LZI Ethyl Glucuronide III Enzyme Immunoassay – EU Only

IVD For In Vitro Diagnostic Use Only

200 ng/mL Cutoff

REF 0530 (100/37.5 mL R_1/R_2 Kit)
0531 (1000/375 mL R_1/R_2 Kit) 2°C 8°C

Lin-Zhi International, Inc.

Intended Use

The LZI Ethyl Glucuronide III Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of ethyl glucuronide in human urine at the cutoff value of 200 ng/mL when calibrated against ethyl glucuronide. The assay is designed for prescription use with a number of automated clinical chemistry analyzers.

The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) or (2) permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

Ethyl glucuronide (EtG) is an ethanol metabolite that is formed from the conjugation of ethanol to glucuronic acid (3, 4). Although EtG is a minor metabolite of ethanol, composing less than 0.05 % of the ingested ethanol dose (4, 5), its long detection time has made it an increasingly popular biomarker for alcohol consumption (6-9). Due to rapid metabolism and excretion, the time frame for alcohol detection in urine is normally less than 12 hours (10). Depending on the dosage of ethanol ingested, EtG has a detection window of up to 4 days following elimination of ethanol from the body (4, 6, 7, 11-13).

EtG can be used to screen for recent alcohol intake as well as chronic alcoholism due to its non-volatile and water-soluble properties (14-18).

Study by Jatlow et al. found sensitivity of ethyl glucuronide at a 500 ng/mL cutoff to be insufficient to identify 'low dose' intake of alcohol and only 50 % sensitivity at 12 hours post consumption. The study also found a 200 ng/mL cutoff was sufficient to offer high sensitivity identification of even 'low consumption alcohol at least 12 hours post consumption (19).

Assay Principle

The LZI Ethyl Glucuronide III Enzyme Immunoassay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between ethyl glucuronide in the sample and ethyl glucuronide-labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (20). Enzyme activity decreases upon binding to the antibody, and the ethyl glucuronide concentration in the sample is measured in terms of enzyme activity. In the absence of ethyl glucuronide in the sample, ethyl glucuronide-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when ethyl glucuronide is present in the sample, antibody would bind to free drug; the unbound ethyl glucuronide-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-ethyl glucuronide antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with ethyl glucuronide in buffer with sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

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

ETHYL GLUCURONIDE 200 ng/mL Cutoff Calibrators	REF
Negative Calibrator	0001
Low Calibrator: Contains 100 ng/mL ethyl glucuronide	0532
Cutoff Calibrator: Contains 200 ng/mL ethyl glucuronide	0533
Intermediate Calibrator: Contains 500 ng/mL ethyl glucuronide	0534
High Calibrator: Contains 1000 ng/mL ethyl glucuronide	0535

ETHYL GLUCURONIDE 200 ng/mL Cutoff Controls	REF
Level 1 Control: Contains 150 ng/mL ethyl glucuronide	0537
Level 2 Control: Contains 250 ng/mL ethyl glucuronide	0538

Precautions and Warning

- *This test is for in vitro diagnostic use. 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 (21).*
- *Do not use the reagents beyond their expiration dates.*

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

Urine samples may be collected in plastic or glass containers. Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for at least seven days (22). Samples should be equilibrated to room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to a laboratory for testing.

Handle all urine 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 340 nm 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 Beckman Coulter AU480. If other instruments are used, performance will need to be validated by the laboratory (23, 24).

Assay Procedure

Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis use the 200 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 150 ng/mL and 250 ng/mL.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took a specific drug and a negative test result does not necessarily mean a person did not take a specific drug. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 200 ng/mL of ethyl glucuronide, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (ΔmAU) equal to or greater than that obtained with the cutoff calibrator is considered a preliminary positive. A sample with a change in absorbance (ΔmAU) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, LC/MS or (2) permitting laboratories to establish quality control procedures.

