SIEMENS

Syva®

Emit® tox™ Serum Benzodiazepine Assay

See shaded sections:
Updated information from 2015-03 version. 10870904_G

Serum Benzodiazepine Assay

1 Intended Use

The Emit® *tox*™ Serum Benzodiazepine Assay is a homogeneous enzyme immunoassay intended for use in the qualitative and semiquantitative analysis of specific benzodiazepines in human serum or plasma. This assay is designed for use with most chemistry analyzers.

The Emit® tox™ Serum Benzodiazepine Assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.*[1] *Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

2 Summary and Explanation of the Test

Benzodiazepines are sedative-hypnotic drugs that are structurally similar and include widely used drugs such as chlordiazepoxide, diazepam, and oxazepam. The different benzodiazepines are absorbed at different rates, and the timing of their psychoactive effects varies with the absorption rate. Benzodiazepines are usually taken orally and are metabolized by the liver. Some benzodiazepine metabolites are pharmacologically active.[2] Benzodiazepines potentiate the effect of other central nervous system depressants, such as ethyl alcohol.[3]

The Emit® *tox*™ assays are intended to be used in emergency situations for the rapid identification of types of commonly abused drugs. The Emit® *tox*™ Serum Benzodiazepine Assay may be used to detect benzodiazepines and benzodiazepine metabolites in human serum or plasma. Positive results for samples containing other compounds structurally unrelated to benzodiazepines have not been observed.

Methods historically used for detecting benzodiazepines in biological fluids include gas chromatography with electron-capture,[4] or flame ionization detection,[5] high-performance liquid chromatography,[6] thin-layer chromatography,[7] fluorescence-TLC densitometry,[8] enzyme immunoassay,[9] and radioimmunoassay.[10]

While confirmation techniques other than GC/MS may be adequate for some drugs of abuse, GC/MS is generally accepted as a vigorous confirmation technique for all drugs, since it provides the best level of confidence in the result.[1]

3 Principle

The Emit® *tox*™ Serum Benzodiazepine Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.[11] 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 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

REF	Product Description	Quantity/Volume*
7B119UL	**Emit® *tox*™ Serum Benzodiazepine Assay Antibody/Substrate Reagent A** sheep antibodies reactive to benzodiazepines (51 µg/mL),† glucose-6-phosphate (66 mM), nicotinamide adenine dinucleotide (40 mM), bulking agents, stabilizers, and preservatives	3 mL
	Enzyme Reagent B benzodiazepines labeled with bacterial glucose-6-phosphate dehydrogenase (0.48 U/mL),† glucose-6-phosphate (32 mM), Tris buffer, bulking agents, stabilizers, and preservatives	3 mL
	Emit® Drug Assay Buffer Concentrate Tris buffer, surfactant, and preservatives	13.3 mL
7B019UL	**Emit® *tox*™ Serum Calibrators** (Negative, Low, and Medium) human serum and preservatives (see below for drug concentrations)	three 3 mL vials

****Reagents and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.***

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

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

The Emit® *tox*™ Serum Calibrators, when reconstituted, contain the following stated diazepam concentrations:

Calibrator	Concentration (µg/mL)
Negative Calibrator	0
Low Calibrator*	0.3
Medium Calibrator*	2

****These calibrators also contain secobarbital, which does not affect the assay. See the Emit® tox™ Serum Calibrators instructions for use.***

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.

Preparation and Storage of Assay Components

To prepare reagents and calibrators:

- Record the date of reconstitution.
- Remove the metal seal and mark the rubber stopper to identify it with the original vial.
- Add the amount of distilled or deionized water listed in Table 1.
- Replace the stopper and gently swirl the vial until the contents are completely dissolved.

After reconstitution, allow the reagents and calibrators to equilibrate at a room temperature of 20–25°C for a minimum of one hour. Alternatively, reconstitute the reagents and calibrators the day before they are to be used and refrigerate them overnight at 2–8°C. After the equilibration period, always store the reagents and calibrators at 2–8°C when not in use and allow them to reach room temperature before use. Store them upright after reconstitution. Do not freeze or expose them to temperatures above 32°C.

Buffer

To prepare the buffer solution from the buffer concentrate:

- Record the date of buffer preparation.
- Pour all of the buffer concentrate into a clean, graduated, plastic or glass container.
- Rinse the concentrate bottle several times with distilled or deionized water, pouring the water into the container each time.
- Add distilled or deionized water to the container until the total volume is 200 mL.* Invert several times to mix thoroughly.

