3 METHODOLOGY

The Emit® 2000 Valproic Acid Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of valproic acid (free and protein-bound) in human serum or plasma. In the performance of the Emit® 2000 Valproic Acid Assay, serum or plasma is mixed with Reagent 1, which contains antibodies to valproic acid and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently, Reagent 2, containing valproic acid labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH), is added. Valproic acid in the sample and valproic acid labeled G6PDH compete for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the valproic acid concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized NAD to NADH, resulting in an absorbance change that can be measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme functions only with the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

4 REAGENTS

Reagents contain the following substances:
- Mouse monoclonal antibodies reactive to valproic acid (2 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), bovine serum albumin, valproic acid labeled with glucose-6-phosphate dehydrogenase (0.39 U/mL), <0.1% sodium azide, Tris buffer, preservatives, and stabilizers.

Precautions
- For in vitro diagnostic use.
- Contains nonsterile mouse monoclonal antibodies and nonsterile bovine serum albumin.
- Reagent 2 contains sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush the drain with a large volume of water to prevent azide buildup.
- Reagents and calibrators contain materials that may cause sensitivity on contact with skin.
- Do not use the kit after the expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.
- Although the reagents contain a blocking agent for human anti-mouse antibody (HAMA), the HAMA in some patient samples may interfere with the method.

Preparation of Reagents
The Emit® 2000 Valproic Acid Assay reagents are provided ready to use; no preparation is necessary.

Storage of Assay Components
- Improper storage of reagents can affect assay performance.
- When not in use, store reagents upright at 2–8°C and with screw caps tightly closed.
- Unopened reagents are stable until the expiration date printed on the label if stored upright at 2–8°C (36–46°F).
- Do not freeze reagents or expose them to temperatures above 32°C.

5 SPECIMEN COLLECTION AND PREPARATION

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, citrate, oxalate/fluoride, and EDTA have been tested and may be used with this assay.
- Sample volume is instrument-dependent. Refer to the analyzer User’s Guide or appropriate Application Sheet.
- To obtain a serum valproic acid concentration that best represents the peak tissue level, draw the sample 1 to 3 hours after an oral dose is given. Collect a trough sample just before the next scheduled dose.†
- Serum or plasma samples may be refrigerated at 2–8°C. For transportation, maintain the sample temperature at 2–8°C. Samples may be frozen (−20°C) for 1 year.†
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, and biological variations affecting drug disposition.†
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious. It is recommended that human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens or other appropriate local practices.†,‡,§

6 PROCEDURE

Materials Provided
Emit® 2000 Valproic Acid Assay
Reagent 1
Reagent 2

Materials Required But Not Provided
Emit® 2000 Valproic Acid Calibrators
Multi-level commercial controls

Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.
Calibration
Recalibrate whenever a new lot of reagents is used or as indicated by control results (See Quality Control, below). If a new set of reagents with the same lot number is used, validate the system by assaying controls.

Quality Control
Each laboratory must establish and follow its own quality control procedures; however, Siemens Healthcare Diagnostics recommends that you at least perform quality control procedures as described below.

Temporary Control Limits. When establishing control limits for the first time, determine a mean control concentration for each control level by running three calibration curves and assaying three replicates of each of multilevel (2 or more) controls. Determine temporary control limits for each control level (± 20% of each mean control concentration). Use the temporary limits for at least 30 days (a minimum of 20 determinations for each level must be completed before establishing permanent control limits).

Permanent Control Limits. After 30 days (and a minimum of 20 determinations), recalculate the mean control concentrations, including all data that are within three standard deviations. Calculate the standard deviation for each control level and multiply by 2.25. Permanent control ranges should be ± 2.25 SD of the mean or ± 12% of the mean, whichever is greater. Establish new permanent control limits whenever a new lot of controls or reagents are used.

Daily Quality Control. Assay at least one control every eight hours, alternating the control levels tested. Ensure that a minimum of two controls is assayed in every 24-hour period. If controls are within their control limits, the calibration is verified. If any control is not within its control limits, rerun that control. If the result is then within the control limits, the calibration is verified. If the control is not within the control limits after repeat testing, recalibrate, and verify the calibration using two or more controls. If any control is not within its control limits after recalibration, check control, calibrators, and reagent handling, and then retest. If a control is still not within its control limits, call for technical assistance.

Diluting High Concentration Samples
To estimate valproic acid concentrations above the assay range, patient samples containing more than 150 µg/mL (1040 µmol/L) valproic acid may be diluted with 1 or 2 parts of distilled or deionized water or Emit® 2000 Valproic Acid Calibrator 0. After diluting the sample, test and multiply the results by the dilution factor.