When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of ethyl glucuronide in the sample may then be estimated from the calibration curve.

Limitations

1. *Use of hand-sanitizers with n-propanol should be avoided for 12 hours prior to collection of the sample.*
2. *Ingestion of medications and foods with alcohol should be avoided for 12 hours prior to collection of the sample.*
3. *Ascorbic Acid, Boric Acid, Citric Acid, and Human Serum Albumin (HSA) may cause false negative results. Ascorbic Acid, Boric Acid, and Citric Acid are not recommended as a preservative for urine.*
4. *Interference was observed with pH 3 and pH 4.*
5. *Urinary tract infections may lead to erroneous results (25, 26)*
6. *A preliminary positive result from this assay indicates only the presence of ethyl glucuronide and does not necessarily correlate with the extent of physiological and psychological effects (e.g., intoxication). This test is not intended for quantifying the individual analytes in samples.*
7. *A negative result does not necessarily mean a person did not abuse drugs.*
8. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
9. *Preliminary positive results should be confirmed by other affirmative, analytical methods (e.g., chromatography), preferably GC/MS or LC/MS.*
10. *The test is designed for use with human urine only.*
11. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Beckman AU480 automated chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results were measured in ng/mL.

EtG Concentration	Within Run (N = 22)			Total Precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	-11.3	6.1	N/A	-11.3	8.3	N/A
50 ng/mL	39.4	6.0	15.8%	39.4	7.3	18.4%
100 ng/mL	88.9	6.6	7.6%	88.9	9.0	10.1%
150 ng/mL	140.7	7.6	5.4%	140.7	9.8	7.0%
200 ng/mL	192.4	7.2	3.8%	192.4	10.6	5.5%
250 ng/mL	250.7	8.8	3.5%	250.7	11.8	4.7%
300 ng/mL	307.6	8.5	2.8%	307.6	12.0	3.9%
350 ng/mL	363.0	7.6	2.1%	363.0	10.9	3.0%
400 ng/mL	413.9	8.3	2.0%	413.9	10.6	2.6%

200 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
EtG Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
50 ng/mL	25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	50.0 %	22	22 Neg	88	88 Neg
150 ng/mL	75.0 %	22	22 Neg	88	88 Neg
200 ng/mL	100.0 %	22	18 Neg/ 4 Pos	88	73 Neg/ 15 Pos
250 ng/mL	125.0 %	22	22 Pos	88	88 Pos
300 ng/mL	150.0 %	22	22 Pos	88	88 Pos
350 ng/mL	175.0 %	22	22 Pos	88	88 Pos
400 ng/mL	200.0 %	22	22 Pos	88	88 Pos

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

EtG Concentration	Within Run (N = 22)			Total Precision (N = 88)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	22.3	2.7	12.5%	22.3	3.8	17.0%
50 ng/mL	43.5	2.9	6.6%	43.5	3.5	8.2%
100 ng/mL	65.8	2.6	4.0%	65.8	4.0	6.1%
150 ng/mL	88.4	2.5	2.9%	88.4	3.8	4.3%
200 ng/mL	111.5	3.0	2.7%	111.5	4.1	3.6%
250 ng/mL	133.1	3.4	2.6%	133.1	4.4	3.3%
300 ng/mL	153.4	3.3	2.2%	153.4	4.6	3.0%
350 ng/mL	173.6	2.9	1.7%	173.6	3.8	2.2%
400 ng/mL	191.8	3.1	1.6%	191.8	4.3	2.2%

Qualitative analysis, continued:

