### 1 INTENDED USE

The Emit® 2000 Digoxin Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of digoxin in human serum or plasma. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

### 2 SUMMARY

Monitoring serum digoxin concentrations, along with careful clinical assessment, is the most effective means of ensuring safe and effective therapy for several reasons:

- Studies have shown a relationship between serum digoxin concentrations and clinical signs of toxicity.
- Clinical manifestations of digoxin toxicity (cardiac disturbances, gastrointestinal problems, and central nervous system disorders) can mimic those of disease processes.
- Concomitant use of other drugs, particularly quinidine, can markedly alter serum digoxin concentrations.
- Digoxin has a narrow range of safe and effective concentrations in serum. Although the therapeutic and toxic concentrations overlap, measurement of digoxin levels helps to maintain effective concentrations and to diagnose and prevent overdosage.

Methods historically used to monitor serum digoxin concentrations include radioimmunoassay, fluorescence polarization immunoassay, and enzyme immunoassay. Because the Emit® 2000 homogeneous enzyme immunoassay uses an enzyme label, it eliminates some difficulties that have been associated with radioimmunoassay techniques.

### 4 REAGENTS

Reagents contain the following substances:
- Rabbit antibodies reactive to digoxin (0.01 µg/mL), glucose-6-phosphate (9.6 mM), nicotinamide adenine dinucleotide (5.6 mM), bovine serum albumin, acidic amphoteric dipetide buffer, digoxin labeled with recombinant glucose-6-phosphate dehydrogenase (0.34 U/mL), HEPES/Tris buffer, preservatives, and stabilizers.

#### Precautions
- For in vitro diagnostic use.
- Contains nonsterile rabbit antibodies.
- Reagents 1 and 2 contain bovine serum albumin.
- Do not use the kit after the expiration date.
- Reagents and calibrators contain a preservative that may cause sensitivity on contact with skin.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

**Preparation of Reagents**

The Emit® 2000 Digoxin Assay reagents are provided ready to use; no preparation is necessary.

### 5 SPECIMEN COLLECTION AND PREPARATION

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, oxalate, and EDTA have been tested in plasma samples containing 1.0 ng/mL digoxin. No discernible difference was observed in digoxin recovery from plasma samples as compared with serum samples.
- Concomitant use of other drugs, particularly quinidine, can markedly alter serum digoxin concentrations.
- Digoxin has a narrow range of safe and effective concentrations in serum. Although the therapeutic and toxic concentrations overlap, measurement of digoxin levels helps to maintain effective concentrations and to diagnose and prevent overdosage.

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### 3 METHODOLOGY

The enzyme in the Emit® 2000 Digoxin Assay is manufactured using recombinant DNA technology. The assay is a homogeneous enzyme immunoassay technique used for the analysis of digoxin and its active metabolites in serum or plasma. The assay is based on competition between drug in the sample and drug labeled with recombinant 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 coenzyme functions only with the recombinant variant of the bacterial (Leuconostoc mesenteroides) enzyme employed in the assay.

#### Catalog Number

- Emit® 2000 Digoxin Assay
- OSR4H618 R1 (Antibody/Substrate Reagent 1)
- OSR4H648 R2 (Enzyme Reagent 2)
- 4H209UL Emit® 2000 Digoxin Calibrators

#### Product Description

- Emit® 2000 Digoxin Assay
- Emit® 2000 Digoxin Calibrators

#### Quantity/Volume

- 2 x 29 mL
- 2 x 13 mL
- 1 x 5 mL
- 5 x 2 mL

*Traceable to USP. Required for calibrating the Emit® 2000 Digoxin Assay. Sold separately.

**Note:** Reagents and calibrators are shipped ready to use in liquid form.

**Note:** Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

### 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.

### Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, oxalate, and EDTA have been tested in plasma samples containing 1.0 ng/mL digoxin. No discernible difference was observed in digoxin recovery from plasma samples as compared with serum samples.
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6 PROCEEDURE

Materials Provided
Emit® 2000 Digoxin Assay
Reagent 1
Reagent 2

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

Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.

Calibration
Calibrate at least once every six months according to the instrument settings or instructions stated in the Application Sheet. 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. Once calibration has been verified, run patient samples.

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, assay 10 replicates of each of multi-level (2 or more) controls to determine a mean control concentration for each control level. Locate each mean control concentration and its corresponding precision limit in Table 1. Ensure that the coefficient of variation (CV) of each mean control concentration is within its precision limit. If any control is not within its precision limit, recalibrate and repeat the 10 replicates at each level. If any level fails to meet the criterion a second time, call for technical assistance.

Table 1 — Within-Run Precision Limits

<table>
<thead>
<tr>
<th>Mean Control Concentration (ng/mL)</th>
<th>Precision Limit (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50–0.80</td>
<td>&lt;15</td>
</tr>
<tr>
<td>0.81–1.20</td>
<td>&lt;12</td>
</tr>
<tr>
<td>&gt;1.2</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Use the mean control concentrations to determine temporary control limits as listed in Table 2.

Table 2 — Control Limits

<table>
<thead>
<tr>
<th>Mean Control Concentration (ng/mL)</th>
<th>Control Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50–0.80</td>
<td>±25%</td>
</tr>
<tr>
<td>0.81–1.20</td>
<td>±25%</td>
</tr>
<tr>
<td>&gt;1.2</td>
<td>±15%</td>
</tr>
</tbody>
</table>

Use these temporary limits for at least 30 days—a minimum of 20 determinations at 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 ± 3 standard deviations. Determine the permanent control limits by referring to Table 2. Establish new permanent control limits whenever a new lot of controls is 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 and that control results fall within your acceptable limits. If any control result is not within the established control limits, despite repeat testing or recalibration, call for technical assistance. Once you have validated the calibration curve, run patient samples.

