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Syva®

Emit® II *Plus* Ethyl Alcohol Assay

See shaded sections:
Updated information from 2019-08 version.

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Ethyl Alcohol Assay

1 Intended Use

The Emit® II Plus Ethyl Alcohol Assay is intended for use in the quantitative analysis of ethyl alcohol (ethanol) in human urine, serum, or plasma. The Emit® II Plus Ethyl Alcohol Assay is designed for use with most clinical chemistry analyzers.

2 Summary and Explanation of the Test

Alcohol (ethyl alcohol, ethanol) is the most frequently performed medicolegal test, and alcohol the most common toxic substance encountered. In addition to beverages, products containing alcohol in significant amounts include mouthwashes, colognes, and medicinal preparations. Measurements of alcohol levels are used to determine legal impairment, for forensic purposes, in the diagnosis and treatment of alcohol dependency, and in emergency settings to detect alcohol poisoning.

Alcohol's deleterious effects are well documented. It has been linked with birth defects (fetal alcohol syndrome), cardiac conditions, high blood pressure, liver disease, and mental deterioration. It is by far the leading cause of death from hepatic failure. Additionally, alcohol-induced behavior is a contributing factor in the majority of accidents and murders.

Within approximately one hour of ingestion, alcohol will have permeated all tissues of the body in proportion to water content. Some alcohol is absorbed while in the stomach, but the principal site of absorption is the upper portion of the small intestine. Rate of absorption is dependent upon emptying time of the stomach, which is subject to various influences. Since alcohol distributes evenly throughout the body water, its concentration in blood following a known dose may be estimated indirectly by measuring concentrations in urine, serum, or plasma.

About 95% of the elimination of alcohol from the body is accomplished by metabolism in the liver. The remainder is excreted unchanged by the lungs, kidneys, and in the feces. Alcohol is rapidly metabolized so that a moderate dose will clear from the blood in approximately one hour.[1,2,3,4]

Frequently used methods for detecting alcohol in biological fluid are flame-ionization gas chromatography, microdiffusion, and enzymatic assay.[1]

The Emit® II Plus Ethyl Alcohol Assay is designed to measure ethyl alcohol in human urine, serum, or plasma. The Emit® II Plus Ethyl Alcohol Assay should be used to detect ethyl alcohol exclusively and not other alcohols such as isopropanol or methanol. Reactivity with compounds structurally unrelated to ethyl alcohol has not been observed.

3 Principle

The Emit® II Plus Ethyl Alcohol Assay is based on an enzymatic reaction.[4] Reagent 1 contains the buffering system. Reagent 2 contains alcohol dehydrogenase (ADH), the coenzyme nicotinamide adenine dinucleotide (NAD), buffer, preservatives, and stabilizers. The ADH catalyzes the oxidation of ethyl alcohol to acetaldehyde. During this reaction, NAD is reduced to NADH.

$$\text{Ethyl alcohol} + \text{NAD} \xrightleftharpoons{\text{ADH}} \text{NADH} + \text{acetaldehyde}$$

The increase in absorbance at 340 nm is proportional to the concentration of alcohol in the specimen.

4 Reagents

REF	Product Description	Volume
9K039UL/	**Emit® II Plus Ethyl Alcohol Assay Reagent 1**	28 mL/
9K309UL/	Tris buffer, surfactant, and preservatives	115 mL/
9K409UL		1000 mL
	Enzyme Reagent 2	12 mL/
	Alcohol dehydrogenase (525 U/mL), NAD (18 mM), MES, Tris buffer, preservatives, and stabilizers	50 mL/ 435 mL

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

Risk and Safety

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

For *in vitro* diagnostic use.

Preparation and Storage of Assay Components

Reagents

The Emit® II Plus Ethyl Alcohol 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. 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

- The assay requires serum, plasma, or urine.
- Be sure the sample tube is kept tightly closed to prevent evaporation of alcohol.
- Fluoride/oxalate tubes preserve alcohol by preventing glycolysis. They are the preferred method for storing blood prior to analysis of plasma specimens. The anticoagulants citrate, EDTA, fluoride/oxalate, and heparin have been tested and may be used with this assay.
- If not analyzed immediately, specimens may be stored refrigerated at 2–8°C for up to 3 days following collection. After 3 days, specimens should be stored frozen. Repeated freeze-thaw cycles should be avoided. For transporting, maintain the specimen temperature at 2–8°C.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Specimens with high turbidity should be centrifuged before analysis.
- Urine specimens within the pH range of 3.0–11.0 do not require prior adjustment of pH.
- Adulteration of the urine specimen may cause erroneous results. If adulteration is suspected, obtain another specimen.
- Specimens should be handled and treated as if they are potentially infectious.

