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Syva®

Emit® tox™ Acetaminophen Assay

See shaded sections:
Updated information from 2015-04 edition. 10870849_E

Acetaminophen Assay

1 Intended Use

The Emit® ***tox***™ Acetaminophen Assay is a homogeneous enzyme immunoassay intended for *in vitro* diagnostic use in the quantitative analysis of acetaminophen in human serum or plasma.

2 Summary and Explanation of the Test

Acetaminophen is a widely used analgesic and antipyretic found in a number of over-the-counter and prescription products. When consumed in overdose quantities, acetaminophen may cause severe liver and kidney damage, or death.[1]

The patient may have few or no symptoms early after acute overdose of acetaminophen. The only reliable early diagnostic indicator is provided by a quantitative measurement of the serum acetaminophen level. Clinical evidence of liver and kidney damage is usually delayed for 24 hours or more after ingestion, well after the time that the prophylactic antidote, acetylcysteine, can be effectively administered.[1] Acetylcysteine is highly effective in preventing liver damage, especially if administered within 8 to 10 hours after overdose, and improves survival in patients with hepatic failure when initiated 12 to 16 hours after overdose.[1]

Measurement of serum acetaminophen may also be used to estimate the drug elimination half-life. Serum half-life is recommended when the time of 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 Principle

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

REF	Product Description	Volume
7A319UL	**Emit® *tox*™ Acetaminophen Assay Antibody/Substrate Reagent 1** sheep antibodies reactive to acetaminophen (73 µg/mL),* G6P (22 mM), NAD (20 mM), bovine serum albumin, preservatives, and stabilizers	28 mL
	Enzyme Reagent 2 acetaminophen labeled with bacterial G6PDH (0.42 U/mL),* Tris buffer, preservatives, bovine serum albumin, and stabilizers	14 mL

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

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

For *in vitro* diagnostic use.

Precautions

- Contains sodium azide (<0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.
- Reagents and calibrators contain materials that may cause sensitivity on contact with skin.

Preparation and Storage of Assay Components

Reagents:

The Emit® ***tox***™ Acetaminophen Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent bottles when not in use.

Note: Caps must always be replaced on the original containers.

When not in use, store reagents at 2–8°C (36–46°F), upright, and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Refer to the analyzer-specific protocols for on-instrument stability information. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C. **Improper storage of reagents can affect assay performance.**

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 analyzer-specific protocol.
- 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

7A409UL Emit® ***tox***™ Acetaminophen Calibrators [0, 10, 25, 50, 100, 200 µg/mL]

Multi-level commercial controls

Instruments

Siemens Healthcare Diagnostics provides instructions for using this assay on a number of chemistry analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for application sheets.

Analyzers must be capable of maintaining a constant reaction temperature, pipetting specimens/reagents and measuring enzyme rates precisely, timing the reaction accurately, and mixing reagents thoroughly.

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

1. Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptance limits as defined by your own laboratory. Once the calibration is validated, run samples.
2. Follow government regulations or accreditation requirements for quality control frequency. At least one 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.
3. 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® *tox*™ Acetaminophen Calibrator 0 or distilled or deionized water. After diluting the sample, test and multiply the results by the dilution factor.

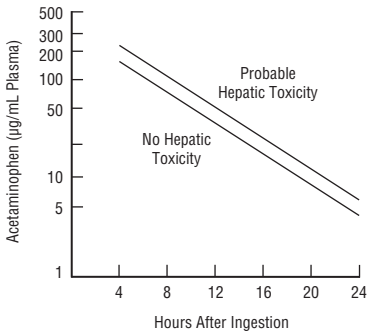
Daily Maintenance

Refer to the instrument operating manual for maintenance instructions.

7 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 analyzer-specific protocol for complete instructions.
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.
- Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.
- 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]**

****Reproduced by permission of* Pediatrics*, vol. 55, p 871–876, © 1975.***

- 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]

8 Limitations

- 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.

9 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 7, Results, for a discussion of overdose cases.

10 Specific Performance Characteristics

The data appearing in this section were collected on the SYVA®-30R Biochemical System.

The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents. Performance may vary depending on the instrumentation used.

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 1 — 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

For additional information, contact Siemens.

Sensitivity

The sensitivity level of the Emit® *tox*™ Acetaminophen Assay is less than 0.25 µg/mL acetaminophen. This level represents the lowest concentration of acetaminophen that can be distinguished from 0 µg/mL with a confidence level of 95%.

Endogenous Substances

No interference was observed in samples containing 50 µg/mL acetaminophen to which 800 mg/dL hemoglobin or 60 mg/dL free bilirubin were added to simulate hemolytic or icteric samples.

No interference was observed in patient samples containing elevated concentrations of endogenous triglyceride (811–1150 mg/dL) and 50 µg/mL acetaminophen.

Calibration Stability

Studies have shown calibration stability of at least 14 days. The *permanent* quality control limits used in these studies were established by following the instructions in Section 6, Procedure, Quality Control. 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.

