LZI Ethyl Alcohol Enzymatic Assay

IVD For In Vitro Diagnostic Use Only

REF 0220 (100/37.5 mL R_1/R_2 Kit)
0221 (1000/375 mL R_1/R_2 Kit)

2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) Ethyl Alcohol Assay is intended for the quantitative determination of ethyl alcohol in human urine, serum or plasma. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

Summary and Explanation of Test

Ethyl alcohol can be found in regular alcoholic liquors, a variety of foods, drinks, candies, and medicinal preparations. When alcohol is ingested, it quickly spreads to the whole body and the majority (> 90 %) is metabolized in the liver and excreted with the remainder in urine and serum.
Alcohol intoxication can lead to severe loss of alertness, stupor, coma and death and frequently causes public safety issues. It can also lead to birth defects (fetal alcohol syndrome) (1-5).
Determination of alcohol concentration is commonly used for measuring legal impairment, forensic judgment, diagnosis/treatment of alcohol dependency, and detection of alcohol intoxication. Many different methods are available for determination of alcohol concentration (1, 2) in biological fluid.

Assay Principle

The LZI Ethyl Alcohol Enzymatic Assay is a set of homogeneous ready-to-use liquid reagents based on the unique enzymatic reaction of alcohol dehydrogenase (ADH). In the presence of nicotinamide adenine dinucleotide (NAD), ADH converts ethyl alcohol to acetaldehyde and reduces NAD to NADH. When ethyl alcohol is absent, ADH has limited enzymatic activity with very little absorbance change at a wavelength of 340 nm. The ethyl alcohol concentration is directly proportional to the ADH activity. The enzyme activity is measured at a 340 nm primary wavelength.

$$\text{Ethyl Alcohol} + \text{NAD} \xleftrightarrow{\text{ADH}} \text{NADH} + \text{Acetaldehyde}$$

Reagents Provided

Buffer Reagent (R_1): Contains Tris-based buffer with sodium azide (0.09 %) as a preservative.
Enzyme Reagent (R_2): Contains alcohol dehydrogenase (ADH), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and controls are sold separately and contain negative human urine with 0.09 % sodium azide as a preservative.*

ETHYL ALCOHOL Calibrators	REF
Negative Calibrator	0001
Calibrator #2: Contains 100 mg/dL (0.10 %) ethyl alcohol	0223

ETHYL ALCOHOL Controls	REF
Level 1 Control: Contains 50 mg/dL (0.05 %) ethyl alcohol	0224
Level 2 Control: Contains 300 mg/dL (0.30 %) ethyl alcohol	0225

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (6).*
- *Do not use the reagents beyond their expiration dates.*
- Rx ONLY *For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use.

Specimen Collection and Handling

Serum, plasma, or urine samples may be used for this assay. Specimens should be collected in plastic or glass containers with a tight cap to prevent alcohol evaporation. Anticoagulants such as citrate, EDTA, fluoride-oxalate, and heparin for plasma specimens may be used for this assay.
Use fresh specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days (7). For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown ethanol analytes in urine are stable at -10ºC for up to 12 months (8). Samples should be at room temperature (18-25ºC) and thoroughly mixed prior to analysis. Specimens with high turbidity should be centrifuged before analysis.
Adulteration of a urine sample may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing.
Handle all specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.
Performance characteristics presented in this package insert have been validated on the Hitachi 717. If other instruments are used, performance will need to be validated by the laboratory (9, 10).

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. Typical assay parameters used for the Hitachi 717 analyzer include a 20 µL sample, 200 µL of buffer reagent (R_1), and 75 µL of enzyme reagent (R_2) at 37ºC incubation temperature, 30-35 reading frames, and a 340 nm primary wavelength.

Calibration and Quality Control

Good laboratory practices recommend the use of control specimens to ensure proper assay performance. Both negative and 100 mg/dL calibrators should be used for calibration of the assay for each batch of samples. Use controls to validate the calibration. Recalibrate the instrument whenever different lots of reagents or calibrators are used.

Expected Results

The LZI Ethyl Alcohol Enzymatic Assay accurately quantifies alcohol concentration in human urine, serum, or plasma containing 3 mg/dL to 600 mg/dL (0.003 - 0.60 %) of alcohol.
The legal definition of alcohol intoxication varies. Alcohol metabolism and excretion vary substantially among individuals and is dependent upon many factors such as sex, age, weight, time elapsed since consumption, and health conditions. The following is a general guideline for the significance of blood alcohol level (4).

Level	Sporadic Drinkers	Chronic Drinkers
100 mg/dL (0.1 %)	Legally intoxicated	Minimal Signs
200-250 mg/dL (0.2-0.25 %)	Alertness lost, lethargy	Effort needed to maintain control
300-350mg/dL (0.30-0.35 %)	Stupor to coma	Drowsy, Slow
>500 mg/dL (>0.50 %)	Death possible	Coma

Limitations

1. *The legal alcohol intoxication level varies by age group and region.*
2. *The test results should be interpreted in light of clinical signs and symptoms.*
3. *Ethyl alcohol is volatile. Precaution is suggested during specimen collection and required to prevent alcohol evaporation from calibrators, controls, and samples.*
4. *The test is designed for use with human urine, serum, and plasma only.*

Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer.

Precision:
The precision of the assay is performed as within-run and run-to-run precision using a Hitachi 717 automated clinical chemistry analyzer.

Concentration	Within Run (N=21)			Run-to-Run (N=12)		
	Mean	SD	% CV	Mean	SD	% CV
50 mg/dL	50.0	0.6	1.2 %	50.5	2.3	4.6 %
100 mg/dL	99.1	0.8	0.8 %	99.2	0.6	0.6 %
200 mg/dL	194.8	1.5	0.8 %	200.5	8.3	4.1 %
300 mg/dL	281.6	3.2	1.1 %	279.9	8.3	3.0 %

Sensitivity: The sensitivity, defined as the lowest concentration that can be differentiated from the negative sample with 95 % confidence, is 3 mg/dL (0.003 %) for urine, serum, and plasma samples.

Accuracy: Urine and serum specimens containing ethyl alcohol were analyzed by the commercial Ethyl Alcohol Assay (A), Ethyl Alcohol Test (B), and by the LZI Ethyl Alcohol Enzymatic Assay. Linear regression analyses of the results are summarized in the following table.

	LZI vs A		LZI vs B	
	Serum	Urine	Serum	Urine
# of Samples	55	110	55	110
Slope	1.059	1.08	0.991	1.003
Intercept	21.03	-7.65	11.03	-0.95
Correlation	0.971	0.998	0.995	0.999

Analytical Recovery: The assay is linear up to a concentration of 600 mg/dL. To demonstrate linearity, drug-free urine samples were spiked with various amounts of ethyl alcohol. A linear regression analysis gave the following equation:
$y = 0.95x + 3.5$, $r^2 = 0.999$

Specificity: Various compounds that are structurally related to ethyl alcohol were tested for cross-reactivity. Levels tested exceed toxic concentrations. Therefore, interference is not clinically significant.

Compound	Target [ ] (mg/dL)	% Cross-Reactivity
Acetaldehyde	2000	0 %
Acetone	2000	0 %
n-Butanol	2000	1.5 %
Ethylene Glycol	2000	0 %
Isopropanol	2000	0 %
Methanol	2000	0 %
n-Propanol	2000	11 %

Endogenous Substance: Grossly hemolyzed (800 mg/dL hemoglobin), icteric (30 mg/dL bilirubin) and lipemic (1000 mg/dL triglycerides) samples were found to have no interference with the assay.

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Additions, deletions, or changes are indicated by a change bar in the margin.

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