3 METHODOLOGY

The Emit® tox™ Salicylic Acid Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of salicylic acid in human serum or plasma. In the performance of the Emit® tox™ Salicylic Acid Assay, serum or plasma is mixed with Reagent 1, which contains antibodies to salicylic acid and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently, Reagent 2, which contains salicylic acid labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH), is added. Salicylic acid in the sample and salicylic acid-labeled G6PDH compete for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the salicylic acid concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized NAD to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous 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 salicylic acid (60.4 µg/mL), *salicylic acid labeled with bacterial G6PDH (0.34 U/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), Tris buffer, bovine serum albumin, preservatives, and stabilizers.

*The antibody titer and enzyme conjugate activity may vary from lot to lot.

For in vitro diagnostic use.

Precautions:
- Reagents contain nonsterile mouse monoclonal antibodies. Reagents contain nonsterile bovine serum albumin.
- Reagent 2 contains sodium azide, which may react with lead or 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.
- Reagents and calibrators contain materials that may cause sensitivity on contact with skin.
- Do not use kit after the expiration date.
- Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents

The Emit® tox™ Salicylic Acid 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 at 2–8°C (36–46°F), upright, and with the 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 EDTA, sodium heparin, citrate, and oxalate/fluoride 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.
- Pharmacokinetic factors influence the relationship between the observed drug level and the time elapsed since drug ingestion. These factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.
- Serum levels drawn less than 6 hours after a toxic dose can be used to confirm overdose, but to use the Done nomogram to predict the severity of the toxic reaction, samples must be drawn at least 8 hours after ingestion of the toxic dose. Repeat testing within 2–3 hours is recommended to ensure that absorption is complete and to determine the effectiveness of the therapeutic intervention.
- After therapeutic doses of salicylates, peak levels are reached at 2 hours.
- Store and transport the serum or plasma refrigerated at 2–8°C.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious. It is recommended that human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens or other appropriate local practices.
### 6 PROCEDURE

#### Materials Provided
- Emit® Salicylic Acid Assay
- Reagent 1
- Reagent 2

#### Materials Required But Not Provided
- Salicylic Acid Calibrators
- Multilevel 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

**Temporary Control Limits.** When establishing control limits for the first time, run 3 calibration curves and assay 3 replicates each of multi-level (2 or more) controls to determine a mean control concentration for each control level. Determine temporary control limits for each control level (a 20% of each mean control concentration). Use the temporary limits for at least 30 days (a minimum of 20 determinations for 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. Calculate the standard deviation for each control level and multiply it by 2.25. Permanent control ranges should be ±2.25 SD of the mean or ±12% of the mean, whichever is greater.

Establish new permanent control limits whenever a new lot of controls or reagents is used.

**Daily Quality Control.** Assay at least 1 control every 8 hours, alternating the control levels tested. Ensure that a minimum of 2 controls is assayed in every 24-hour period. If controls are within their control limits, calibration is verified. If any control is not within its control limits, rerun that control. If the result is then within the control limits, calibration is verified. If the control is not within the control limits after repeat testing, recalibrate according to the instructions in the Calibration section and verify calibration using 2 or more controls.

If, after recalibration, any control is not within its control limits, check the handling of the control, calibrator, and reagent, and then retest. If a control is still not within its control limits, call the Technical Assistance Center in the USA or your local Siemens Healthcare Diagnostics representative.

Each laboratory must establish and follow its own quality control procedures. At a minimum, perform the quality control procedures recommended by Siemens as described above. Ensure that the quality control results meet the acceptance criteria before reporting patient results.

#### Diluting High Concentration Samples
To estimate salicylic acid concentrations above the assay range, patient samples containing more than 80 mg/dL (5792 µmol/L) salicylic acid may be diluted with one or two parts of distilled or deionized water or Emit® Salicylic Acid Calibrator 0. After dilution, test the sample and multiply the results by the dilution factor. See the analyzer User’s Guide or appropriate Application Sheet for instructions.

#### Evaluation and Interpretation of Results
- Results are calculated automatically by the analyzers. No additional manipulation of data is required unless samples have been manually diluted.
- Consult the appropriate instrument operating manual and Application Sheet for complete instructions.
- The concentration of salicylic acid in serum or plasma depends on the time of drug ingestion; concomitant drug therapy; sample condition; time of sample collection; and individual variation in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.
- In acute aspirin overdose in pediatric patients, a single serum or plasma level plotted on the Done nomogram provides a good indication of the severity of intoxication.

