Ethyl Glucuronide (ETG)
Urine Rapid Test Strip Instructions

EZ Testing Solutions, Inc.

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INTENDED USE

The ETG Rapid Test Strip (Urine) is a rapid visual immunoassay for the qualitative presumptive detection of Ethyl Glucuronide in human urine specimens at the cut-off concentrations listed below:

Parameter	Calibrator	Cut-off (ng/mL)
ETG	Ethyl Glucuronide	500

INTRODUCTION

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 glucuronosyl transferase. ETG is a product of metabolic process about of Ingested alcohol (ethanol) rapidly metabolized in the body, which is excreted in the blood, hair and urine. By using The ETG Rapid Test Strip (Urine), can detect ETG 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 rehabilitation programs

PRINCIPLE

The ETG Rapid Test Strip (Urine) has been designed to detect ETG through visual interpretation of color development in the device. The membrane was immobilized with ETG conjugates on the test region, and the sample pad was pre-coated with colored anti-ETG antibodies colloidal gold conjugates. After specimens were added, the gold-conjugates move along the membrane chromatographically by capillary action and antibodies get to the test region. If there is no drug molecule in the urine the antibody gold conjugate would attach to the drug conjugate to form a visible line. Therefore, the formation of a visible precipitant in the test region occurs when the urine is negative for the drug. If ETG are present in the urine, the drug antigen competes with the immobilized drug conjugate on the test region for limited antibody sites. In case of sufficient concentration of the drug, it fills the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate to the drug conjugate zone on the test region. Therefore, absence of the colored band on the test region indicates a positive result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

Each test consists of a reagent strip mounted in a plastic housing. The amount of each antigen and/or antibody coated on the Strip is less than 0.001 mg for antigen conjugates and goat anti-rabbit IgG antibodies, and less than 0.0015 mg for antibody components. The control zone of each test contains goat anti-rabbit IgG antibody. The test zone of each test contains drug-bovine protein antigen conjugate, and the conjugate pad of each test contains monoclonal anti-drug antibody and rabbit antibody-colored particle complex.

MATERIAL

Materials provided

- Test strips (individually pouched or in canisters
- Package insert

Materials Required but Not provided

- Positive and negative controls
- Timer
- Centrifuge

PRECAUTIONS

- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch or canister is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded in accordance with local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C/36-86°F until the expiry date printed on the sealed pouch or canister.
- The test must remain in the sealed pouch or closed canister until use.
- **Do not freeze.**
- Kits should be kept out of direct sunlight.
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

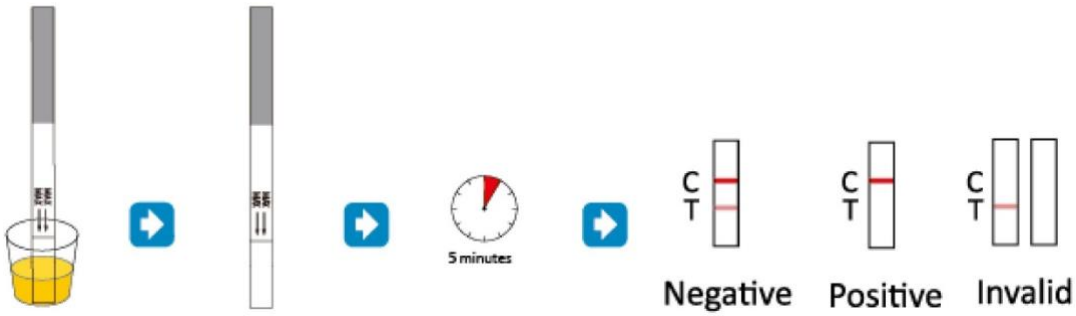
SPECIMEN COLLECTION AND STORAGE

- The ETG Rapid Test Strip (Urine) is intended for use with human urine specimens only.
- Urine collected at any time of the day may be used.
- Urine specimens must be collected in clean, dry containers.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Urine specimens may be stored at 2-8°C/36-46°F for up to 2 days. For long term storage, specimens should be kept below -20°C/-4°F.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C/59-86°F) before use.