6 Procedure

Materials Provided

Emit® II Plus Ethyl Alcohol Assay
- Reagent 1
- Reagent 2

Materials Required But Not Provided

9K029UL	Emit® Ethyl Alcohol Negative Calibrator
9K059UL	Emit® Ethyl Alcohol 100 mg/dL Calibrator
9K049UL	Emit® Ethyl Alcohol Low Control
9K079UL	Emit® Ethyl Alcohol High Control

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.

Daily Maintenance

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

Assay Sequence

To run the assay, see the instrument operator's manual and the analyzer-specific protocol from Siemens.

Calibration

Note: These reagents are qualified for use with these calibrators only. However, other material may be used for quality control purposes.

Table 1 — Alcohol Concentrations in Emit® Ethyl Alcohol Calibrators and Controls

	Concentration (mg/dL)	Concentration (%)	Concentration (g/L)
Negative Calibrator	0	0.00	0.00
100 mg/dL Calibrator	100	0.10	1.00
Low Control	36–44	0.036–0.044	0.36–0.44
High Control	270–330	0.27–0.33	2.70–3.30

Run the Emit® Ethyl Alcohol Negative Calibrator and the Emit® Ethyl Alcohol 100 mg/dL Calibrator with each new set of reagents and as indicated by control results. Validate the calibration by running controls (see Quality Control). Refer to the Emit® Ethyl Alcohol Calibrators and Controls instructions for use and the analyzer-specific protocol for additional information and instrument settings. Recalibrate as indicated by control results.

Quality Control

Validate the calibration by assaying controls. Ensure that the control results fall within acceptable limits as defined by your own laboratory. Refer to the instrument operating manual(s) for appropriate instrument checks.

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 ethanol concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.

Once the calibration is validated, run specimens.

Diluting High Concentration Specimens

The assay is linear to a concentration of 600 mg/dL (0.60%, 6.0 g/L) alcohol. Patient specimens containing more than 600 mg/dL (0.60%, 6.0 g/L) alcohol may be diluted with either 1 or 2 parts of the Emit® Negative Alcohol Calibrator or deionized, distilled water. After diluting the specimen, repeat the entire assay sequence and multiply the result by the dilution factor to obtain the true concentration.

Note: Use the original specimen for dilution. Do not take specimen from cup.

7 Results

- Significance of the alcohol level varies on an individual basis and is dependent on factors such as age, weight, sex, adiposity, concurrent presence of other drugs, stomach contents, presence of hypoglycemia, and degree of tolerance. Alcohol levels are directly related to time elapsed since ingestion, type of sample, and, in the case of serum or plasma, site of sampling. Results should be interpreted in light of clinical signs and symptoms.[2]
- 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

- When diluting patient specimens containing high alcohol concentrations, the following factors can affect the result: diluting with the correct fluid (Emit® Ethyl Alcohol Negative Calibrator or deionized, distilled water), and the accuracy of dilution.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

9 Expected Values

The Emit® II Plus Ethyl Alcohol Assay accurately quantifies alcohol concentration in human urine, serum, or plasma containing 10–600 mg/dL (0.01–0.60%, 0.1–6.0 g/L) alcohol.

Note: To convert mg/dL to g/L ethyl alcohol, multiply by 0.01.

Lethal dosage for children has been established at 3 g/kg body weight, but a smaller amount can be lethal in the presence of induced hypoglycemia or drug interactions. Alcohol-tolerant adults have been observed to survive blood concentrations of 1500 mg/dL (1.50%, 15 g/L) with supportive treatment.[2] See Table 2 for further information.

Table 2 — Blood Alcohol Levels[2]

Level	Sporadic Drinkers	Chronic Drinkers
100 mg/dL (0.10%, 1.0 g/L)	Legally intoxicated*	Minimal signs
200–250 mg/dL (0.20–0.25%, 2.0–2.5 g/L)	Alertness lost, becoming lethargic	Effort needed to maintain emotional and motor control
300–350 mg/dL (0.30–0.35%, 3.0–3.5 g/L)	Stupor to coma	Drowsy and slow
>500 mg/dL (>0.50%, >5.0 g/L)	Death possible	Coma

****The legal definition of intoxication varies.***

10 Specific Performance Characteristics

The data appearing in this section were collected on the SYVA®-30R Biochemical System using the Emit® II Plus Ethyl Alcohol Assay and the Emit® Ethyl Alcohol Assay (comparative method).

Accuracy

Urine and serum specimens containing ethyl alcohol were analyzed by the Emit® II Plus Ethyl Alcohol Assay and by the Emit® Ethyl Alcohol Assay. The results were then compared. In a separate study, the Emit® Ethyl Alcohol Assay was compared to gas chromotography (GC). Data are summarized in Table 3.

