Ethylglucoronide (ETG) Dip Card Tests

A rapid, one-step screening test for the qualitative detection of Ethylglucoronide (ETG) in human urine.

Intended Use

The Ethylglucoronide Dip Card Test is a lateral flow chromatographic immunoassay for the qualitative detection of Ethylglucoronide in human urine at the following cut-off concentration:

Test	Calibrator	Cut-off
Ethylglucoronide (ETG)	Ethylglucoronide	500 ng/mL

The tests are used to obtain visual qualitative results and are intended **for forensic use only** to assist in the determination of alcohol compliance.

This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed assay result. Liquid Chromatography/Mass Spectrometry (LC/MS) is the preferred confirmatory methods.

Summary and Explanation

Ethylglucoronide (ETG), a metabolite of ethyl alcohol formed by glucoronidation, is created in the body after contact with ethanol from activities such as drinking alcoholic beverages. The metabolite ETG is used as a biomarker to test for ethanol use and to track alcohol abstinence in conditions where drinking alcohol is prohibited, such as military, school, and addiction recovery programs. The presence of ETG indicates that ethanol alcohol was ingested within the past three to four days, or roughly 80 hours after the ethanol alcohol has been metabolized by the human body. ETG can also be used to monitor the amount of alcohol consumed over time through detection in hair and nails.

Test Principle

The ETG test is a one-step competitive lateral flow immunoassay in which chemically modified drugs (ETG conjugates) compete for limited antibody binding sites with the ETG which may be present in urine. The test device contains membrane strips which are pre-coated with ETG-protein conjugates on the test band. The ETG antibody-colloidal gold conjugate pad is placed at one end of the membrane on each test strip. In the absence of ETG in the urine, the solution of the colored antibody-colloidal gold conjugate moves along with the sample solution chromatographically by capillary action across the membrane to the immobilized ETG-protein conjugate zone on the test band region. The colored antibody-gold conjugate then attaches to the ETG-protein conjugates to form visible lines as the antibody complex with the ETG conjugate. Therefore, the formation of the visible precipitant in the test zone occurs when the test urine is negative for ETG. When ETG is present in the urine, the drug/metabolite antigen competes with drug-protein conjugate on the test band region for the limited antibody. When a sufficient concentration of ETG is present, it will fill the limited antibody binding sites which will prevent attachment of the colored antibody (drug-protein conjugate)-colloidal gold conjugate to the drug-protein conjugate zone on the test band region. Therefore, absence of the color band on the test region indicates a positive result.

A control band with a different antigen/antibody reaction is added to the membrane strip at the control region (C) to indicate that the test has performed properly. This control line should always appear regardless of the presence of drug or metabolite. If the control line does not appear the test device should be discarded.

Materials Provided

Each ETG Test Kit contains:

1. Package Insert (PI)
2. Single-Panel ETG Dip Card

25 devices are packaged in each kit. Each test device contains the reagent strip housed in a separate strip channel in the plastic holder.

Materials Required But Not Provided

1. Specimen collection container
2. Timer
3. External urine controls (optional)

Warnings and Precautions

- FOR FORENSIC USE ONLY
- The pouch containing the device should be sealed. Discard the test device if package is ripped or torn.
- Urine specimens may be potentially hazardous and should be handled in the same manner as an infectious agent.
- Avoid cross-contamination of urine samples by using a new container for a different urine sample. Do not reuse the container for different urine sample collection.

Product Storage

The pouched ETG device should be stored at normal humidity and room temperature or refrigerated (2-30°C) until the expiration date stated on the pouch. The product is sensitive to humidity and should be used immediately after being opened. Any test in an improperly sealed pouch should be discarded.

Specimen Collection and Handling

Urine Assay: The urine specimen must be collected in a clean and dry container. The urine sample can be collected and tested at any time of the day. Fresh urine does not require any special handling or pretreatment.

Urine Storage: It is recommended that the collected fresh urine be tested immediately. Fresh urine may be stored at room temperature (25°C) for up to 4 hours or stored refrigerated (2-8°C) for up to 48 hours prior to performing the test. Specimens that have been refrigerated must be brought to room temperature prior to testing.