200 ng/mL Cutoff		Within Run (N = 22)		Run-to-Run (N = 88)	
EtG Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0.0 %	22	22 Neg	88	88 Neg
50 ng/mL	25.0 %	22	22 Neg	88	88 Neg
100 ng/mL	50.0 %	22	22 Neg	88	88 Neg
150 ng/mL	75.0 %	22	22 Neg	88	88 Neg
200 ng/mL	100.0 %	22	20 Neg/ 2 Pos	88	75 Neg/ 13 Pos
250 ng/mL	125.0 %	22	22 Pos	88	88 Pos
300 ng/mL	150.0 %	22	22 Pos	88	88 Pos
350 ng/mL	175.0 %	22	22 Pos	88	88 Pos
400 ng/mL	200.0 %	22	22 Pos	88	88 Pos

Accuracy: Three hundred and twenty-six (326) unaltered clinical urine specimens were tested with the LZI Ethyl Glucuronide III Enzyme Immunoassay and confirmed by LC/MS. Specimens having an ethyl glucuronide concentration greater than 200 ng/mL by LC/MS are defined as positive, and specimens with an ethyl glucuronide concentration below 200 ng/mL by LC/MS are defined as negative in the table below. Near cutoff samples are defined as ±50 % of the cutoff value. Adjusted LC/MS values have been corrected for cross-reactivity. The correlation results are summarized as follows:

Semi-Quantitative Accuracy Study:

200 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreeent
Positive	0	0	5*	30	177	97.6 %
Negative	28	36	45	5**	0	95.6 %

The following table summarizes the result for the discordant samples:

200 ng/mL Cutoff	LC/MS	Pos/Neg Result	LZI EIA (ng/mL)	Pos/Neg Result
92*	168	-	213.3	+
99*	180	-	288.7	+
102*	184	-	242.8	+
104*	185	-	214	+
106*	189	-	246.8	+
115**	204	+	127.1	-
116**	206.1	+	153.7	-
119**	213	+	135.4	-
121**	229	+	158.6	-
131**	267	+	187	-

Qualitative Accuracy Study:

200 ng/mL Cutoff	Neg	< 50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	High Pos	% Agreemet
Positive	0	0	6*	31	177	98.1 %
Negative	28	36	44	4**	0	94.7 %

The following table summarizes the result for the discordant samples:

200 ng/mL Cutoff	LC/MS	Pos/Neg Result	LZI EIA (ng/mL)	Pos/Neg Result	Cutoff Rate
92*	168	-	126.0	+	121.4
99*	180	-	157.0	+	121.4
102*	184	-	135.4	+	121.8
104*	185	-	129.9	+	121.4
106*	189	-	143.4	+	121.4
113*	196	-	131.5	+	121.8
115**	204	+	87.4	-	120.1
116**	206.1	+	104.0	-	122.3
119**	213	+	98.4	-	120.4
121**	229	+	95.4	-	120.1

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section) of the entire assay range, pooled negative human urine spiked with ethyl glucuronide at 1000 ng/mL was serially diluted. Each sample was run in 10 replicates and the average was used to determine percent recovery compared to the expected target value. The recovery within the linear range of the assay ranged from 92.8 % to 107.7 %.

Target Concentration (ng/mL)	Determined (ng/mL)	% Recovery
1000	1022.8	102.3%
900	954.6	106.1%
800	857.9	107.2%
700	754.1	107.7%
600	626.1	104.4%
500	492.6	98.5%
400	395.6	98.9%
300	288.8	96.3%
200	185.6	92.8%
100	95.5	95.5%
50	42.5	85.0%
0	4.6	N/A

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into pooled negative human urine to various concentrations and evaluated against the cutoff calibrator.

The following table lists the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator (as negative). Compounds tested at high concentration with results below the cutoff value were listed as Not Detected (ND).