Table 3 — Summary of Comparative Analysis Data

	Emit® II Plus vs Emit®		Emit® vs GC	
	Serum*	Urine	Serum	Urine
Slope	0.986	0.997	0.991	1.004
Intercept (mg/dL)	-1.02	1.19	3.013	3.0
Correlation	1.000	1.000	0.999	0.992
Standard Error of the Estimate (mg/dL)	1.90	1.67	3.5	9.0
N	98	97	42	57

****Serum and plasma samples were collected from 5 individuals and showed equivalent recovery when spiked with 100 mg/dL of ethanol.***

Precision

Within-run precision was determined using the Emit® Ethyl Alcohol 100 mg/dL Calibrator, Low Control, and High Control. Total precision was determined using data from the calibrator and controls run over a period of 20 days. Precision data were calculated according to the National Committee of Clinical Laboratory Standards (NCCLS) Guideline EP5-A (February 1999). The data are summarized in Table 4.

Table 4 — Analysis of Precision

Calibrator or Control	Mean (mg/dL)	SD (mg/dL)	CV (%)	N
Within-Run Precision				
100 mg/dL Calibrator	98.9	1.44	1.46	80
Low Control	39.8	0.55	1.39	80
High Control	296.2	4.58	1.55	80
Total Precision				
100 mg/dL Calibrator	98.9	1.70	1.72	80
Low Control	39.8	0.71	1.78	80
High Control	296.2	4.78	1.61	80

Specificity

The Emit® II Plus Ethyl Alcohol Assay is designed to detect ethyl alcohol exclusively and not other alcohols such as isopropanol or methanol. Reactivity with compounds structurally unrelated to ethyl alcohol has not been observed.

The assay specificity was tested by conducting studies on the compounds listed in Table 5. An ethyl alcohol-free aqueous matrix was used. Levels tested exceed toxic concentrations. Therefore, interference is not considered to be clinically significant.

Table 5 — Specificity

Compound	Level Tested (mg/dL)	Measured Ethyl Alcohol (mg/dL)	Reactivity*
Acetaldehyde	2000	0	< 1
Acetone	2000	0	< 1
n-Butanol	1000	37	3.7
Ethylene Glycol	2000	0	< 1
Isopropanol	2000	8.5	< 1
Methanol	2000	0	< 1
n-Propanol	1500	213	14.2
Propylene glycol	2000	0	< 1

$$\text{*\% reactivity} = \frac{\text{100 x measured ethyl alcohol (mg/dL)}}{\text{compound tested}}$$

Sensitivity

The sensitivity level of the Emit® II Plus Ethyl Alcohol Assay is less than 3.0 mg/dL (0.003%, 0.03 g/L) for urine, serum, and plasma. This level represents the lowest concentration of ethyl alcohol that can be distinguished from 0 mg/dL with a confidence level of 95%.

Endogenous Substances

No clinically significant interference has been found in specimens to which 800 mg/dL (8.0 g/L) hemoglobin, 750 mg/dL (7.5 g/L) triglycerides, 30 mg/dL (0.3 g/L) bilirubin, or 250 U/mL lactate dehydrogenase (LDH) plus 100 mM lactate were added to simulate hemolytic, lipemic, icteric, or post-mortem specimens.

11 Bibliography

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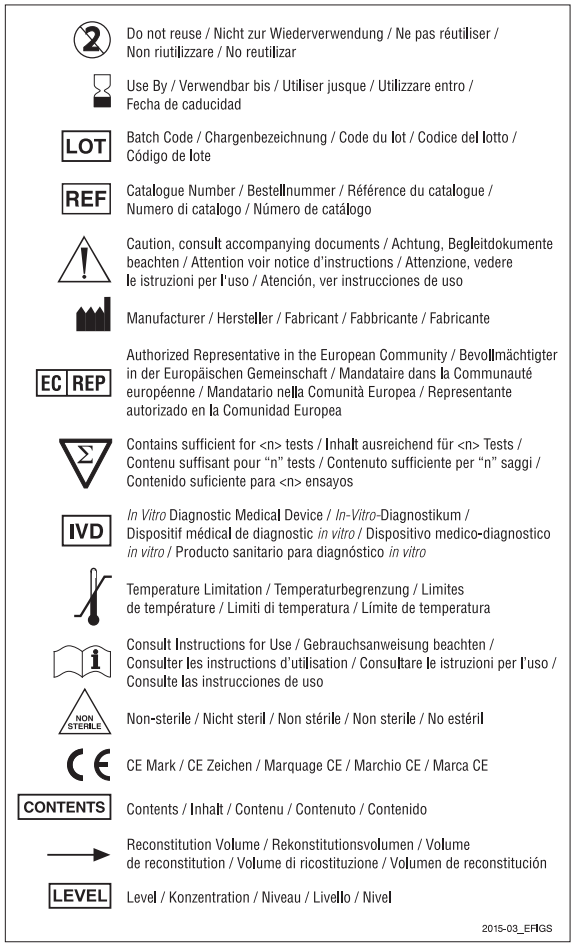
12 Symbols Key



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

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