****Refer to analyzer specific protocol for buffer dilution total volume.***

Table 1 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temperature	Recon Volume	Minimum Recon Time 20–25°C	Unopened Stability	Opened Stability
Reagents A and B	2–8°C	3 mL	1 h	Exp date	12 wk
Calibrators	2–8°C	3 mL	1 h	Exp date	12 wk
Buffer	Unopened: 2–8°C Diluted: 20–25°C	200 mL*	None	Exp date	12 wk

Refer to analyzer specific protocol for buffer dilution total volume.

5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, oxalate, and EDTA have been tested and may be used with this assay.
- Sample volume is instrument dependent. Refer to the appropriate analyzer-specific protocol.
- Store the serum or plasma refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.

6 Procedure

Materials Provided

Emit® *tox*™ Serum Benzodiazepine Assay

Reagent A
Reagent B
Buffer

Materials Required But Not Provided in Assay Kit

Emit® *tox*™ Serum Calibrators

Negative Calibrator
Low Calibrator
Medium Calibrator

Other Items:

Graduated container, accuracy within 1% of volume
Distilled or deionized water

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

At the beginning of each workday, assay the Emit® *tox*™ Serum Calibrators. For the rest of the workday, use the Emit® Low Calibrator for qualitative analysis and all three calibrators for semi-quantitative analysis. Recalibrate if you change reagents or as indicated by control results. Refer to the Emit® *tox*™ Serum Calibrators instructions for use or the analyzer-specific protocol.

Quality Control

1. 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 own laboratory. Once the calibration is validated, run samples.
2. Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of Quality Control (QC) material with known serum benzodiazepine 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.

Daily Maintenance

Refer to the instrument operator's guide for maintenance instructions.

7 Results

Emit® *tox*™ Serum Low Calibrator, which contains a stated concentration of 0.3 µg/mL diazepam, is used as a reference for distinguishing "positive" from "negative" samples.

Positive Results

A sample that gives a change in absorbance (ΔA) value equal to or higher than the Low Calibrator's ΔA value is interpreted as positive: The sample contains benzodiazepines.

Negative Results

A sample that gives a change in absorbance (ΔA) value lower than the Low Calibrator's ΔA value is interpreted as negative. Either the sample does not contain any of the benzodiazepines that are detected by this assay, or benzodiazepines are present which produce rates that are less than the rate produced by the Low Calibrator.

Semiquantitative Results

Using the Emit® *tox*™ Serum Benzodiazepine Assay, semiquantitative determinations of benzodiazepines are possible. Where estimates of relative total drug concentrations are desired, a standard curve should be prepared by plotting the ΔA values of the Emit® *tox*™ Serum Negative Calibrator, Low Calibrator, and Medium Calibrator against the calibrator diazepam concentrations. The ΔA values of positive samples may then be compared to this standard curve.

Immunoassays which produce a single result in the presence of multiple components cannot fully quantitate the concentration of individual components. A more specific alternative chemical method must be used to obtain a confirmed analytical result (see Section 1, Intended Use).

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.

8 Limitations

- The assay is designed for use only with human serum or plasma.
- A positive result from the assay indicates the presence of benzodiazepines but does not indicate or measure intoxication.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.
- Severely hemolytic, lipemic, or icteric samples may cause poor reproducibility and questionable results. When such samples are encountered, another specimen should be obtained.

9 Expected Values

When the Emit® *tox*™ Serum Benzodiazepine Assay is used as a qualitative assay, the amount of drugs detected by the assay in any given sample cannot be estimated. The assay results distinguish positive from negative samples—positive indicating samples that contain benzodiazepines.

When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drug and metabolites detected by the assay (see Section 7, Results).

10 Specific Performance Characteristics

Data appearing in this section were collected on the Syva® ETS ® Plus Analyzer.

Performance characteristics of the Emit® *tox*™ Serum Benzodiazepine Assay are affected by all parameters of the measurement. The following information represents total system performance and should not be interpreted to pertain only to reagents.

Sensitivity

The sensitivity of the Emit® *tox*™ Serum Benzodiazepine Assay is 0.07 µg/mL for serum and plasma. Sensitivity is defined as the lowest concentration of diazepam that can be distinguished from 0 µg/mL with a confidence level of 95%.