Structurally Related Compounds:

Cross-reactant	Concentration (ng/mL)	% Cross-reactivity
Ethyl Glucuronide (EtG)	200	104.3%
Acetaldehyde	1,000,000	0.0%
Ethyl Sulfate (EtS)	1,000,000	0.0%
Butanol	1,000,000	0.0%
2-Chloroethanol	1,000,000	0.0%
Ethanol	1,000,000	0.0%
Ethylene Glycol	1,000,000	0.0%
Isopropanol	1,000,000	0.0%
Methanol	1,000,000	0.0%
n-Propanol	1,000,000	0.0%
Alprazolam Glucuronide	100,000	0.0%
Acetaminophen-D-glucuronide	100,000	0.0%
Buprenophine-3β-D-glucuronide	10,000	1.0%
Butyl-β-D-glucopyranoside	100,000	0.0%
d-Glucose	30,000,000	0.0%
Ethyl α-D-glucopyranoside	100,000	0.0%
Ethyl β-D-glucopyranoside	100,000	0.3%
Glucuronic Acid	1,000,000	0.0%
Isopropyl-β-D-glucuronide	7,600	3.1%
Methyl-β-D-glucuronide	7,000	3.5%
Morphine-3-glucuronide	100,000	0.0%
Morphine-6-glucuronide	100,000	0.0%
4-Nitrophenyl-β-D-glucuronide	100,000	0.0%
NorBuprenorphine-glucuronide	10,000	0.5%
n-Propyl-β-D-glucuronide	1,960	10.3%
Oxazepam Glucuronide	10,000	1.0%
Phenyl-β-D-glucuronide	100,000	0.0%
Termazepam Glucuronide	10,000	0.9%
Trichloroethyl β-D-glucuronide	100,000	0.0%

Structurally Unrelated Compounds:

The following structurally unrelated compounds were spiked into pooled negative human urine and the two levels of controls (150 ng/mL and 250 ng/mL) for the assay. The spiked solution was evaluated against the assay's calibration curve.

Interference was observed with benzoylecgonine, codeine, hydromorphone, MDA, MDEA, MDMA, and morphine. No other major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 200 ng/mL. Results are summarized in the following table:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Ethyl Glucuronide Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Acetaminophen	100,000	ND	Neg	Pos
6-Acetylmorphine	10,000	ND	Neg	Pos
Acetylsalicylic Acid	100,000	ND	Neg	Pos
Amitriptyline	100,000	ND	Neg	Pos
Amlodipine Besylate	100,000	ND	Neg	Pos
Amoxicillin	100,000	ND	Neg	Pos
d-Amphetamine	100,000	ND	Neg	Pos
Atorvastatin	20,000	ND	Neg	Pos
Benzoylecgonine	100,000	ND	Pos	Pos
Buprenorphine	15,000	ND	Neg	Pos
Bupropion	100,000	ND	Neg	Pos
Caffeine	100,000	ND	Neg	Pos
Carbamazepine	100,000	ND	Neg	Pos
Cetirizine	100,000	ND	Neg	Pos
Chlorpheniramine	100,000	ND	Neg	Pos
Chlorpromazine	100,000	ND	Neg	Pos
Clomipramine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Pos	Pos
Desipramine	100,000	ND	Neg	Pos
Diphenhydramine	100,000	ND	Neg	Pos
Duloxetine	100,000	ND	Neg	Pos
Fentanyl	10,000	ND	Neg	Pos
Fluoxetine	100,000	ND	Neg	Pos
Fluphenazine	100,000	ND	Neg	Pos
Gabapentin	100,000	ND	Neg	Pos
Hydrocodone	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Pos	Pos
Ibuprofen	100,000	ND	Neg	Pos
Imipramine	100,000	ND	Neg	Pos
Lisinopril	100,000	ND	Neg	Pos
Losartan	10,000	ND	Neg	Pos
Loratidine	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Pos	Pos
MDEA	100,000	ND	Pos	Pos
MDMA (3,4-methylenedioxy-methamphetamine)	100,000	ND	Pos	Pos
Meperidine	100,000	ND	Neg	Pos

