EtG Urine Dip-Card

One Step Assay
Rapid Visual Results
For Qualitative In Vitro Diagnostic Use

INTENDED USE
The EtG Alcohol Urine Dip-Card Test is intended to be used in vitro for the examination of urine specimen solely for the purpose of screening of Ethyl Glucuronide at a cutoff concentration of 500 ng/ml.

This device is one-step immunoassay intended to provide qualitative rapid detection of Ethyl Glucuronide at a cutoff concentration of 500 ng/ml in human urine. It is for health care professional use only.

This assay provides only a preliminary result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas Chromatography / Mass Spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are obtained.

CANADA: In Canada this test is for laboratory use only.

SUMMARY AND EXPLANATION OF THE TEST
Ethyl Glucuronide (EtG) is a direct metabolite of ethanol (ethyl alcohol), when ethanol is presence in the bloodstream; it is conjugated in the liver with glucuronic acid to form ethyl glucuronide. The EtG can be detected directly from the urine as an indication of recent alcohol consumption, even after the ethyl alcohol is no longer measurable. In another word, the presence of EtG in the urine is a definitive indicator that alcohol has been ingested. Traditional laboratory practices typically measure the amount of alcohol present in the body. Depending on the amount of alcohol that has been consumed, this method usually reveals alcohol ingestion within the past few hours. The presence of EtG in the urine, on the other hand, demonstrates that ethyl alcohol was ingested within the past three or four days, or roughly 80 hours after the ethyl alcohol has been metabolized by the body. As a result, it can be determined that a urine alcohol test employing EtG is more accurate than the traditional laboratory test which simply measures the short period existence of ethyl alcohol in the urine.

PRINCIPLE OF THE PROCEDURE
This assay is a one-step lateral flow chromatographic immunoassay. The test strip includes 1) a burgundy-colored conjugate pad containing anti-EtG antibodies coupled to colloidal gold; and 2) nitrocellulose membrane containing a Test (T) line and a Control (C) line. The Test line is coated with EtG-BSA, and the Control line is coated with goat anti-rabbit IgG antibody.

This test is a competitive binding immunoassay. The Ethyl Glucuronide in the urine specimen competes with the monoclonal anti-EtG antibody coated on the nitrocellulose membrane for the limited binding sites of the conjugated ethyl glucuronide-protein.

When an adequate amount of urine specimen is applied to the sample pad of the device, the urine specimen migrates by capillary action through the test strip. If the level of Ethyl Glucuronide in the urine specimen is below the cutoff (500 ng/ml), the Test line appears as a visible burgundy line. If the level of Ethyl Glucuronide in the urine specimen is at or above the cutoff, no Test line develops.

The C line binds to the gold-conjugated rabbit IgG and forms a burgundy color line, regardless of the presence of Ethyl Glucuronide.

REAGENTS AND MATERIAL SUPPLIED
- 25 Individually Pouched Devices
- 1 Insert, Instructions for Use

MATERIAL REQUIRED BUT NOT PROVIDED
- Specimen collection containers
- Timer

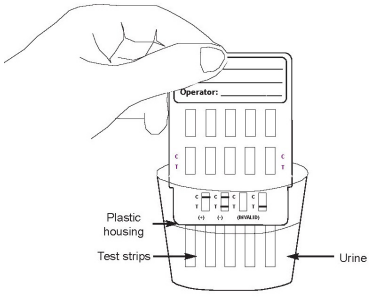
STORAGE AND STABILITY
Store the kit at room temperature 15-30°C (59-86°F). Each device may be used until the expiration date printed on the label if it remains sealed in its foil pouch containing a desiccant.

Do not freeze and/or expose the kit to temperatures over 30°C (86°F).

SPECIMEN COLLECTION
1. Each urine specimen must be collected in a clean container. Do not mix specimens.
2. Specimens may be kept at 15-30°C (59-86°F) for at least 8 hours, at 2-8°C for up to 3 days and at -20°C or lower for long term storage.

PRECAUTION
1. The instructions must be followed exactly to obtain accurate results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Dispose of all specimens and used assay materials as potentially biohazardous.

ASSAY PROCEDURE
IMPORTANT: YOU MUST EQUILIBRATE REFRIGERATED SPECIMENS TO ROOM TEMPERATURE BEFORE TESTING.
1. Bring the test device in sealed pouch to room temperature.
2. Immediately before use, open the pouch.
3. Remove test device from the pouch.
4. Label device with specimen identification.
5. Remove the cap from the device.
6. Dip the test strip into the urine specimen.

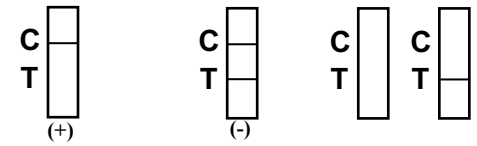


Note: You must dip the test strip into the specimen completely ensuring that the plastic housing remains above the specimen surface. See picture to the left.

7. Start the timer.
8. Remove the device from the specimen when pink migration starts in the result window. It should take about 10-15 seconds.
9. Replace the cap back onto the device.
10. Set the device on a clean and level surface
11. Read results at 4-7 minutes.

