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 specimens at the cut-off level of 300ng/mL. This assay is intended for forensic use only. The test is provided by ELITEST Diagnostics as 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 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. Therefore, EtG can be a useful diagnostic biomarker for determining recent alcohol use and in monitoring abstinence in alcoholics in alcohol withdrawal treatment programs.1,2 Ethanol can be produced in vitro due to fermentation of urine samples containing sugars, bacteria or yeast when samples are exposed to warm temperatures.2 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.2

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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 for 15 minutes to remove any particles before testing. It is recommended to shake the urine specimen at least two times prior to testing to ensure homogeneous dispersion of the specimen. 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 for 15 minutes to remove any particles before testing. It is recommended to shake the urine specimen at least two times prior to testing to ensure homogeneous dispersion of the specimen.

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 membrane surface.
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.

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.

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.

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

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 within-run and between-run precision. The correlation with expected results for the solutions targeted to 0% and 20% of the cut-off level was > 95% across all lots.

Ethyl Glucuronide (EtG) Test

<table>
<thead>
<tr>
<th>Ethyl Glucuronide Concentration (ng/mL)</th>
<th>Number of Test Devices</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lot 1</td>
<td>Lot 2</td>
<td>Lot 3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>450</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>600</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Precision

- For Forensic Use Only.
- Do not use after the expiration date.
- 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.

Interpretation

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 mononclonal antibody specific to ethyl glucuronide.
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:

### Analytical Sensitivity

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.

<table>
<thead>
<tr>
<th>Percent of Cut-off EtG Concentration in ng/mL</th>
<th>Test Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Cut-off (0ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>+50% Cut-off (150ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>+25% Cut-off (75ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>-25% Cut-off (37.5ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>-50% Cut-off (15ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>Cut-off (30ng/mL)</td>
<td>0</td>
</tr>
<tr>
<td>&lt;25% Cut-off (7.5ng/mL)</td>
<td>1</td>
</tr>
<tr>
<td>≥50% Cut-off (150ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>≥25% Cut-off (75ng/mL)</td>
<td>30</td>
</tr>
<tr>
<td>2X Cut-off (600ng/mL)</td>
<td>30</td>
</tr>
</tbody>
</table>

### 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.

### Potentially 100µg/mL, interfering substances
None of the following substances at the concentration tested interfered with the One Step Ethyl Glucuronide (EtG) Test.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetobutaldiol</td>
<td>300</td>
</tr>
</tbody>
</table>

### INTERFERENCE

**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.

### 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.

### REFERENCES


**Effectiveness**

Manufactured by:
W.H.P.M., Inc.
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www.whpm.com

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