Precision

Precision values were obtained using the SYVA®-30R Biochemical System. Precision was determined by measuring 2 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) Guidelines EP5-A (February 1999). Results of these studies are summarized in Table 2.

Table 2 — Precision

Control	Number of Replicates	Mean (µg/mL)	Standard Deviation (µg/mL)	Coefficient of Variation (%)
Within-Run				
1	80	30.5	1.16	3.8
2	80	60.1	1.44	2.4
3	80	170.7	4.62	2.7
Total				
1	80	30.5	1.6	5.3
2	80	60.1	3.06	5.1
3	80	170.7	6.18	3.6

Accuracy

Patient serum samples with acetaminophen concentrations ranging from 2.7 µg/mL to 328 µg/mL were analyzed on the SYVA®-30R Biochemical System by the Emit® ***tox***™ Acetaminophen Assay and by the Emit® Acetaminophen Assay. Samples with acetaminophen concentrations greater than the assay range (200 µg/mL) were diluted and tested, and the test results were multiplied by the dilution factor. The patient sample results from the Emit® ***tox***™ Acetaminophen Assay and the Emit® Acetaminophen Assay were compared. Data are summarized in Table 3.

Table 3 — Accuracy

	Emit® ***tox***™ vs Emit®
Slope	1.01
Intercept (µg/mL)	-0.9
Mean (µg/mL)	
Emit® ***tox***™ Assay	59.4
Comparison Method	59.6
Standard Error of the Estimate (µg/mL)	4
Correlation Coefficient	0.995
Number of Samples	100

11 Risk and Safety

H317
P280, P272, P302 + P352, P333 + P313, P501
Warning!
May cause an allergic skin reaction.

Wear protective gloves/protective clothing/eye protection/face protection. Contaminated work clothing should not be allowed out of the workplace. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents and container in accordance with all local, regional, and national regulations.

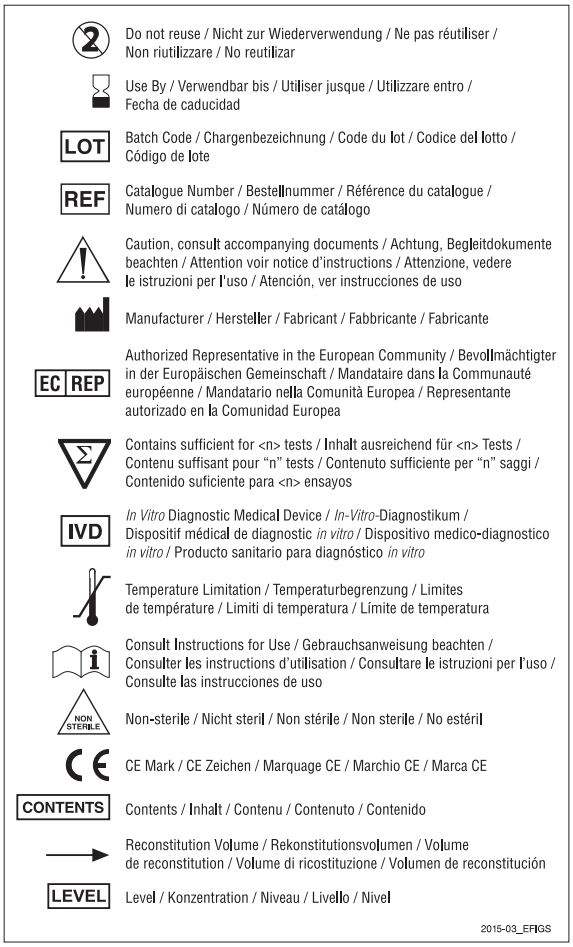
Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone.

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

12 Bibliography

1. Olson KR. Management of poisoning and drug overdose. In: Dale DC, Federman DD, eds. *Scientific American Medicine*. New York, NY: Scientific American Inc; 2000: sec 8(I).
2. Burtis CA, Ashwood ER, eds. *Tietz Textbook of Clinical Chemistry*. 2nd ed. Philadelphia, Pennsylvania: W.B. Saunders Co; 1994:1167–1169.
3. Jacobs DS, DeMott WR, Grady HJ, Horvat RT, Huestis DW, Kasten BL Jr. *Laboratory Test Handbook*. 2nd ed. Stow, Ohio: Lexi-Comp Inc; 1990:768.
4. Occupational exposure to bloodborne pathogens (29 CFR 1910.1030). *Federal Register*. December 06, 1991;56:64004; amended April 13, 1992;57:12717; July 01, 1992;57:29206; February 13, 1996;61:5507.
5. World Health Organization. *Laboratory Biosafety Manual*. 2nd ed. Geneva: World Health Organization; 1993.
6. Rumack BH et al: Acetaminophen overdose: 662 cases with evaluations of oral acetylcysteine treatment. Arch Intern Med 1981;141:380–385.

13 Symbols Key



For technical assistance, call Siemens Healthcare Diagnostics:
1-800-227-8994 in the USA
1-800-264-0083 in Canada
Outside the USA and Canada, call your local Siemens representative.

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