The Emit® 2000 Carbamazepine Assay is a homogeneous enzyme immunoassay technique used to measure specific compounds in biological fluids.5,6 The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the enzyme employed in the assay.

### 4 REAGENTS

Reagents contain the following substances:

Mouse monoclonal antibodies reactive to carbamazepine (10 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), carbamazepine labeled with glucose-6-phosphate dehydrogenase (0.22 U/mL), 0.1% sodium azide, Tris buffer, preservatives, and stabilizers.

### Precautions

- For in vitro diagnostic use.
- Contains nonsterile mouse monoclonal antibodies.
- Assay components contain 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.
- Do not use the kit after the expiration date.
- This kit contains streptomycin sulfate. Please dispose of appropriately.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

### 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.
- 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, and EDTA have been tested and may be used with this assay. Some sample dilution may occur when samples are collected in tubes containing citrate anticoagulant. The amount of dilution and the possible need to correct for it should be considered when interpreting assay results for these samples.
- Store the serum or plasma refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C. Samples can be stored refrigerated at 2–8°C for up to one month or stored frozen for up to three months.
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.
- Measure the steady-state serum concentration representing the trough level just before the next scheduled dose.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.

### 6 PROCEDURE

**Materials Provided**

- Emit® 2000 Carbamazepine Assay
- Reagent 1
- Reagent 2

**Materials Required But Not Provided**

- Emit® 2000 Carbamazepine Calibrators
- Multi-level commercial controls

**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**

- Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.
- Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known carbamazepine concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
- Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.
Diluting High Concentration Samples
To estimate carbamazepine concentrations above the assay range, patient samples containing more than 20 µg/mL (85 µmol/L) carbamazepine may be diluted with one or two parts distilled or deionized water or Emit® 2000 Carbamazepine Calibrator 0. After diluting the sample, repeat the entire assay sequence and multiply the results by the dilution factor. Some analyzers dilute and retest high concentration samples automatically. See the analyzer User’s Guide or appropriate Application Sheet for instructions.

Evaluation and Interpretation of Results
- This assay uses Math Model No. 1.
- Results are automatically calculated; no additional manipulation of data is required.
- The factors that can influence the relationship between carbamazepine serum or plasma concentrations and clinical response include the type and severity of seizures, age, general state of health and use of other drugs.
- The concentration of carbamazepine in serum or plasma depends on the time of the last drug dose; mode of administration; concomitant drug therapy; sample condition; time of sample collection; and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.1,2
- Results of this test should always be interpreted in conjunction with the patient’s medical history, clinical presentation and other findings.

7 LIMITATIONS OF THE PROCEDURE
This assay has no specific limitations.

8 EXPECTED VALUES
The Emit® 2000 Carbamazepine Assay accurately quantitates carbamazepine concentrations in human serum or plasma containing 2.0–20 µg/mL (8.5–85 µmol/L) carbamazepine. The desired therapeutic effect is usually achieved in the serum concentration range of 4.0–12 µg/mL (17–51 µmol/L). Further, peak concentrations above 12 µg/mL (51 µmol/L) are often associated with toxicity.2,3

Note: To convert from µg/mL to µmol/L carbamazepine, multiply by 4.23.

For effective treatment, some patients may require serum levels outside these ranges. 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 (see Section 6, Procedure, Evaluation and Interpretation of Results).

9 SPECIFIC PERFORMANCE CHARACTERISTICS
The information presented in this section is based on Emit® 2000 Carbamazepine Assay studies performed on the AU4000i/AU600i 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, 750 mg/dL triglycerides, or 30 mg/dL bilirubin were added to simulate hemolytic, lipemic, or icteric samples.

Precision
Within-run precision was determined by assaying 20 replicates of each level of a tri-level control. Table 1 summarizes the data.

Table 1 — 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>4.72</td>
<td>10.49</td>
<td>15.33</td>
</tr>
<tr>
<td>%CV</td>
<td>2.9</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Total precision was calculated according to NCCLS guideline EPS-T2 using data collected from controls run in duplicate twice daily over 20 days. Table 2 summarizes the data.

Table 2 — 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>4.42</td>
<td>10.15</td>
<td>14.97</td>
</tr>
<tr>
<td>%CV</td>
<td>6.3</td>
<td>4.8</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Comparative Analysis
In this study, patient samples were analyzed on the TDx analyzer and on the AU600 Clinical Chemistry System. A summary of the results is as follows:

<table>
<thead>
<tr>
<th></th>
<th>Slope</th>
<th>Intercept</th>
<th>Mean</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDx</td>
<td>1.03</td>
<td>0.35</td>
<td>7.22</td>
<td>0.99</td>
</tr>
<tr>
<td>AU600</td>
<td>7.79</td>
<td>8.05</td>
<td>7.79</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Specificity
The Emit® 2000 Carbamazepine Assay measures the total (protein-bound plus unbound) carbamazepine 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 3 do not interfere with the Emit® 2000 Carbamazepine Assay when tested in the presence of 8.0 µg/mL carbamazepine. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 3 — Compounds That Do Not Interfere
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>5</td>
</tr>
<tr>
<td>Carbamazepine-10.11-epoxide</td>
<td>50</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>8</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>500</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>500</td>
</tr>
<tr>
<td>Primidone</td>
<td>200</td>
</tr>
<tr>
<td>Valproic Acid</td>
<td>1000</td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity level of the Emit® 2000 Carbamazepine Assay is 0.5 µg/mL. This level represents the lowest measurable concentration of carbamazepine that can be distinguished from 0 µg/mL with a confidence level of 95%.

Calibration Stability
Studies have shown calibration stability of more than two weeks. When proper reagent handling, instrument maintenance, and operating procedures are followed, the calibration should remain stable for at least two weeks.

10 REFERENCES