Structurally Unrelated Compounds, continued:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Ethyl Glucuronide Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Metformin	100,000	ND	Neg	Pos
Metoprolol	100,000	ND	Neg	Pos
Methadone	100,000	ND	Neg	Pos
d-Methamphetamine	100,000	ND	Neg	Pos
Morphine	100,000	ND	Pos	Pos
Nicotine	100,000	ND	Neg	Pos
Nortriptyline	100,000	ND	Neg	Pos
Omeprazole	100,000	ND	Neg	Pos
Oxazepam	100,000	ND	Neg	Pos
Oxycodone	100,000	ND	Neg	Pos
Oxymorphone	100,000	ND	Neg	Pos
Phenobarbital	100,000	ND	Neg	Pos
(1S,2S)-(+) Pseudoephedrine	100,000	ND	Neg	Pos
Quetiapine	100,000	ND	Neg	Pos
Ranitidine	100,000	ND	Neg	Pos
Salbutamol (Albuterol)	100,000	ND	Neg	Pos
Sertraline	100,000	ND	Neg	Pos
THC-COOH (11-Nor-Δ^9-THC-9-carboxylic acid)	1,000	ND	Neg	Pos
l-Thyroxine	10,000	ND	Neg	Pos
Tramadol	100,000	ND	Neg	Pos
Zolpidem	10,000	ND	Neg	Pos

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

The following structurally unrelated compounds which showed interference at ±25 % of cutoff concentrations were then spiked into pooled negative human urine at ±50 % of cutoff concentrations (100 ng/mL and 300 ng/mL) for the 200 ng/mL cutoff. No interference was observed at ±50 % of cutoff concentrations. Results are summarized in the following table:

Cross-reactant	Spiked [ ] (ng/mL)	Spiked Ethyl Glucuronide Concentration		
		0 ng/mL	100 ng/mL Control	300 ng/mL Control
Benzoylecgonine	100,000	ND	Neg	Pos
Codeine	100,000	ND	Neg	Pos
Hydromorphone	100,000	ND	Neg	Pos
MDA (3,4-methylenedioxyamphetamine)	100,000	ND	Neg	Pos
MDEA	100,000	ND	Neg	Pos
MDMA (3,4-methylene-dioxymethamphetamine)	100,000	ND	Neg	Pos
Morphine	100,000	ND	Neg	Pos

Endogenous and Preservative Compound Interference Study:

The following endogenous compounds were spiked into pooled negative human urine and the two levels of controls (150 ng/mL and 250 ng/mL) for the assay. The spiked solution was evaluated against the assay's calibration curve.

Interference was observed with Acetone, Ascorbic Acid, Boric Acid, Citric Acid, Human Serum Albumin (HSA), and Sodium Fluoride. No other major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct corresponding preliminary positive/negative results against the cutoff value of 200 ng/mL. Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked EtG Concentration		
		0 ng/mL	150 ng/mL Control	250 ng/mL Control
Acetone	1000	Neg	Pos	Pos
Ascorbic Acid	1500	Neg	Neg	Neg
Bilirubin	2	Neg	Neg	Pos
Boric Acid	1000	Neg	Neg	Neg
Calcium Chloride ($CaCl_2$)	300	Neg	Neg	Pos
Citric Acid (pH 3)	800	Neg	Neg	Neg
Creatinine	500	Neg	Neg	Pos
Ethanol	1000	Neg	Neg	Pos
Galactose	10	Neg	Neg	Pos
γ-Globulin	500	Neg	Neg	Pos
Glucose	3000	Neg	Neg	Pos
Hemoglobin	300	Neg	Neg	Pos
β-hydroxybutyric Acid	100	Neg	Neg	Pos
Human Serum Albumin	500	Neg	Pos	Pos
Oxalic Acid	100	Neg	Neg	Pos
Potassium Chloride	6000	Neg	Neg	Pos
Riboflavin	7.5	Neg	Neg	Pos
Urea	6000	Neg	Neg	Pos
Uric Acid	10	Neg	Neg	Pos
Sodium Azide	1000	Neg	Neg	Pos
Sodium Chloride	6000	Neg	Neg	Pos
Sodium Fluoride	1000	Neg	Pos	Pos
Sodium Phosphate	300	Neg	Neg	Pos

