can be reported as > 150 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) - conc. of Core TDM Multi-Cal Low Calibrator

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

µg/mL x 6.93 = µmol/L
µmol/L x 0.144 = µg/mL

The therapeutic efficacy and toxic effects are closely related to the serum drug concentration. The effective serum VPA concentraiton for seizure control has been reported as 50-100 µg/mL.[13-15] However, a number of factors may complicate interpretation of serum VPA levels,[3] including the time interval between drug administrations, the type of seizures, albumin concentration, and the presence of other anti-epileptic drugs. Therefore, adjustments to drug dosage should be based on both clinical and laboratory data.

Limitations

1. 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 procainamide results that do not fit the clinical profile.
2. 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.[16] The results obtained in your laboratory may differ from these data.

Precision

Control sera and pooled human sera 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	120	120	119	120	120	119
$\bar{x}$ (µg/mL)	24.4	95.0	136.8	24.4	95.0	136.8
SD (µg/mL)	0.59	1.43	1.81	0.83	1.93	2.48
CV %	2.4	1.5	1.3	3.4	2.0	1.8

Method Comparison

A comparison using the CEDIA VPA assay (y) with a commercial fluorescence polarization immunoassay (x) gave the following correlation:

Linear regression
y = 1.056x + 0.023
r = 0.995

Number of samples measured: 113

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
100.0	130.1	-	-
83.3	108.3	112.7	104
66.7	86.7	87.5	101
50.0	65.0	67.4	104
33.3	43.3	43.3	100
16.7	21.7	20.9	96
0.0	0	-	-

Recovery

Valproic acid in the form of 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
100.0	141.1	-	-
83.3	117.7	116.0	99
66.7	94.2	93.0	99
50.0	70.7	67.3	95
33.3	47.1	44.2	94
16.7	23.6	21.6	92
0.0	0.1	-	-

Specificity

The following compounds have been tested for cross-reactivity with the CEDIA Valproic Acid II assay.

Compound	% Crossreactivity
3-Hydroxy-2-propylpentanoic acid	4.4
4-Hydroxy-2-propylpentanoic acid	4.4
5-Hydroxy-2-propylpentanoic acid	5.8
3-Oxo-2-propylpentanoic acid	3.8
2-Phenyl-2-ethylmalonamide (PEMA)	< 0.16
2-Propyl-2,3'-pentadienoic acid	14.2
2-Propyl-2-pentanoic acid	1.0
2-Propyl-4-pentanoic acid	22.3
2-Propylglutaric acid	< 0.4
2-Propylsuccinic acid	< 0.9
Carbamazepine	< 0.04
Carbamazepine-10,11-epoxide	< 0.9
Clonazepam	< 0.3
Diazepam	< 0.3
Phenobarbital	< 0.06
Phenytoin	< 0.04
Primidone	< 1.0
Salicylic acid	< 0.004

No interference was found in CEDIA VPA assay with:

Substance	Concentration	Substance	Concentration
Bilirubin	≤ 60 mg/dL	IgM	≤ 840 mg/dL
Hemoglobin	≤ 1.0 g/dL	Rheumatoid factor	≤ 200 IU/mL
IgA	≤ 790 mg/dL	Total Protein	≤ 10 g/dL
IgG	≤ 4.3 g/dL	Triglyceride	≤ 1.0 g/dL

Sensitivity

The minimum detectable concentration of the CEDIA VPA assay is 3.0 µg/mL (20.8 µmol/L).

References

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

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