Note: Urine specimens and all materials coming in contact with them should be handled and disposed of as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

Test Procedure

IMPORTANT: The test device and patient's sample, or controls should be brought to room temperature (15-30°C) prior to testing. Do not open pouches until ready to perform the assay.

Remove the test device from the sealed pouch and use it as soon as possible.

1. Remove the cap from the end of test card. Label the device with patient ID or control number.
2. With arrows pointing toward the urine specimen, immerse the strip(s) of the test card vertically in the urine specimen for at least 10 to 15 seconds.
3. Replace the cap and place the test card on a non-absorbent flat surface. Start the timer and wait for the colored line(s) to appear.
4. The result(s) should be read at 5 minutes. Do not interpret the result(s) after 8 minutes.

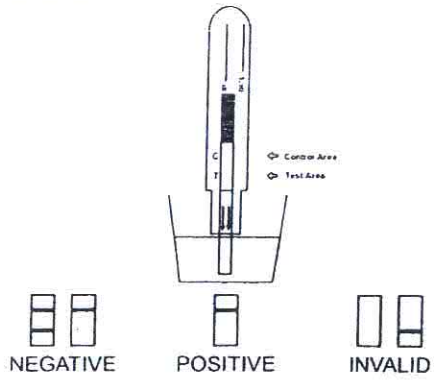
Positive test results must be confirmed by another test method. Send the entire urine specimen to a toxicology laboratory for confirmation.

Interpretation of Results

Negative: A colored line appears in the control (C) region and a colored line appears in the test region (T). This negative result indicates that the ETG concentration in the urine specimen is below the designated cut-off levels for the ETG tested. The color intensity of the line for the ETG may be weaker or stronger than that of the control line.

Positive: A colored line(s) appears in the control region (C). The absence of a colored line in the test region (T) indicates a positive result.

Invalid: No line appears in the control region (C). Under no circumstances should a positive sample be identified until the control line (C) forms in the viewing area. If the control line (C) does not form, the test result is inconclusive and the assay should be repeated with a new device.



Quality Control

A built-in procedural control is included in the test by using a different antigen/antibody reaction at the control region (C) on each test strip. This control line should always appear regardless of the presence of metabolite. If the control line does not appear, the test device should be discarded. The presence of this control line in the control region serves as 1) verification that sufficient volume is added and 2) that proper flow is obtained.

Good Laboratory Practice recommends the use of control materials to ensure proper device performance. External controls are not provided in the kit. However, they are available from commercial sources and it is recommended that positive and negative controls be used to verify proper test performance. Use the same assay procedure as with a urine specimen. Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

Limitations of Procedure

- The assay is designed for use with human urine only.
- A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication.
- There is a possibility that technical or procedural error as well other substances, as factors not listed, may interfere with the test and cause false results. See SPECIFICITY for lists of substances that will produce either positive results, or that do not interfere with test performance.
- If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of alcohol use.

Performance Characteristics

Cutoff Characterization - Accuracy

The sensitivity of the ETG Dip Card Tests is determined to be 500 ng/mL of ETG metabolite. The ETG test was compared with a predicate device to verify the accuracy of the ETG test. The results demonstrate that the ETG test is equivalent to the predicate device which is currently being distributed to the U.S. market. Other ETG compounds listed in the Specificity section can be detected at the indicated concentration level.