INTERPRETATION OF RESULTS

***IMPORTANT*: Do not read test results after seven (7) minutes. The T Line should always be interpreted independently of the C Line.**



PRELIMINARY POSITIVE | **NEGATIVE** | **INVALID**

Positive:
If only the C line appears, the test indicates that the EtG level in the sample is at a cutoff of 500 ng/ml or higher.
Samples with preliminary positive results should be confirmed with a more specific method before a positive conclusion is made.

Negative:
If both the C line and T line appear, the test indicates that the EtG level is below 500 ng/ml.
Note: A very faint T line should be considered negative.

Invalid:
If no Control (C) line develops within 5 minutes, repeat the assay with a new test device.

QUALITY CONTROL
- **Built-in Control Features**
  This test contains a built-in control feature, the C line. The appearance of the burgundy C line indicates an adequate volume of specimen has been absorbed and the capillary flow has occurred. The C line should always

appear. If the Control line does not develop within 5 minutes, review the entire procedure and repeat test with a new device.

- **External Quality Control**
  Users should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls. SAMHSA recommends that the concentration of drug in positive and negative controls be approximately 25% above and below the cutoff concentration of the assay.

LIMITATIONS

1. This test is for *professional in vitro* diagnostic use only.
2. Results obtained by this device provide only a preliminary qualitative result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.
3. This product is designed for testing human urine only.
4. Adulterants such as bleach or other strong oxidizing agents may produce erroneous test results. When suspected, collect a fresh specimen and repeat the test with a new device.
5. A positive result does not indicate level or intoxication, administration route or concentration in urine.
6. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
7. The test does not distinguish between drugs of abuse and certain medications.

EXPECTED VALUES

This test is capable of detecting EtG at a cutoff level of 500 ng/ml or higher.

PERFORMANCE CHARACTERISTICS

1. Accuracy

The accuracy was determined by comparing the results from the EtG *Urine Test* with the GC/MS data. This study was carried out in house, using eighty (120) blind labeled clinical urine specimens. The detailed data is shown in the table in this section.

The overall agreement was 97.5%.

		EtG Test		Total	Agreement
		Positive	Negative		
GC/MS (ng/ml)	Drug-free	0	40	40	100%
	<75% (0-750)	0	20	20	100%
	75%-Cutoff (750-1000)	1	19	20	95%
	Cutoff-125% (1000-1250)	18	2	20	90%
	Positive (>1250)	20	0	20	100%
Total		39	81	120	97.5%

2. Reproducibility

In-house evaluation

This study was conducted with three different lots. Specimens used in this study were the same used for the outside evaluation. The devices were tested for five consecutive days five times each, for a total of 25 assays for each standard.

The results were in 100% agreement among the replicates within each lot. No significant inter-lot or inter-day variation occured across the three different lots of devices.

3. Cross-Reactivity

A study was conducted to evaluate the cross-reactivity of compounds structurally related to EtG. The following compounds, when spiked into known drug-free urine pools and then tested, showed a positive response at the concentration listed.

Description	*Conc.(ng/ml)*	*Description*	*Conc.(ng/ml)*
Ethyl- β-D-glucuronide	500	Ethyl- β-D-glucuronide-D5	500

4. Interference

To evaluate the possible interference of structurally unrelated compounds, the following analytes, usually found in urine and commonly prescribed therapeutic drugs, were spiked in drug-free urine pools, as well as EtG positive (spiked with EtG to the level of 500 ng/ml) urine pools, and then tested. No significant interference with either negative or positive results was observed at the concentrations listed in the following table:

Compounds tested and found not to interfere with the test at 1.0 mg/ml concentration in urine	
Acetylsalicylic Acid	*Cortisone*
Amikacin	*Dextromethorphan*
Ampicillin	*Methadone*
Arterenal	*Methanol*
Aspirin	*Oxalic Acid*
Atropine	*Penicillin-G (Benzylpenicillin)*
Benzoic Acid	*Pheniramine*
Benzoylecgonine	*Phenylpropanalamine*
Caffeine	*Ranitidine*
(+)-Chlorpheniramine	*Salicylic Acid*
Cocaine	*Thioridazine*
Codeine	*Trifluoperazine*

Biological analytes tested and found no interference with the test at the concentrations listed	
Biological Analytes	**Concentration**
Albumin	*2 mg/ml*
Bilirubin	*1 mg/ml*
Creatine	*1 mg/ml*
Glucose	*2 mg/ml*
Hemoglobin	*1 mg/ml*
PH	*4.5 – 8.5*
Uric Acid	*1 mg/ml*
Vitamin C (L-Ascorbic Acid)	*1 mg/ml*

There is a possibility that other substances and/or factors not listed, may interfere with the test and cause false results.

Effect of Specific Gravity: Eight (8) human urine specimens with the specific gravity ranging from 1.002 to 1.035 g/ml were collected in house. Each was spiked with nortriptyline to three levels, 750, 1,500, and 2,000 ng/ml. All those specimens were tested, separately. The results indicated that the specific gravity of urine, ranging from 1.002 to 1.035, did not affect the performance.

REFERENCES

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	Temperature limitation		Use by YYYY-MM
LOT	Batch/Lot code	IVD	*In vitro* diagnostic medical device
	Manufacturer	REF	Catalog number
	Contains sufficient for < n > tests		Consult instructions for use
	Do not reuse		Caution, consult accompanying documents

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