

### Emit® 2000 Digoxin Assay

#### 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. Measurements obtained from this device are used in the diagnosis and treatment of digoxin overdose or in monitoring 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. Measurements obtained from this device are used in the diagnosis and treatment of digoxin overdose or in monitoring levels of digoxin to ensure appropriate therapy.

#### 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:\(^\text{1-3}\)

- 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.\(^\text{1,2}\) Because the Emit® 2000 homogeneous enzyme immunoassay uses an enzyme label, it eliminates some difficulties that have been associated with radioimmunoassay techniques.\(^\text{4}\)

#### 3 METHODOLOGY

The enzyme in the Emit® 2000 Digoxin Assay is manufactured using recombinant DNA technology. The assay is a homogeneous enzymatic immunoassay technique used for the analysis of digoxin and its active metabolites in serum or plasma.\(^\text{3}\) The assay is based on competition between drug in the sample and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) 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.

#### 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 ampholytic didecapeptide 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. Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

**Preparation of Reagents**

The Emit® 2000 Digoxin 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, 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. i.e., the concentrations of the plasma samples agreed with the concentrations of the serum samples within the precision limits of the assay at the 1.0 ng/mL level.
- Sample volume is instrument-dependent. Refer to the appropriate User’s Guide or Application Sheet for volumes.
- Use fresh samples. If samples are to be tested within 8 hours of collection, they may be stored at room temperature (20–25°C). For transporting, maintain the sample temperature at 2–8°C. Samples can be stored refrigerated at 2–8°C for up to 7 days or stored frozen (-20°C) for up to 6 months. Repeated freeze-thaw cycles should be avoided.
- Samples that contain particulate matter, fibrous material, or gel-like masses; appear unusual; or are frozen require preparation. Use the following instructions to prepare such samples:
  1. If the sample is frozen, thaw at room temperature (20–25°C).
  2. Vigorously mix sample in a vortex for at least 30 seconds.
  3. Centrifuge sample at ≥2000 rpm for 15 minutes.
  4. Collect a specimen from the middle portion of the sample. Avoid collecting lipids from the top portion or particulate matter from the bottom portion.
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose; factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.\(^\text{1,2}\)
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.
- For reliable interpretation of results, collect samples either after the drug’s distribution phase or immediately before the next oral dose (at least 6 hours after administration). Samples drawn before the drug has completed its distribution phase will not accurately reflect the level of drug in the myocardium. These samples cannot be used to evaluate cardiac response because serum levels do not represent tissue levels until at least 6 hours after oral dose or 4 hours after an intravenous dose. To evaluate maintenance doses, collect samples when digoxin levels are at steady state—the time to reach steady state is normally from three to five elimination half-lives but may be prolonged in patients with impaired renal function.\(^\text{1,3}\)

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**Catalog Numbers and Description**

- **OSR4H229**
  - **Emit® 2000 Digoxin Assay**
  - **OSR4H618 R1 (Antibody/Substrate Reagent 1)**
  - **OSR4H648 R2 (Enzyme Reagent 2)**

- **4H209UL**
  - **Emit® 2000 Digoxin Calibrators**

**Calibrator Concentrations**

<table>
<thead>
<tr>
<th>Level</th>
<th>ng/mL</th>
<th>ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Preparation of Reagents**

- 1 x 5 mL,†
- 2 x 13 mL

**Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics**

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**References**


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**Updated information from March 2008 edition.**

See shaded sections:

- Updated information from March 2008 edition.

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**Shaded Sections**

- Updated information from March 2008 edition.
6 PROCEDURE

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.

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.1,3
• 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
• 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
• 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 0.3–5.0 ng/mL (0.38–6.4 nmol/L) digoxin. The therapeutic range of 0.5–2.0 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.1,3 Digoxin toxicity is commonly associated with serum levels > 2.0 ng/mL (2.65 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 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, 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 level 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>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>%CV</td>
<td>8.0</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Table 2 — Total Precision

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>%CV</td>
<td>10.4</td>
<td>9.0</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>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 (µg/ml)</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous Substances and Synthetic Hormones</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>50</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>100</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>100</td>
</tr>
<tr>
<td>Procanamide</td>
<td>100</td>
</tr>
<tr>
<td>Propranolol</td>
<td>100</td>
</tr>
<tr>
<td>Quinidine</td>
<td>100</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>100</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
</tr>
<tr>
<td>Cortisol</td>
<td>10</td>
</tr>
<tr>
<td>Cortisone</td>
<td>10</td>
</tr>
<tr>
<td>Estriol</td>
<td>10</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
</tr>
<tr>
<td>Prednisone</td>
<td>10</td>
</tr>
<tr>
<td>Progesterone</td>
<td>10</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5</td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity level of the Emit® 2000 Digoxin Assay is 0.3 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