One Step Ethyl Glucuronide (EtG) Test

(Dip Card)

For Forensic Use Only

INTENDED USE

The **One Step Ethyl Glucuronide (EtG) Test** is a lateral flow chromatographic immunoassay for the qualitative detection of Ethyl Glucuronide (EtG) in human urine specimen at the cut-off level of 300ng/mL. This assay is intended for forensic use only.
This assay provides only a preliminary qualitative test result. A more specific confirmatory reference method, such as liquid chromatography tandem mass spectrometry (LC/MS/MS) or gas chromatography/mass spectrometry (GC/MS) must be used in order to obtain a confirmed analytical result.

SUMMARY AND EXPLANATION OF THE TEST

Ethyl Glucuronide (EtG) is a direct metabolite of ethanol, which is formed by enzymatic conjugation of ethanol with glucuronic acid.[1, 2] Alcohol in urine is normally detected for only a few hours, whereas EtG can be detected up to several days even after complete elimination of alcohol from the body.[3] Therefore, EtG can be a useful diagnostic biomarker for determining recent alcohol use and in monitoring abstinence in alcoholics in alcohol withdrawal treatment programs.[4-7] Ethanol can be produced *in vitro* due to fermentation of urine samples containing sugars, bacteria or yeast when samples are exposed to warm temperatures.[8] In such cases, EtG test can be used, as a confirmatory test to determine if the alcohol in the sample is due to consumption of alcohol or it is formed *in vitro* as a result of fermentation. Currently EtG is monitor by GC/MS and LC/MS/MS.[9-10]
Ethyl glucuronide (EtG) is a minor non-oxidative metabolite of ethyl alcohol formed by the in vivo conjugation of ethanol with glucuronic acid with UDP glucuronosyltransferase. EtG is a product of metabolic process of ingested alcohol (ethanol) rapidly metabolized in the body, which is excrete in the blood, hair and urine. By using, the **One Step Ethyl Glucuronide (EtG) Test** EtG can be detect in urine, confirming the consumption of alcohol. The EtG metabolite remains in the body longer and therefore has a more useful window of detection of 8 to 80 hours. EtG testing is an excellent option for zero-tolerance alcohol consumption or for rehabilitation programs.
The **One Step Ethyl Glucuronide (EtG) Test** yields a positive result when the concentration of Ethyl Glucuronide in urine exceeds 300ng/mL.

PRINCIPLE

The **One Step Ethyl Glucuronide (EtG) Test** is an immunoassay based on the principle of competitive binding. Metabolite of ethyl alcohol analyte, which may be present in the urine specimen, compete against its respective ethyl glucuronide conjugate for binding sites on its specific antibody.
During testing, urine specimen migrates upward by capillary action. If ethyl glucuronide is present in the urine specimen below its cut-off concentration, it will not saturate the binding sites of its specific antibody. The antibody will then react with the ethyl glucuronide conjugate and a visible colored line will show up in the test line region of the specific ethyl glucuronide strip. The presence of ethyl glucuronide above the cut-off concentration will saturate the binding sites of its antibody. Therefore, the colored line will not form in the test line region.
An ethyl glucuronide-positive urine specimen will not generate a colored line in the specific test line region of the strip because of competitive binding, while an ethyl glucuronide-negative urine specimen will generate a line in the test line region because of the absence of competitive binding. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been applied and membrane wicking has occurred.

REAGENTS

The test contains a membrane strip coated with ethyl glucuronide conjugate (purified bovine albumin) at the test line, a goat polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with rabbit monoclonal antibody specific to ethyl glucuronide.

PN:Y0311146601

PRECAUTIONS

- For Forensic Use Only.
- For single use only.
- Do not use after the expiration date.
- The dip card should remain in the sealed pouch until ready to use.
- Use of gloves is recommended to avoid unnecessary contact with the specimen.
- The used dip card and urine specimen should be discarded according to federal, state and local regulations.

STORAGE AND STABILITY

Store in original package at 2°C - 30°C (36°F - 86°F). DO NOT FREEZE. The test is stable through the expiration date printed on the labels.

SPECIMEN COLLECTION AND PREPARATION

The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible precipitates should be allowed to settle to obtain a clear specimen for testing.

SPECIMEN STORAGE

Urine specimen collected for later testing may be stored at 2°C - 8°C (36°F - 46°F) for up to 48 hours. For prolonged storage, specimens may be frozen and stored at below -20°C. Frozen specimens should be thawed and mixed well before testing.