Evaluation and Interpretation of Results
- This assay uses Math Model No. 1.
- Results are calculated by the analyzers; no additional manipulation of data is required unless samples have been manually diluted.
- Consult the appropriate instrument User’s Guide and Application Sheet for complete instructions.
- The concentration of valproic acid in serum or plasma depends on the time of the last drug dose; time of sample collection; disease states that affect drug clearance; age; concomitant drug therapy; and individual variations in absorption, distribution, and elimination. These parameters must be considered when interpreting results.1
- An increase of the biologically active free fraction of the drug, caused by saturation of the protein binding or disease states that alter protein binding, can influence the relationship between serum or plasma valproic acid concentration and clinical response. Although the total drug concentration is within the therapeutic range, the patient may exhibit toxic symptoms.1

7 LIMITATIONS OF THE PROCEDURE
When diluting patient samples containing high valproic acid concentrations, the following factors can affect the accuracy of the result: the use of the correct diluent (Emit® 2000 Valproic Acid Calibrator 0 or distilled or deionized water), the accuracy of the dilution, and the specificity of the assay to drug metabolites.

8 EXPECTED VALUES
The Emit® 2000 Valproic Acid Assay accurately quantitates valproic acid concentrations in human serum or plasma up to 150 µg/mL (1040 µmol/L). In most patients, valproic acid serum concentrations of 50–100 µg/mL (347–693 µmol/L) effectively control generalized and partial seizures. Seizure control may improve at levels greater than 100 µg/mL (693 µmol/L), but toxicity may occur at levels of 100–150 µg/mL (693–1040 µmol/L).2

Note: To convert from µg/mL to µmol/L valproic acid, multiply by 6.93.
For effective treatment, some patients may require serum levels outside this range. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms.

9 SPECIFIC PERFORMANCE CHARACTERISTICS
The information presented in this section is based on Emit® 2000 Valproic Acid Assay studies performed on the AU4000®/AU600® Clinical Chemistry System. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

Endogenous Substances
No clinically significant interference has been found in samples to which 800 mg/dL hemoglobin, 30 mg/dL bilirubin, or 750 mg/dL triglycerides were added to simulate hemolytic, icteric, or lipemic samples.

Precision
Within-run precision was calculated according to NCCLS Guideline EPS-A by assaying two replicates of each level of a tri-level control twice a day for 20 days (N=80). Total precision was also calculated from these data. Table 1 summarizes within-run precision results, and Table 2 summarizes total precision results.

Table 1 — Summary of Within-Run Precision

<table>
<thead>
<tr>
<th>Level</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/mL)</td>
<td>26.2</td>
<td>79.0</td>
<td>130.2</td>
</tr>
<tr>
<td>%CV</td>
<td>2.6</td>
<td>2.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table 2 — Summary of Total Precision

<table>
<thead>
<tr>
<th>Level</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/mL)</td>
<td>26.2</td>
<td>79.0</td>
<td>130.2</td>
</tr>
<tr>
<td>%CV</td>
<td>4.3</td>
<td>3.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Comparative Analysis
In this study, patient samples were analyzed on the SYVA®-30R Biochemical System and on the AU800 Clinical Chemistry System. Table 3 summarizes the results.

Table 3 — Summary of Comparative Analysis

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept</th>
<th>Mean (µg/mL)</th>
<th>Correlation Coefficient</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.01</td>
<td>-0.83</td>
<td>SYVA®-30R 70.9</td>
<td>0.98</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU800 70.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Specificity
The Emit® 2000 Valproic Acid Assay measures the total (protein-bound plus unbound) valproic acid concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested. The compounds listed in Table 4 do not interfere with the Emit® 2000 Valproic Acid Assay when tested in the presence of 50 µg/mL valproic acid. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 4 — Compounds That Do Not Interfere

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>1000</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>100</td>
</tr>
<tr>
<td>Diazepam</td>
<td>100</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>1000</td>
</tr>
<tr>
<td>2-N-Propyl-3-hydroxy-pentanoic acid</td>
<td>100</td>
</tr>
<tr>
<td>2-N-Propyl-4-hydroxy-pentanoic acid</td>
<td>100</td>
</tr>
<tr>
<td>2-N-Propyl-5-hydroxy-pentanoic acid</td>
<td>50</td>
</tr>
<tr>
<td>2-N-Propyl-3-oxo-pentanoic acid</td>
<td>100</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>750</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>1000</td>
</tr>
<tr>
<td>Primidone</td>
<td>1000</td>
</tr>
<tr>
<td>2-Propyl glutaric acid</td>
<td>400</td>
</tr>
<tr>
<td>2-Propyl-2-pentenoic acid</td>
<td>20</td>
</tr>
<tr>
<td>2-Propyl-4-pentenoic acid</td>
<td>10</td>
</tr>
<tr>
<td>2-Propyl succinic acid</td>
<td>500</td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity level of the Emit® 2000 Valproic Acid Assay is 3.98 µg/mL. This level represents the lowest measurable concentration of valproic acid that can be distinguished from 0 µg/mL with a confidence level of 95%.

Calibration Stability
Studies have shown calibration stability of at least 14 days. Calibration stability may vary from laboratory to laboratory depending on handling of reagents, maintenance of instruments, adherence to operating procedures, establishment of control limits, and verification of calibration.
REFERENCES