Endogenous and Preservative Compound Interference Study, continued:
The following endogenous compounds which showed interference at ±25 % of cutoff concentrations were then spiked into pooled negative human urine at ±50 % of cutoff concentrations (100 ng/mL and 300 ng/mL) for the assay. Interference was still observed with Ascorbic Acid, Boric Acid, Citric Acid, and Human Serum Albumin (HSA). Results are summarized in the following table:

Endogenous Substance	Spiked [ ] (mg/dL)	Spiked Ethyl Glucuronide Concentration		
		0 ng/mL	100 ng/mL	300 ng/mL
Acetone	1000	Neg	Neg	Pos
Ascorbic Acid	1500	Neg	Neg	Neg
Boric Acid	1000	Neg	Neg	Neg
Citric Acid (pH 3)	800	Neg	Neg	Neg
HSA	500	Neg	Pos	Pos
Sodium Fluoride	1000	Neg	Neg	Pos

pH Interference Study:
Pooled negative human urine and urine spiked with analyte to the two levels of controls (150 ng/mL and 250 ng/mL) were adjusted to the following pH levels and tested by the assay. The pH adjusted solutions were evaluated against the assay's calibration curve.
Interference was observed with pH 3 and pH 4. All other pH adjusted levels gave correct corresponding preliminary positive/negative results against the cutoff value of 200 ng/mL. Results are summarized in the following table:

pH	Spiked Ethyl Glucuronide Concentration		
	0 ng/mL	150 ng/mL Control	250 ng/mL Control
pH 3	Neg	Neg	Neg
pH 4	Neg	Neg	Neg
pH 5	Neg	Neg	Pos
pH 6	Neg	Neg	Pos
pH 7	Neg	Neg	Pos
pH 8	Neg	Neg	Pos
pH 9	Neg	Neg	Pos
pH 10	Neg	Neg	Pos
pH 11	Neg	Neg	Pos

Specific Gravity: Samples ranging in specific gravity from 1.000 to 1.030 were split into three portions each and either left un-spiked or further spiked to a final ethyl glucuronide concentration of either 150 ng/mL or 250 ng/mL (the negative and positive control concentrations, respectively). These samples were then evaluated in semi-quantitative and qualitative modes. No interference was observed.

Bibliography:

1. Urine Testing for Drug of Abuse, National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Mandatory Guidelines for Federal Workplace Drug Testing Program, National Institute on Drug Abuse, Federal Register, **53** (69):11970 (1988).
3. Schmitt G., Aderjan R., Keller, T., Wu, M., Ethyl glucuronide: an unusual ethanol metabolite in humans. Synthesis, analytical data, and determination in serum and urine, *J. Anal. Toxicol.*, **19**(2):91-4 (1995).
4. Dahl, H., Stephanson, N., Beck, O., and Helander, A., Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide, *J Anal Toxicol*, **26**(4):201-4 (2002).
5. Helander, A., and Beck, O., Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake, *J Anal Toxicol.*, **29**(5):270-4 (2005).
6. Bergström, J., Helander, A., and Jones, A.W**.,** Ethyl glucuronide concentrations in two successive urinary voids from drinking drivers: relationship to creatinine content and blood and urine ethanol concentrations, *Forensic Sci Intl.*, **133**(1-2):86-94 (2003).
7. Wurst, F.M., and Metzger, J.**,** The ethanol conjugate ethyl glucuronide is a useful marker of recent alcohol consumption, *Alcohol Clin Exp Res.*, **26**(7):1114-9 (2002).
8. Jones, A.W., Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship, *Toxicol Rev.*, **25**(1):15-35 (2006).
9. Høiseth, G., Bernard, J.P., Karinen, R., Johnsen, L., Helander, A., Christophersen, A.S., and Mørland, J., A pharmacokinetic study of ethyl glucuronide in blood and urine: applications to forensic toxicology, *Forensic Sci Intl.*, **172**(2-3):119-24 (2007).
10. Helander, A., Beck, O., and Jones, A.W., Laboratory testing for recent alcohol consumption: comparison of ethanol, methanol, and 5-hydroxytryptophol, *Clin. Chem.*, **42**(4):618-24 (1996).
11. Schmitt, G., Droenner, P., Skopp, G., and Aderjan, R, Ethyl glucuronide concentration in serum of human volunteers, teetotalers, and suspected drinking drivers, *J. Forensic Sci.* **42**(6):1099-102 (1997).
12. Borucki, K., et al., Detection of recent ethanol intake with new markers: comparison of fatty acid ethyl esters in serum and of ethyl glucuronide and the ratio of 5-hydroxytryptophol to 5-hydroxyindole acetic acid in urine, *Alcohol Clin Exp Res.* **29**(5):781-7 (2005).