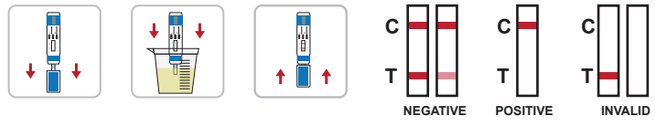
MATERIALS

Materials Provided:
- Dip Cards
- Desiccants
- Package Insert

Materials Required But Not Provided:
- Specimen Collection Container
- Disposable Gloves
- Timer

DIRECTIONS FOR USE

1) Remove the dip card from the foil pouch.
2) Remove the cap from the dip card. Label the device with patient or control identifications.
3) Immerse the absorbent tip into the urine sample for 5 seconds. Urine sample should not touch the plastic device.
4) Replace the cap over the absorbent tip and lay the dip card on a clean, flat, and non-absorptive surface.
5) Read result at 5 minutes. **DO NOT INTERPRET RESULT AFTER 10 MINUTES.**



INTERPRETATION OF RESULTS

(Please refer to the previous illustration)
NEGATIVE: Two colored lines appear, one in the control region (C), and another one in the adjacent test region (T).* This negative result indicates that the ethyl glucuronide concentration is below the detectable level.
*NOTE: The shade of color of the line(s) may vary, but it should be considered negative whenever there is even a faint distinguishable color line.

POSITIVE: One colored line appears in the control region (C). No line appears in the test region (T).This positive result indicates that the ethyl glucuronide concentration is above the detectable level.

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test dip card. If the problem persists, discontinue using the lot immediately and contact your supplier.

QUALITY CONTROL

A procedural control is included in the test. A color line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

LIMITATIONS

1. The **One Step Ethyl Glucuronide (EtG) Test** provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Liquid chromatography tandem mass spectrometry (LC/MS/MS) or gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.
2. There is a possibility that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. A positive result does not indicate intoxication of the donor, the concentration of ethyl glucuronide in the urine.
4. A negative result may not necessarily indicate ethyl glucuronide-free urine. Negative results can be obtained when ethyl glucuronide is present but below the cut-off level of the test.
5. If adulteration is suspected, the test should be repeated with a new urine specimen and a new dip card.
6. Apply clinical and professional judgment to ethyl glucuronide test result, particularly when preliminary positive result is obtained.

PERFORMANCE CHARACTERISTICS

Precision

A study was conducted in an effort to determine the precision of the **One Step Ethyl Glucuronide (EtG) Test**. Testing was conducted using three different lots of product to demonstrate the within-run and between-run precision. The correlation with expected results for the solutions targeted to ±50% and 2X of the cut-off level was > 99% across all lots.

Ethyl Glucuronide（EtG）

Ethyl Glucuronide Concentration (ng/mL)	Number of Test Samples	Positive			Negative		
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
No Drug Present	70	0	0	0	50	10	10
150	70	0	0	0	50	10	10
450	70	50	10	10	0	0	0
600	70	50	10	10	0	0	0

Analytical Sensitivity

The cut-off concentration of the **One Step Ethyl Glucuronide (EtG) Test** is determined to be 300 ng/mL. Testing should be run in 30 replicates with negative urine and standard control at ±25% cut-off, ±50% cut-off and 2X cut-off concentration levels. Test results are summarized below.

Percent of Cut-off EtG Concentration in ng/mL	n	Test Result	
		Negative	Positive
0% Cut-off (0ng/mL)	30	30	0
-50% Cut-off (150ng/mL)	30	30	0
-25% Cut-off (225ng/mL)	30	30	0
Cut-off (300ng/mL)	30	3	27
+25% Cut-off (375ng/mL)	30	1	29
+50% Cut-off (450ng/mL)	30	0	30
2X Cut-off (600ng/mL)	30	0	30

Analytical Specificity

To evaluate the specificity of the test, ethyl glucuronide metabolites and other structurally related compounds which are likely to be present in the urine specimen were added to ethyl glucuronide-free normal human urine and tested with the **One Step Ethyl Glucuronide (EtG) Test**. Positive results were produced when the concentrations are equal to or greater than the levels listed below for each compound.

Compound	Concentration (ng/mL)
Ethyl-β-D-glucuronide	300

INTERFERENCE

Potentially 100µg/mL interfering substances were also added to ethyl glucuronide-free urine. None of the following substances at the concentration tested interfered with the **One Step Ethyl Glucuronide (EtG) Test**.