Diluting High Concentration Samples
To estimate digoxin concentrations above the assay range, patient samples containing more than 5.0 ng/mL (6.4 nmol/L) digoxin may be diluted with one part Emit® 2000 Digoxin 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. Refer to the appropriate analyzer User’s Guide or Application Sheet for additional instructions.

Evaluation and Interpretation of Results
• This assay uses Math Model No. 1.
• Results are automatically calculated; no additional manipulation of the data is required.
• The factors that can influence the relationship between the measured digoxin serum or plasma concentrations and clinical response include kidney function, age, electrolyte balance, tissue oxygenation, thyroid status, autonomic nervous system tone, type and severity of heart disease, and coadministered drugs.7
• The concentration of digoxin in serum or plasma depends on the time of the last drug dose; dosage form; 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.1,3

7 LIMITATIONS OF THE PROCEDURE

• Severely lipemic and hemolyzed samples should be avoided as they may cause poor reproducibility and questionable quantitation.
• Endogenous, digoxin-like immunoreactive factors (DLIF) have been detected in the serum and plasma of neonates, pregnant women, and patients in renal and hepatic failure. Several studies have established that these factors can cause falsely elevated digoxin measurements when assayed by commercially available immunoassays.6
• In rare instances, individuals have antibodies that interfere with the assay by depressing its enzymatic rate. This rate depression may cause low test results.
• Fab fragments of antidigoxin antibodies, found in the serum and plasma of individuals being treated for digoxin intoxication, have the potential to interfere with any immunoassay in which they are not separated from digoxin before testing.7

8 EXPECTED VALUES

The Emit® 2000 Digoxin Assay measures digoxin concentrations in human serum or plasma containing 0.2–5.0 ng/mL (0.3–8.4 nmol/L) digoxin. The therapeutic range of 0.8–2.0 ng/mL [1.02–2.56 nmol/L] includes effective serum concentrations for a wide range of patient populations, although lower concentrations of 0.5–1.2 ng/mL [0.64–1.54 nmol/L] have been found to be more appropriate in certain populations such as chronic heart failure patients.4,5
• Digoxin toxicity is commonly associated with serum levels > 2.0 ng/mL (2.6 nmol/L) but may occur with lower digoxin levels. Significant overlap of toxic and nontoxic values has been reported. Consequently, analysis of serum concentrations alone is not sufficient for optimization of digoxin therapy. Additional factors such as age, thyroid condition, electrolyte balance, hepatic and renal functions, and other clinical symptoms must be considered.
Each laboratory should determine the appropriateness of this range for the diagnostic evaluation of patient results.

Note: To convert from ng/mL to nmol/L digoxin, multiply by 1.28.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 Digoxin 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 contain only to reagents.

Endogenous Substances
No clinically significant interference has been found in samples to which 300 mg/dL hemoglobin, 30 mg/dL bilirubin, or 80 mg/mL gamma globulin were added to simulate hemolytic, icteric, or hypergammaglobulinemic samples.

Precision
Within-run precision was determined by assaying 2 replicates of each of a tri-level control twice a day for twenty days (N=80). Total precision data were also calculated from these data. Tables 1 and 2 summarize the findings.

Table 1 — Within-Run Precision

<table>
<thead>
<tr>
<th>Mean (ng/mL)</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÇV</td>
<td>8.0</td>
<td>6.9</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 2 — Total Precision

<table>
<thead>
<tr>
<th>Mean (ng/mL)</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ÇV</td>
<td>10.4</td>
<td>9.0</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Comparative Analysis
In this study, patient samples were analyzed on the SYVA®-30R Biochemical System and on the AU600 Clinical Chemistry System. Table 3 summarizes the results.

Table 3 — Comparative Analysis Results

<table>
<thead>
<tr>
<th>Slope</th>
<th>1.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept (ng/mL)</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mean (ng/mL) SYVA®-30R</td>
<td>1.54</td>
</tr>
<tr>
<td>Mean (ng/mL) AU600</td>
<td>1.57</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.998</td>
</tr>
<tr>
<td>Number</td>
<td>50</td>
</tr>
</tbody>
</table>
Specificity
The Emit® 2000 Digoxin Assay measures the total (protein-bound plus unbound) digoxin 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 Digoxin Assay when tested in the presence of 1.0 ng/mL digoxin. 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>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic Drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>50</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>100</td>
<td>Cortisol</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100</td>
<td>Cortisone</td>
</tr>
<tr>
<td>Phenytion</td>
<td>100</td>
<td>Estriol</td>
</tr>
<tr>
<td>Procainamide</td>
<td>100</td>
<td>Prednisolone</td>
</tr>
<tr>
<td>Propranolol</td>
<td>100</td>
<td>Prednisone</td>
</tr>
<tr>
<td>Quinidine</td>
<td>100</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>100</td>
<td>Testosterone</td>
</tr>
<tr>
<td>Spiromolactone</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Endogenous Substances and Synthetic Hormones</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity level of the Emit® 2000 Digoxin Assay is 0.2 ng/mL. This level represents the lowest measurable concentration of digoxin that can be distinguished from 0 ng/mL with a confidence level of 95%.

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

10 REFERENCES


For technical assistance:
Beckman Coulter customers contact their technical assistance center.
1-800-223-0130

Siemens Healthcare Diagnostics customers contact their technical assistance center.
1-800-227-8994 in the USA
1-800-264-0083 in Canada

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