1. Remove the test from its sealed pouch, or remove one strip from the canister, and use it as soon as possible. For best results, the assay should be performed within one hour. Canisters should be closed tightly after removing strips.
2. Hold the strip by the end, where the product name is printed. To avoid contamination, do not touch the strip membrane.
3. Holding the strip vertically, dip the test strip in the urine specimen for at least 10-15 seconds. Do not immerse past the maximum line (MAX) on the test strip.
4. After the test has finished running, remove the strip from the specimen and place it on anon-absorbent flat surface. Start the timer and wait for the colored band(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes.



INTERPRETATION OF RESULTS

POSITIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

NEGATIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region (T) should be considered negative. Please note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region(C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The ETG Rapid Test Strip (Urine) is for professional in vitro diagnostic use and should be only used for the qualitative detection of Ethyl Glucuronide.
2. This assay provides a preliminary analytical test result only. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) has been established as the preferred confirmatory method by the National Institute on Drug Abuse (NIDA). Clinical consideration and professional judgment should be applied to any test result, particularly when preliminary positive results are indicated.
3. There is a possibility that technical or procedural errors as well as other substances and factors may interfere with the test and cause false results.
4. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. Therefore, please preclude the possibility of urine adulteration prior to testing.
5. A positive result indicates the presence of an Ethyl Glucuronide only and does not indicate or measure intoxication.
6. A negative result does not at any time rule out the presence of Ethyl Glucuronide in urine, as they may be present below the minimum detection level of the test.

PERFORMANCE CHARACTERISTICS

A. Accuracy

The accuracy of the ETG Rapid Test Strip (Urine) was compared and checked against commercially available tests with a threshold value at the same cut-off levels. Urine samples taken from volunteers claiming to be non-users were examined under both tests. The results were >99.9% in agreement.

B. Reproducibility

The reproducibility of the ETG Rapid Test Strip (Urine) was verified by blind tests performed at four different locations. Samples with ETG concentrations at 50% of the cut-off were all determined to be negative, while samples with ETG concentrations at 200% of the cut-off were all determined to be positive.

C. Precision

Test precision was determined by blind tests with control solutions. Controls with ETG concentrations at 50% of the cut-off yielded negative results, and controls with ETG concentrations at 150% of the cut-off yielded positive results.

D. Cross-reactivity

The following tables list the concentrations of compounds (ng/mL) above which the ETG Rapid Test Strip (Urine) identified positive results at 5 minutes.

ETG 500 related compounds	Concentration (ng/ml)
Ethyl Glucuronide	500

The following compounds yielded negative results up to a concentration of 100 µg/mL:

(-)-Ephedrine
(+)-Naproxen
(+/-)-Ephedrine
4-Dimethyllaminoantiyrine
Acetaminophen
Acetone
Albumin
Alcohol
Amitriptyline
Ampicillin
Aspartame
Aspirin
Benzocaine
Bilirubin
b-Phenylethyl-amine
Caffeine
Chloroquine
Chlorpheniramine
Creatine
Dextromethorphan
Dextrorphan tartrate
Dopamine
Erythromycin
Ethanol
Furosemide
Glucose
Guaiacol Glyceryl Ether
Hemoglobin
Ibuprofen
Imipramine
Isoproterenol
Lidocaine
Methadone
Oxalic Acid
Penicillin-G
Pheniramine
Phenothiazine
Procaine
Protonix
Pseudoephedrine
Quinidine
Ranitidine
Sertraline
Tyramine
Vitamin C (Ascorbic Acid)
Trimeprazine
Venlafaxine

LITERATURE REFERENCES

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GLOSSARY OF SYMBOLS

	Catalog number		Temperature limitation
	Consult instructions for use		Batch code
	In vitro diagnostic medical device		Use by
	Manufacturer		Do not reuse

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