Emit® II Plus Ethyl Alcohol Assay

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Catalog Number	Product Description	Quantity/ Volume
OSR9K229	**Emit® II Plus Ethyl Alcohol Assay**	
	OSR9K618 R1 (Reagent 1)	2 x 30 mL
	OSR9K648 R2 (Enzyme Reagent 2)	2 x 14 mL
9K029UL	**Emit® Ethyl Alcohol Negative Calibrator***	1 x 3 mL
9K059UL	**Emit® Ethyl Alcohol 100 mg/dL Calibrator***	1 x 3 mL
9K049UL	**Emit® Ethyl Alcohol Low Control***	1 x 3 mL
9K079UL	**Emit® Ethyl Alcohol High Control***	1 x 3 mL

Required for use with the Emit® II Plus Ethyl Alcohol Assay. Sold separately. See Table 1 for alcohol concentrations.

Note: Reagents, calibrators, and controls are shipped ready to use in liquid form. No reconstitution is required.

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

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

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. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

2 SUMMARY

Alcohol (ethyl alcohol, ethanol) is the most frequently performed medicolegal test, and alcohol is 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 principle 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 (see Section 9 under Specificity).

3 METHODOLOGY

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

4 REAGENTS

Reagents contain the following substances:

Tris buffer, surfactant, alcohol dehydrogenase (525 U/mL), NAD (18 mM), MES, preservatives, and stabilizers

Precautions

- For *in vitro* diagnostic use.
- Do not leave alcohol-containing solutions uncapped longer than absolutely necessary. Store tightly capped.
- Do not use the calibrators after the expiration date.

Preparation of Reagents

The Emit® II Plus Ethyl Alcohol 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 upright at 2–8°C and with 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

- The assay requires serum, plasma, or urine.
- Never use alcohol or other volatile disinfectants when collecting or storing blood specimens. Use aqueous zephiran (benzalkonium chloride), iodine, or other suitable aqueous disinfectants.
- 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.
- Preservatives are not required with urine samples. Urine samples should be stored refrigerated with as little dead air space in the sample container as possible. Before opening, invert the container gently several times.
- 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

Emit® Ethyl Alcohol Negative Calibrator
Emit® Ethyl Alcohol 100 mg/dL Calibrator
Emit® Ethyl Alcohol Low Control
Emit® Ethyl Alcohol High Control

Refer to the instrument User's Guide for appropriate instrument checks and maintenance instructions.

Calibration

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 for additional information, and refer to the instrument User's Guide or the Application Sheet for 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. Once the calibration is verified, run specimens.

Diluting High Concentration Samples

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 the analyzer sample tube.

Evaluation and Interpretation of 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]

7 LIMITATIONS OF THE PROCEDURE

- 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 accuracy of the dilution.
- Other substances and/or factors not listed (eg, technical or procedural errors) may interfere with the test and cause false results.

8 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.***

9 SPECIFIC PERFORMANCE CHARACTERISTICS

The information presented in this section is based on Emit® II Plus Ethyl Alcohol Assay studies performed on the AU400®/AU600® Clinical Chemistry System. Positive specimens were confirmed by GC/MS. Refer to the Application Sheets 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 refer only to reagents.

Precision

Within-run precision was calculated according to NCCLS Guideline EP5-A by running two replicates of the 100 mg/dL calibrator with positive and negative controls twice a day for 20 days (N=80). Total precision was also calculated from these data. The data (in mg/dL) are presented in Table 3.

Table 3 — Summary of Within-Run and Total Precision

	Within-Run Precision			Total Precision		
	100 mg/dL	40 mg/dL	300 mg/dL	100 mg/dL	40 mg/dL	300 mg/dL
Mean	100	40	300	100	40	296
SD	0.9	0.5	2.5	2.4	1.7	5.6
%CV	0.8	1.1	0.8	2.4	4.1	1.9

Comparative Analysis

Clinical urine and serum specimens were tested using the Emit® II Plus Ethyl Alcohol Assay on the AU400/AU600 Clinical Chemistry System using the corresponding Emit® II assay on the SYVA®-30R Biochemical System. Results are presented in Table 4.

Table 4 — Comparative Analysis with Emit® II Plus Ethyl Alcohol Assay

		Urine	Serum
Slope		0.969	1.052
Intercept		2.899	-9.617
Mean	SYVA®-30R	114.2	168.8
	AU600	113.5	167.9
Correlation Coefficient		1.000	0.993
Number		50	50

Analytical Recovery

Negative human urine and serum specimens were spiked with ethyl alcohol at concentrations throughout the assay range. Recovery results on the AU600 are listed below.

Table 5 — Analytical Recovery of Emit® II Plus Ethyl Alcohol Assay

Spiked Concentration (mg/dL)	Mean (mg/dL) Urine	Mean (mg/dL) Serum
25	27	28
80	82	80
200	208	190
400	370	375

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 6. An ethyl alcohol-free aqueous matrix was used. Levels tested exceed toxic concentrations; therefore, interference is not considered to be clinically significant.

Table 6 — 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

**** % Reactivity = 100 x (Measured Ethyl Alcohol (mg/dL)/Compound Tested)***

Sensitivity

The minimum detection limit at 95% confidence for the Emit® II Plus Ethyl Alcohol Assay is <10 mg/dL.

10 REFERENCES

1. Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man.* 3rd ed. Chicago, IL: Year Book Medical Publishers Inc; 1989:322–324.
2. Ellenhorn MJ, Barceloux DG. *Medical Toxicology.* New York, NY: Elsevier Science Publishing Company, Inc; 1988:525-526, 782–796.
3. Wyngaarden JB, Smith LH Jr, eds. *Cecil Textbook of Medicine.* Philadelphia, PA: WB Saunders Co; 1988:48–52.
4. Tietz NW, ed. *Textbook of Clinical Chemistry.* Philadelphia, PA: WB Saunders Co; 1986: 1692–1694, 1704–1706.

Symbols Key

	Do not reuse
	Use By
LOT	Batch Code
REF	Catalogue Number
	Caution, consult accompanying documents
	Manufacturer
EC REP	Authorized Representative in the European Community
	Contains sufficient for <n> tests
IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation
	Consult Instructions for Use
NON STERILE	Non-sterile
CE	CE Mark
CONTENTS	Contents
	Reconstitution Volume
LEVEL	Level

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