Precision

Within-run precision was determined using the Emit® *tox*™ Serum Negative Calibrator, Low Calibrator, and Medium Calibrator. Results are in Table 2.

Table 2 — Within-Run Precision

	Study	N	Mean (ΔA)	Standard Deviation (ΔA)	Coefficient of Variation (%)
Negative Calibrator	1	16	315	3	1
(0 µg/mL diazepam)	2	16	316	2.1	0.7
Low Calibrator	1	16	438	5	1.1
(0.3 µg/mL diazepam)	2	16	404	5.2	1.3
Medium Calibrator	1	16	685	14.7	2.1
(2 µg/mL diazepam)	2	16	709	5.3	0.7

Specificity

The Emit® *tox*™ Serum Benzodiazepine Assay detects specific benzodiazepines in serum or plasma. Table 3 lists the concentrations of compounds that will produce a positive result.

Table 3 — Compounds Detected

Compound	Concentration (µg/mL)	Compound	Concentration (µg/mL)
Alprazolam	0.4	Flunitrazepam	0.55
Bromazepam	1.5	Lorazepam	3
Chlordiazepoxide	5	Medazepam	1
Clonazepam	2	Midazolam	0.9
Demoxepam	3	Nitrazepam	1
Desalkylflurazepam	1	Oxazepam	1
N-Desmethyldiazepam	1	Prazepam	1
Diazepam	0.3	Temazepam	1
Flurazepam	3	Triazolam	0.55

Table 4 lists the compounds that are not detected by the assay. Positive results for samples containing other compounds structurally unrelated to benzodiazepines have not been observed.

Table 4 — Compounds Not Detected

Compound	Concentration Tested (µg/mL)	Compound	Concentration Tested (µg/mL)
Acetaminophen	1500	Methaqualone	1000
Acetylsalicylic acid	1000	Morphine	25
Amitriptyline	100	Phencyclidine	1000
Amphetamine	100	Phenytoin	100
Ethchlorvynol	300	Propoxyphene	100
Glutethimide	200	Secobarbital	100
Imipramine	100		

For additional information, contact the Technical Assistance Center or your local Siemens representative.

11 Risk and Safety

H312, H319, H315, H334, H317, H412
P280, P273, P342 + P311, P302 + P312, P501

Danger!

Harmful in contact with skin. Causes serious eye irritation. Causes skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin reaction. Harmful to aquatic life with long lasting effects.

Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF ON SKIN: Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Sodium azide; 2-methyl-3(2h)-isothiazolone, hydrochloride

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

12 Bibliography

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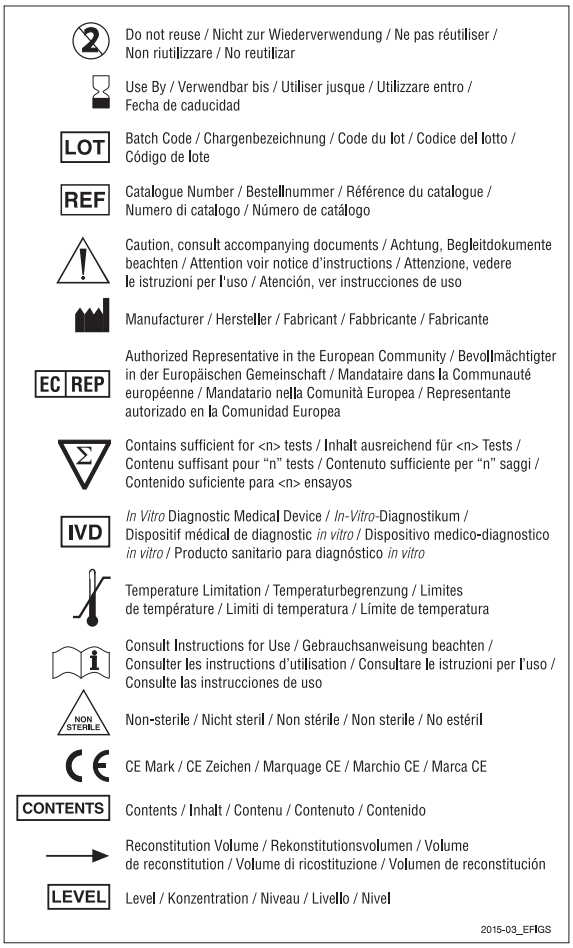
13 Symbols Key



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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