Phenytoin Assay

Methods historically used to monitor serum phenytoin concentrations include chromatographic assays and immunoassays.

4 REAGENTS

Reagents contain the following substances:

Mouse monoclonal antibodies reactive to phenytoin (53.4 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (10 mM), phenytoin labeled with glucose-6-phosphate dehydrogenase (0.24 U/mL), Tris buffer, 0.1% sodium azide, 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 it 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.

Preparation of Reagents

The Emit® 2000 Phenytoin 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.
• 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.
• Sample volume is instrument-dependent. Refer to the appropriate Application Sheet for specific volumes.
• 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. 1–3
• Draw a sample within two to four hours after an intravenous loading dose and, at steady state, collect a specimen representing the trough level just before the next scheduled dose. 1–3
• Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.

6 PROCEDURE

Materials Provided

Emit® 2000 Phenytoin Assay
Reagent 1
Reagent 2

Materials Required But Not Provided

Emit® 2000 Phenytoin 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.
• Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.
7 LIMITATIONS OF THE PROCEDURE

This assay has no specific limitations.

8 EXPECTED VALUES

The Emit® 2000 Phenytoin Assay accurately quantitates phenytoin concentrations in human serum or plasma containing 2.5–40 µg/mL (10–158 µmol/L) phenytoin. Most patients achieve a satisfactory therapeutic response in the serum concentration range of 10–20 µg/mL (40–79 µmol/L). Further, peak concentrations above 20 µg/mL (79 µmol/L) are often associated with toxicity.9,10 For patients being treated with fosphenytoin (Gerebrex®), it is important not to collect samples for phenytoin analysis until at least 2 hours after the completion of intravenous infusion, or 4 hours after intramuscular injection, when conversion of the prodrug to phenytoin can be expected to be essentially complete.9,11

Note: To convert from µg/mL to µmol/L phenytoin, multiply by 3.96.

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.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 Phenytoin Assay studies performed on the AU400®/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, 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 — Summary of Within-Run Precision

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/mL)</td>
<td>4.23</td>
<td>12.87</td>
</tr>
<tr>
<td>%CV</td>
<td>3.8</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Total precision was calculated according to NCCLS guideline EP5-T2 using data collected from controls run in duplicate twice daily over twenty (20) days. Table 2 summarizes the data.

Table 2 — Summary of Total Precision

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/mL)</td>
<td>4.03</td>
<td>11.71</td>
</tr>
<tr>
<td>%CV</td>
<td>6.0</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Sensitivity

The Emit® 2000 Phenytoin Assay is 0.5 µg/mL. This level represents the lowest measurable concentration of phenytoin 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.
REFERENCES


