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.

3 METHODOLOGY

The Emit® tox™ Acetaminophen Assay is a homogeneous enzyme immunoassay 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),
- 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.
- 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.

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

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/fluoride 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.
- If the time of ingestion is not known, the acetaminophen half-life, an indicator of metabolic hepatoxicity, may be estimated by drawing 2 or more blood samples at intervals of 2 to 3 hours.
- 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 for Bloodborne Pathogens or other appropriate local practices.
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 controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your laboratory. Once the calibration is validated, run samples.
- Follow government regulations or accreditation requirements for quality control frequency. At least once on each day of use, analyze two levels of a Quality Control (QC) material with known acetaminophen concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
- Refer to the instrument operator’s manual for appropriate instrument checks.

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® 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.6


- Alcoholics are at risk for toxicity at lower doses. Enhanced susceptibility to toxic effects has also been reported in persons receiving long-term anticonvulsant therapy and patients taking isoniazid.1
- The acetaminophen half-life may also be used to assess potential hepatotoxicity, and can be estimated without reference to the time of drug ingestion. Hepatic necrosis should be anticipated if the half-life exceeds 4 hours, and hepatic coma is likely if the half-life exceeds 12 hours.2
- Results of this test should always be interpreted in conjunction with the patient’s medical history, clinical presentation and other findings.
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 3 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
Compounds That Do Not Interfere

| Acetaminophen cysteine | Cysteine |
| Acetaminophen glucuronide | Diazepam |
| Acetaminophen mercapturate | Methionine |
| Acetaminophen sulfate | Phenacetin |
| Acetylcysteine | Phenylephrine hydrochloride |
| Amitriptyline | Propoxyphene |
| Caffeine | Salicylic acid |
| Codeine | Secobarbital |

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

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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