Acebutolol Hydrochloride
Acepromazine-d6 Hydrochloride
Acetaminophen
N-Acetylprocainamide
Acetophenetidin
Amoxicillin
Ampicillin
Amitriptyline Hydrochloride
S(-)-Amphetamine
R(-)-Amphetamine
Amobarbital
(±)Amphetamine
R-(-)-Apomorphine Hydrochloride Hemihydrate
Aspirin
Aspartame
L-Ascorbic Acid
Atropine
Benzphetamine HCL
Benzilic Acid
Benzoylecgonine
SS Benzoic Acid
Bilirubin, Mixed Isomers
Brompheniramine Maleate
Buspirone Hydrochloride
Butabarbital
Cannabidiol
Cannabinol
Caffeine
Chlordiazepoxide HCL
Chlorothiazide
Chloroquine Diphosphate
Chlorpheniramine Maleate
Chlorpromazine Hydrochloride
Chloramphenicol
Chloral Hydrate
Cholesterol
Chlorothiazide
Clomipramine Hydrochloride
Clonidine Hydrochloride
(-) Cotinine
Cocaethylene
Cocaine Hydrochloride
Codeine
Cortisone
Creatinine
Dextromethorphan
Diazepam
Diclofenac Sodium
Dicyclomine
Diflunisal
Digoxin
4-Dimethylaminoantipyrine
5,5-Diphenylhydantoin
Diphenhydramine
Dopamine Hydrochloride
Doxylamine Succinate Salt
Ecgonine Methyl Ester
Ecgonine HCL
Efavirenz
Emetine Dihydrochloride Hydrate
(-)-Epinephrine
Ephedrine-(±) Hydrochloride
(-)-Ephedrine HCL
(1R,2S)-(-)-Ephedrine
Erythromycin
Estradiol
Estrone-3-Sulfate Potassium Salt
Ethyl-P-Aminobenzoate
Fenoprofen Calcium Salt Hydrate
Furosemide
Gentisic Acid
D-Glucuronic Acid
Glutethimide
Guaifenesin (Guaiacol Glyceryl Ether)
Hemoglobin Porcine
Hippuric Acid
Hydralazine Hydrochloride
Hydrocodone
α-Hydroxyhippuric Acid
21-Hydroxyprogesterone
p-Hydroxymethamphetamine
Hydrocortisone
Hydrochlorothiazide
(±)- 4-Hydroxyamphetamine HCL
Ibuprofen
Imipramine HCL
Iprazid
Isoxsuprine Hydrochloride
Isoproterenol Hydrochloride
Ketamine Hydrochloride
Ketoprofen
Labetalol Hydrochloride
Levorphanol
Loperamide Hydrochloride
Loxapine Succinate Salt
Maprotiline Hydrochloride
(±)-3,4-Methylenedioxyethylamphetamine
(±)-3,4-Methylenedioxyamphetamine
Meperidine
Meprobamate
Methamphetamine Hydrochloride
(±)Methadone
S(+)-Methamphetamine
L-methamphetamine
Methoxyphenamine Hydrochloride
Methylphenidate
(±)-3,4-Methylenedioxymethamphetamine
Methyprylon
Morphine-3-β-D-Glucuronide
Morphine Sulfate Salt Solution
Nalidixic Acid
Nalorphine Hydrochloride
Naproxen
Naloxone
Naltrexone Hydrochloride
Nicotinamide (Vitamin B3)
Nimesulide
Nifedipine
Norcodeine
Nordoxepin Hydrochloride
Norethisterone
D-Norpropoxyphene Maleate Salt
Noscapine HCL Hydrate
Noroxymorphone HCL
Nylidrin Hydrochloride
(±)-Octopamine HCL
Oxalic Acid
Oxazepam
Oxolinic Acid
Oxycodone
Oxymetazoline Hydrochloride
Papaverine Hydrochloride
Phencyclidine
Pentobarbital
Pentazocine
Perphenazine
Penicillin G Sodium Salt
Phenelzine Sulfate Salt
Phenobarbital
Phentermine HCL
Phenylethylamine
L-Phenylephrine
Phenylpropanolamine Hydrochloride
Prednisolone
Prednisone Acetate
Procaine HCL
Promazine Hydrochloride
Promethazine
D-Propoxyphene
Propranolol Hydrochloride
Pseudoephedrine HCL
Quinine
Quinidine
Quinacrine Dihydrochloride
Ranitidine Hydrochloride
Salicylic Acid
Secobarbital
Serotonin HCL
Sertraline HCL
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrozoline Hydrochloride
Tetrahydrocortisone 3-(β-D-Glucuronide)
Thebaine
Theophylline
Thioridazine
Thiamine, (Vitamin B1) HCL
L-Thyroxine
Tolbutamide
Trimethoprim
Trazodone Hydrochloride
Triamterene
Trimipramine
Tryptamine
Trifluoperazine Dihydrochloride
DL-Tryptophan
Trans-2-Phenylcyclopropylamine Hydrochloride
DL-Tyrosine
Tyramine
Uric Acid
Verapamil Hydrochloride
Zomepirac Sodium Salt

EFFECT OF SPECIMEN SPECIFIC GRAVITY

The urine samples of normal, high, and low specific gravity ranges from 1.005-1.030 were spiked with ethyl glucuronide analyte at 50% below and 50% above cut-off level respectively and tested using the **One Step Ethyl Glucuronide (EtG) Test**. The results demonstrate that varying ranges of specimen specific gravity do not interfere with the performance of the test.

EFFECT OF SPECIMEN PH

The pH of an aliquot negative urine pool was adjusted to pH ranges of 4.0 - 9.0, and spiked with ethyl glucuronide at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with the **One Step Ethyl Glucuronide (EtG) Test**. The results demonstrate that varying ranges of specimen pH do not interfere with the performance of the test.

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