Acetaminophen (µmol/L) 0 66 166 331 662 1324
Acetaminophen (µg/mL) 0 10 25 50 100 200

with hepatic failure when initiated 12 to 16 hours after overdose.1 especially if administered within 8 to 10 hours after overdose, and improves survival in patients

7A409UL

and colorimetric immunoassay.3 The methods historically used to monitor serum acetaminophen concentrations are

high-performance liquid chromatography, gas-liquid chromatography, UV spectrophotometry,

Measurement of serum acetaminophen may also be used to estimate the drug elimination half-life. Serum half-life is recommended when the time of the ingestion is not known. Acetaminophen half-life is used to judge toxicity and may be a better predictor of hepatotoxicity than a single serum measurement.2 The methods historically used to monitor serum acetaminophen concentrations are high-performance liquid chromatography, gas-liquid chromatography, UV spectrophotometry, and colorimetric immunoassay.3

3 METHODOLOGY
The Emit® tox™ Acetaminophen Assay is a homogeneous enzyme immunoassay technique used for the quantitative analysis of acetaminophen in human serum or plasma. In the performance of the Emit® tox™ Acetaminophen Assay, serum or plasma is mixed with Reagent 1, which contains antibodies to acetaminophen and the coenzyme nicotinamide adenine dinucleotide (NAD). Subsequently, Reagent 2, containing acetaminophen labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH), is added. Acetaminophen in the sample and acetaminophen labeled G6PDH compete for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the acetaminophen 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 can be 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:

Sheep antibodies reactive to acetaminophen (73 µg/mL),* acetaminophen labeled with bacterial G6PDH (0.42 U/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (20 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
• Reagent 1 contains nonsterile sheep antibodies. Reagent 2 contains nonsterile mouse monoclonal antibodies. Reagents and calibrators contain nonsterile bovine serum albumin.
• Reagent 2 contains sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush the drain 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™ Acetaminophen 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, heparin, citrate, and oxalate/fluridate have been tested and may be used with this assay.
• Sample volume is instrument-dependent. Refer to the appropriate application sheet.
• Draw a sample at least 4 hours after drug ingestion to ensure that the plasma or serum concentrations have peaked. Ingestion of massive quantities of acetaminophen or of a modified-release preparation may result in delayed peak serum acetaminophen levels. In such cases, repeated serum concentrations should be obtained.1
• If the time of ingestion is not known, the acetaminophen half-life, an indicator of potential hepatotoxicity, may be estimated by drawing 2 or more blood samples at intervals of 2 to 3 hours.2
• Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, concomitant drug therapy, and biological variations affecting drug disposition.
• Store and transport samples 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.4,5
6 PROCEDURE

Materials Provided
Emit® tox™ Acetaminophen Assay
Reagent 1
Reagent 2

Materials Required But Not Provided
Emit® tox™ Acetaminophen 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 (± 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 and verify calibration using 2 or more controls.

If, after recalibration, any control is not within its control limits, check control, calibrator, and reagent handling, 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 quality control procedures as 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 acetaminophen concentrations above the assay range, patient samples containing more than 200 µg/mL (1324 µmol/L) acetaminophen may be diluted with 1 or 2 parts of Emit® tox™ Acetaminophen Calibrator 0 or distilled or deionized water. After diluting the sample, test 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 analyzer. 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 acetaminophen in serum or plasma depends on the time of drug ingestion: 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.
• In acute acetaminophen overdose, a single serum or plasma level determination, plotted on the Rumack-Matthew nomogram (see below), provides a good indication of whether overdose therapy is required. Values above the lower line indicate that treatment should be initiated.1

Semi-Logarithmic Plot of Plasma Acetaminophen Levels vs Time*

The lower line defines acetaminophen concentrations 25% below those expected to cause hepatic toxicity. It allows for possible errors in assay values or in estimating time elapsed since ingestion of the drug overdose.6


7 LIMITATIONS OF THE PROCEDURE

• When diluting patient samples containing high acetaminophen concentrations, the following factors can affect the accuracy of the result: diluting with the correct fluid (Emit® tox™ Acetaminophen Calibrator 0 or distilled or deionized water), the accuracy of the dilution, and the assay’s specificity to drug metabolites.

• In rare cases, patients may have antibodies that interfere with the assay. This may cause erroneous results.

8 EXPECTED VALUES

The Emit® tox™ Acetaminophen Assay accurately quantitates serum or plasma acetaminophen concentrations up to 200 µg/mL (1324 µmol/L). Samples quantitating above the assay range should be reported as having concentrations greater than 200 µg/mL.

Note: To convert from µg/mL to µmol/L acetaminophen, multiply by 6.62.

Normal therapeutic doses of acetaminophen result in serum concentrations of 10–30 µg/mL (66–199 µmol/L) in healthy adults.3 See Section 6, Procedure, Evaluation and Interpretation of Results, for a discussion of overdose cases.

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® tox™ Acetaminophen Assay studies performed on the AU4000®/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 (NCCLS) Guideline EP5-A (February 1999). Table 1 summarizes the results.

Table 1 — Precision

<table>
<thead>
<tr>
<th>Control</th>
<th>Mean (µg/mL)</th>
<th>Standard Deviation (µg/mL)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within-Run</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>45.2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>135.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Comparative Analysis
In this study, patient samples containing acetaminophen were analyzed on the SYVA®-30R Biochemical System and the AU600 Clinical Chemistry System, and the results were compared. Table 2 summarizes the results.

Table 2 — Comparative Analysis Results

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.982</td>
<td>-1.342</td>
</tr>
<tr>
<td>Intercept (µg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (µg/mL) SYVA®-30R</td>
<td>55.5</td>
<td>53.1</td>
</tr>
<tr>
<td>AU600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.992</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
Specificity
The Emit® tox™ Acetaminophen Assay measures the total (protein-bound plus unbound) acetaminophen concentration in serum or plasma. Compounds whose chemical structure would suggest possible cross-reactivity, concurrent therapeutics, and other compounds commonly present in acetaminophen specimens have been tested.

The compounds listed in Table 1 do not interfere with the Emit® tox™ Acetaminophen Assay at maximum pharmacological or physiological concentrations when tested in the presence of 50 µg/mL acetaminophen.

Table 3 — Specificity

<table>
<thead>
<tr>
<th>Compounds That Do Not Interfere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen cysteine</td>
</tr>
<tr>
<td>Acetaminophen glucuronide</td>
</tr>
<tr>
<td>Acetaminophen mercapturate</td>
</tr>
<tr>
<td>Acetaminophen sulfate</td>
</tr>
<tr>
<td>Acetylcycteine</td>
</tr>
<tr>
<td>Amitriptyline</td>
</tr>
<tr>
<td>Caffeine</td>
</tr>
<tr>
<td>Codeine</td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity level of the Emit® tox™ Acetaminophen Assay is 0.12 µg/mL. This level represents the lowest concentration of acetaminophen that can be distinguished from 0 µg/mL 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