Precision

The study data demonstrates that the ETG Rapid Tests are able to produce consistent results. The precision study was evaluated in a blind study with prepared control solutions. Controls at concentrations of 50%, 75%, cutoff, 125%, and 150% of the cutoff yielded accurate results. The urine controls listed below were tested by 3 operators over 10 non-consecutive days. The data is shown below:

Control Level (Cut-Off Range)	ETG 500 Total (n = 216)	
	+	-
Negative	0	36
50% cutoff	0	36
75% cutoff	0	36
Cutoff	36	0
125% cutoff	36	0
150% cutoff	36	0

Specificity

The specificity for the ETG Dip Card test has been tested by adding various drugs, drug metabolites, and other structurally related compounds that are likely to be present in normal human urine. The following compounds were found to produce positive results when tested at levels greater than the concentrations (in ng/mL) listed below:

Ethyl-β-D-glucuronide related compounds	Concentration (ng/mL)
Ethyl-β-D-glucuronide	500 ng/mL
Ethyl-β-D-glucuronide-D5	500 ng/mL

Interference

The following compounds were found not to cross-react when tested at concentrations at 100 µg/mL in ±50% of the drug cut-off concentrations.

Endogenous Compounds:

Albumin	Creatinine	Riboflavin
Bilirubin	Glucose	Sodium Chloride
Cholesterol	Hemoglobin	Uric Acid

Un-structurally related compound:

Acetaminophen	Cyproheptadine	Meprobamate	Promazine
Acetylsalicylic Acid	Deoxycorticosterone	Methapyrilene	Promethazine
Amobarbital	Dextromethorphan	Methylphenidate	d-Propoxyphene
Amoxicillin	Diazepam	Nalidixic Acid	d,l-Propranolol
R-(-)-Apomorphine	Diclofenac	Naloxone	d-Pseudoephedrine
L-Ascorbic Acid	Diflunisal	Naltrexone	Pyridoxal-5-phosphate
Atropine	4-Dimethyl-aminoantipyrine	(+)-Naproxen	Pyridoxine
Baclofen	Diphenhydramine	Niacinamide	Pyrilamine
Barbital	5, 5-Diphenylhydantoin	Nicotinic Acid	Pyrogallol
Benzocaine	Dopamine	Nifedipine	Quinine
Benzoic Acid	Doxylamine	Nitrazepam	Quinidine
Buprenorphine	(-) -ephedrine	19-Norethindrone	Quinolinic Acid
Cannabidiol	l-Erythromycin,	Norpropoxyphene	Sertraline
Carisoprodol	Estradiol	Nortriptyline	Salicylic Acid
Chloral Hydrate	Estrone	Noscapine	Secobarbital Sodium
Chloramphenicol	Ethanol	Octopamine	Suldamethazine
Chlordiazepoxide	Fenofibrate	Oxalic Acid	Sulindac
(+)-Chlorpheniramine	Fentanyl	Oxazepam	Tetracycline
Chlorpromazine	Fotemustine	Papaverine	Tetrahydrozoline
Chlorprothixene	Furosemide	Perphenazine	Thiamine
Clofibrate	Gemfibrozil	Phenelzine	Thioridazine
Clonazepam	Guaiacolglyceryl ether	Pheniramine	Tramadol
Clonidine	Gentisic acid	Phenobarbital	Triazolam
Cortisone	Hydralazine	L-Phenylephrine	Trifluoperazine
(-)-Cotinine	Hydrocortisone	Phenylethylamine	Tryptamine
Creatine Hydrate	3-Hydroxytyramine	Phenylpropanolamine	Tyramine
Cyclobenzaprine	(+/-)-Isoproterenol	Prednisone	Zomepirac sodium salt
Cyclodextrin-r	Ketamine		

Effect of Urine pH

The pH ranges of 2.0 to 9.0 were prepared by adjusting the ETG urine controls at ±25% cut-off levels. The testing results demonstrate that the varying ranges of urine pH do not affect the test performance.

Effect of Urine Specific Gravity

The specific gravity (SG) ranges of 1.001, 1.010, 1.015, 1.020, 1.025 and 1.030 were prepared by adjusting the drug urine controls at ±25% and ±50% cut-off levels, respectively. The testing results with the ETG tests demonstrate that the varying ranges of urine SG do not affect the test results.

Bibliography of Suggested Reading

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Manufactured by:
Chemtron Biotech, Inc.
9245 Brown Deer Road, Suite B
San Diego, CA 92121, USA
Phone number: (858) 450-0044
Email address: support@uschemtronbio.com

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