Procainamide concentrations together helps to achieve an optimum anti-arrhythmic effect and concentrations include chromatographic assay and immunoassay.1–3 Because the ratio varies from patient to patient, measuring serum N-acetylprocainamide and procainamide concentrations together helps to achieve an optimum anti-arrhythmic effect and reduce the risk of toxicity. Methods historically used to monitor serum N-acetylprocainamide and procainamide concentrations include chromatographic assay and immunoassay.1–3

**Table 1**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Product Description</th>
<th>Quantity/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSR4N229</td>
<td>Emit® 2000 N-Acetylprocainamide Assay</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OSR4N518 R1 (Antibody/Substrate Reagent 1)</td>
<td>2 x 23 mL.</td>
</tr>
<tr>
<td></td>
<td>OSR4N548 R2 (Enzyme Reagent 2)</td>
<td>2 x 13 mL.</td>
</tr>
<tr>
<td>4N109UL</td>
<td>Emit® 2000 N-Acetylprocainamide Calibrators*</td>
<td>1 x 5 mL†, 5 x 2 mL</td>
</tr>
</tbody>
</table>

†Additional negative calibrator is provided.
Note: Reagents and calibrators are shipped ready to use in liquid form.

1 INTENDED USE
The Emit® 2000 N-Acetylprocainamide Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of N-acetylprocainamide in human serum or plasma. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

2 SUMMARY
Measurements of the antiarrhythmic drug procainamide in serum may not accurately reflect the drug’s complete pharmacologic activity in the body without corresponding measurements of the active metabolite N-acetylprocainamide. Serum levels of N-acetylprocainamide increase in patients on chronic procainamide therapy, particularly in those with renal impairment. The average serum concentration ratio of N-acetylprocainamide to procainamide is 0.8 or 1.2, depending on a genetically determined tendency to acetylate procainamide rapidly or slowly. Because the ratio varies from patient to patient, measuring serum N-acetylprocainamide and procainamide concentrations together helps to achieve an optimum anti-arrhythmic effect and reduce the risk of toxicity.

3 METHODOLOGY
The Emit® 2000 N-Acetylprocainamide Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.1–3 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 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 N-acetylprocainamide (3.3 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), N-acetylprocainamide labeled with glucose-6-phosphate dehydrogenase (0.22 U/mL), Tris buffer, preservatives, and stabilizers.

Precautions
• For in vitro diagnostic use.
• Contains nonsterile mouse monoclonal antibodies.
• Do not use the kit after the expiration date.
• Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents
The Emit® 2000 N-Acetylprocainamide 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 7 days or stored frozen (-20°C) for up to 1 month.
• 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.1,2
• 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 N-Acetylprocainamide Assay
Reagent 1
Reagent 2

**Materials Required But Not Provided**
Emit® 2000 N-Acetylprocainamide 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 (eg, low, medium, and high) controls in every run. 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.

Diluting High Concentration Samples
To estimate N-acetylprocainamide concentrations above the assay range, patient samples containing more than 16 µg/mL (58 pmol/L) N-acetylprocainamide may be diluted with one or two parts distilled or deionized water or Emit® 2000 N-Acetylprocainamide 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 N-acetylprocainamide serum or plasma concentrations and clinical response include renal and circulatory function, rate of acetylation, the severity and type of cardiac arrhythmia, general state of health, and use of other drugs.
- The concentration of N-acetylprocainamide 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, biotransformation, distribution, and excretion. These parameters must be considered when interpreting results.1,2

7 LIMITATIONS OF THE PROCEDURE

This assay has no specific limitations.

8 EXPECTED VALUES

The Emit® 2000 N-Acetylprocainamide Assay accurately quantitates N-acetylprocainamide concentrations in human serum or plasma containing 1.0–16 µg/mL (3.6–58 pmol/L) N-acetylprocainamide. Since N-acetylprocainamide is a metabolite of procainamide, no therapeutic range has been established exclusively for it. However, most patients achieve satisfactory therapeutic response when the sum of procainamide and N-acetylprocainamide concentrations in serum is 10–30 µg/mL.1,2

Note: To convert from µg/mL to µmol/L N-acetylprocainamide, multiply by 3.61.

Because of patient-to-patient differences in metabolic activity, renal function, and type and severity of cardiac arrhythmia, 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 (see Section 6, Procedure, Evaluation and Interpretation of Results).

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® 2000 N-Acetylprocainamide Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Refer to the Application Sheet 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, 1000 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>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>100</td>
<td>N-(2-diethylaminoethyl)</td>
<td>100</td>
</tr>
<tr>
<td>Desethyl-N-acetylprocainamide</td>
<td>100</td>
<td>Isonicotinamide</td>
<td>100</td>
</tr>
<tr>
<td>(DENAPA)</td>
<td>p-Acetamidobenzoic acid</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Diphenoxylate</td>
<td>0.1</td>
<td>p-Aminobenzoic Acid (PABA)</td>
<td>100</td>
</tr>
<tr>
<td>Diphenylhydantoin</td>
<td>100</td>
<td>Phenytin</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>100</td>
<td>Procainamide</td>
<td>100</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>100</td>
<td>Procaine</td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>Pranoprofen</td>
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</tr>
<tr>
<td>Glyburide</td>
<td>100</td>
<td>Quinidine</td>
<td>100</td>
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<tr>
<td>Hydrocortisone</td>
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<td>Quinidine</td>
<td>100</td>
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<tr>
<td>Isoproterenol</td>
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<td>Tocainide</td>
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<tr>
<td>Lidocaine</td>
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</tr>
<tr>
<td>Monoethylglycinexylidide</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

2 4N05D_B

Sensitivity
The sensitivity level of the Emit® 2000 N-Acetylprocainamide Assay is 0.25 µg/mL. This level represents the lowest measurable concentration of N-acetylprocainamide 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