### 7 LIMITATIONS OF THE PROCEDURE

When diluting patient samples containing high salicylic acid concentrations, the following factors can affect the accuracy of the result: the use of the correct diluent (Emit® Salicylic Acid Calibrator 0 or distilled or deionized water) and the accuracy of the dilution.

### 8 EXPECTED VALUES

The Emit® Salicylic Acid Assay accurately quantitates salicylic acid concentrations in human serum or plasma containing up to 80 mg/dL (5792 µmol/L) salicylic acid.

The therapeutic range for analgesic and antipyretic effects is below 6 mg/dL (434 µmol/L) salicylic acid. The therapeutic range for anti-inflammatory effects is 15–30 mg/dL (1088–2172 µmol/L) salicylic acid. Salicylic acid are frequently maintained to manage acute rheumatic fever; these levels are within the toxic range for salicylates, which starts at 30 mg/dL (2172 µmol/L). Hemodialysis is indicated when serum salicylic acid concentrations exceed 100 mg/dL (7240 µmol/L). See Section 6, Procedure, Evaluation and Interpretation of Results, for a discussion of overdose cases.

**Note:** To convert from mg/dL to µmol/L salicylic acid, multiply by 72.4.

For effective treatment, some patients may require serum or plasma levels outside these ranges. Therefore, the expected ranges are provided only as guidelines, 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® Salicylic Acid Assay studies performed on the AU6000 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 significant interference has been found in samples to which 800 mg/dL hemoglobin, 30 mg/dL bilirubin, or 750 mg/dL triglycerides were added to simulate hemolytic, icteric, or lipemic samples.

#### Precision

Precision was determined by assaying two replicates each of in-house tri-level controls on 20 days with 2 runs per day. Precision was calculated according to National Committee for Clinical Laboratory Standards (NCCCLS) Guideline EP5-A (February 1999). Table 1 summarises the results.

**Table 1 — Precision**

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean (mg/dL)</th>
<th>Standard Deviation (mg/dL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-Run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.3</td>
<td>0.19</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>19.3</td>
<td>0.74</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>49.2</td>
<td>1.23</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.3</td>
<td>0.30</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>19.3</td>
<td>0.95</td>
<td>5.0</td>
</tr>
<tr>
<td>3</td>
<td>49.2</td>
<td>2.29</td>
<td>4.7</td>
</tr>
</tbody>
</table>

**Comparative Analysis**

In this study, samples from patients taking salicylic acid and samples spiked with salicylic acid were analyzed on the SYVA®-30R Biochemical System and the AU6000 Clinical Chemistry System, and the results were compared. Table 2 summarises the results.

**Table 2 — Comparative Analysis Results**

<table>
<thead>
<tr>
<th>Slope</th>
<th>Intercept (mg/dL)</th>
<th>Mean (mg/dL)</th>
<th>SYVA®-30R</th>
<th>AU6000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.049</td>
<td>0.175</td>
<td>26.1</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Samples</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Specificity

The Emit® Salicylic Acid Assay measures the total (protein-bound plus unbound) salicylic acid 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 1 do not interfere with the Emit® Salicylic Acid Assay when tested in the presence of 30 mg/dL salicylic acid. 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>Acetaminophen</td>
<td>1000</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>1000</td>
</tr>
<tr>
<td>p-Aminosalicylic Acid</td>
<td>400</td>
</tr>
<tr>
<td>Benzoic Acid</td>
<td>1000</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1000</td>
</tr>
<tr>
<td>Codeine</td>
<td>100</td>
</tr>
<tr>
<td>Diflunisal</td>
<td>600</td>
</tr>
<tr>
<td>2,3-Dihydroxybenzoic Acid</td>
<td>500</td>
</tr>
<tr>
<td>Fenoprofen</td>
<td>1000</td>
</tr>
<tr>
<td>Gentisic Acid</td>
<td>10</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1000</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1000</td>
</tr>
<tr>
<td>Methyl Salicylate</td>
<td>500</td>
</tr>
<tr>
<td>Naproxen</td>
<td>150</td>
</tr>
<tr>
<td>Salicylamide</td>
<td>500</td>
</tr>
<tr>
<td>Salicylsalicylic Acid</td>
<td>1000</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>1000</td>
</tr>
</tbody>
</table>
Sensitivity
The sensitivity level of the Emit® tox™ Salicylic Acid Assay is 0.2 mg/dL salicylic acid. This level represents the lowest concentration of salicylic acid that can be distinguished from 0 mg/dL with a confidence level of 95%.

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

10 REFERENCES