Bibliography, continued:

13. Stephanson, N., Dahl, H., Helander, A., and Beck, O., Direct quantification of ethylglucuronide in clinical urine samples by liquid chromatography-mass spectrometry, *Ther Drug Monit*, **24**(5):645–51 (2002).
14. Seidi, S., et al., Ethyl glucuronide – A Biological Marker for recent alcohol consumption, *Addiction Biology*, **6**(3):205-212 (2001).
15. Kipper, G.E., et al., Ethyl glucuronide: A Biomarker to identify Alcohol use by Health Professionals Recovering from Substance use Disorders, *Alcohol and Alcoholism*, **39**(5): 445-449 (2004).
16. Wurst, F.M., et al., Ethyl glucuronide – A marker of Recent Alcohol Consumption with Clinical and Forensic Implications, *Alcohol*, **20**(2):111-116 (2000).
17. Skipper, G.E., et al., Ethyl glucuronide (EtG): A new marker to detect Alcohol use in recovering physicians, *Journal of Medical Licensure and Discipline*, **90**(2):14-17 (2004).
18. Wurst, F.M., et al., Ethyl glucuronide Discloses Recent Covert Alcohol Use Not Detected by Standard Testing in Forensic Psychiatric Inpatients, *Alcoholism: Clinical and Experimental Research*, **20**(3):471-476 (2003).
19. Jatlow, M.D., et al., Ethylglucuronide and Ethyl Sulfate Assays in Clinical Trials, Interpretation and Limitations: Results of a Dose Ranging Alcohol Challenge Study and Two Clinical Trials. *Alcohol Clin Exp Res.* **38**(7):2056-2065 (2014).
20. Rubenstein, K.E., Schneider, R.S., and Ullman, E.F., Homogeneous Enzyme Immunoassay: A New Immunochemical Technique, *Biochem Biophys Res Commun*, **47**:46 (1972).
21. Sodium Azide. National Institute for Occupational Safety (NIOSH). Pocket Guide to Chemical Hazards. Third Printing, September 2007. Available online at: https://www.cdc.gov/niosh/npg/default.html
22. Schloegl, H., et al., Stability of ethyl glucuronide in urine, post-mortem tissue and blood samples, *International journal of legal medicine,***120**(2):83-88 (2006).
23. Nichols, J., Instrument Validation: The Road to Success. CLN's Lab 2004: From Basic to Advanced Series. 14-16 (2004).
24. CDRH Guidance for Industry and FDA Staff: Replacement Reagent and Instrument Family Policy (2003).
25. Walsham, N.E. and Sherwood, R.A. Ethyl glucuronide. *Ann Clin Biochem* **49**(2):110–117 (2012).
26. Helander, A. and Dahl, H. Urinary tract infection: a risk factor for false negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. *Clin Chem* **51**(9):1728–30 (2005).

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