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 formulation information.

NOTE 4: To confirm sufficient 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 Phenobarbital II 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 adapt to analyzer within 24 hours of another collector. If the assay cannot be performed within 24 hours, or if the sample is to be shipped, keep it frozen. Store samples at -20°C and assay within 2 weeks. To protect the integrity of the sample do not induce foaming and avoid repeated freezing and thawing. Centrifuge specimens containing particulate matter.

Some therapeutic drug concentrations are reduced when sample is stored in separator tube for a prolonged period of time (> 2 hours).13

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. Two (2) point calibration is used with this assay. In the case of Hitachi analyzers, use CEDIA Core TDM Multi-Cal Low Calibrator as S1, and CEDIA Core TDM Multi-Cal High Calibrator as S2. For exact analyzer application parameter settings, refer to each instrument specific application sheet, which is available from Microgenics Customer Technical Support.

Hitachi 911 analyzer: If the barcode 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

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. NOTE: Reassess control targets and ranges following a change of reagent lot.

Calibration Frequency: Two point calibration is recommended:

* after reagent bottle change
* after reagent lot change
* as required following quality control procedures

Results and Expected Values

In most patients, a therapeutic response is achieved with phenobarbital concentrations in the 15-40 µg/mL (65-172 µmol/L) range.5 Some patients may require phenobarbital concentrations outside this range to obtain effective seizure control. Therefore, therapeutic range is provided only as a guide and individual patient results should be interpreted in conjunction with other clinical symptoms and individual clinical history.

The CEDIA Phenobarbital II Assay is designed to quantitate patient sample phenobarbital concentrations between 1.2 µg/mL and 80 µg/mL (CEDIA TDM Multi-Cal High Calibrator). Specimen results outside this range should be reported as either > 80 µg/mL or < 1.2 µg/mL (assay sensitivity limit).

Specimens quantitating greater than 80 µg/mL can also be diluted one part sample with one part Multi-Cal Low Calibrator and reassayed. The value obtained on reassay should be derived as:

Actual Value = (2 x diluted value) − Multi-Cal Low Calibrator value

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

µg/mL x 4.31 = µmol/L

µmol/L x 0.232 = µg/mL

Limitations

1. The CEDIA Phenobarbital II Assay performance has not been established with body fluids other than human serum and plasma (Na or Li heparin; Na EDTA).

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 Microgenics representative for an alternative procedure.

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.

Precautions and Warnings

1. For In Vitro Diagnostic Use Only.

2. The reagents contain less than 1% 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.

3. This product contains chemical(s) known to the State of California to cause cancer and/or birth defects or other reproductive harm.

4. This product was derived as:

   R1 Enzyme donor solution: Connect Bottle 1a (ED Reagent) to Bottle 1 (ED 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.

   R2 Enzyme acceptor solution: Connect Bottle 2a (EA Reagent) to Bottle 2 (EA 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.

   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 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 formulation information.

   NOTE 4: To confirm sufficient 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.

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   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 Microgenics representative for an alternative procedure.

   Specific Performance Characteristics

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

   Precision

   Measured precision studies using packaged reagents, pooled human serum and control sera yielded the following results in µg/mL with a Hitachi 911 analyzer following NCCLS modified replication experiment guidelines:

<table>
<thead>
<tr>
<th>Method</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
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   Precautions and Warnings

   1. For In Vitro Diagnostic Use Only.

   2. The reagents contain less than 1% 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.

   3. This product contains chemical(s) known to the State of California to cause cancer and/or birth defects or other reproductive harm.
A comparison using the CEDIA Phenobarbital II assay (y) with a commercial fluorescence polarization immunoassay (x) gave the following correlation (µg/mL):

Deming’s regression

\[ y = -2.01 + 1.08x \]

Linear regression

\[ y = -1.69 + 1.06x \]

To assess the recovery of the assay, phenobarbital was added to a low phenobarbital sample and then diluted with an analyte-free sample. The percent recovery was then determined by dividing the assayed value by the expected value.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (µg/mL)</th>
<th>Assayed Value (µg/mL)</th>
<th>% Recovery</th>
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<tbody>
<tr>
<td>Hemoglobin</td>
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<td></td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein</td>
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No interference was found in CEDIA Phenobarbital II Assay with:

- Amobarbital (> 20%) and Mepobarbital (> 100%) show significant interference with the CEDIA Phenobarbital II Assay.

Sensitivity

The minimum detectable concentration of the CEDIA Phenobarbital II Assay is 1.2 µg/mL (5.2 µmol/L). This value was determined by calculating the concentration of phenobarbital which would give a response equal to two standard deviations above that of the Core TDM Multi-Cal Low Calibrator.

Recovery

The following compounds have been tested for cross-reactivity in the CEDIA Phenobarbital II Assay.

- 1,3-dimethylbarbituric acid
- 2-Phenyl-2-ethylmalonamide
- 5-(p-Hydroxyphenyl)-5-phenylhydantoin
- Amitriptyline
- Aporobarbital
- Barbital
- Butalbarbital
- Carbamazepine –10, 11-epoxide
- Carbamazepine
- Chlorazepate
- Chlorpromazine
- Diazepam
- Ethothon
- Ethosuximide
- Glutethimide
- Imipramine
- Mephenytoin
- Methsuximide
- Phentobarbital
- Phenytoin
- p-Hydroxyphenobarbital
- Primidone
- Promethazine
- Secobarbital
- Sulthiame
- Valproic Acid

Cross-reactivity (%):< 0.12

Amobarbital (> 20%) and Mepobarbital (> 100%) show significant interference with the CEDIA Phenobarbital II Assay.

Method Comparison

To assess the linearity of the assay, a high sample was diluted with the Core TDM Multi-Cal Low Calibrator.

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