

# Rapid Benzodiazepine Urine Test

Ref. No. 0539

## INTENDED USE

The Acro Rapid Benzodiazepine Urine Test is a rapid immunoassay for the qualitative detection of benzodiazepine and its metabolites in human urine at 200 ng/mL cut-off concentration. This assay is used to obtain a visual, qualitative result and is intended for professional use only.

This assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to a drug test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectroscopy (GC/MS) is the preferred confirmation method.

## SUMMARY AND EXPLANATION

Benzodiazepines are medications that are frequently prescribed for the symptomatic treatment of anxiety and sleep disorders. They produce their effects via specific receptors involving a neurochemical called gamma aminobutyric acid (GABA). Because they are safer and more effective, Benzodiazepines have replaced barbiturates in the treatment of both anxiety and insomnia. Benzodiazepines are also used as sedative before some medical procedures, and for the treatment of seizure disorders and alcohol withdrawal.

Risk of physical dependence increases if Benzodiazepines are taken regularly (e.g., daily) for more than a few months, especially at higher than normal doses. Stopping abruptly can bring on such symptoms as trouble sleeping, gastrointestinal upset, feeling unwell, loss of appetite, sweating, trembling, weakness, anxiety and changes in perception.

Only trace amounts (less than 1%) of most Benzodiazepines are excreted unaltered in the urine; most of the concentration in urine is conjugated drug. The detection period for the Benzodiazepines in the urine is 3-7 days.

The length of time following drug use for which a positive result may occur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life, the drug user's age, weight, activity and diet.

## PRINCIPLE OF THE TEST

The Acro Rapid Benzodiazepine Urine Test is based on the principle of competitive immunochemical reaction between a chemically labeled drug (drug-protein conjugate) and the drug or drug metabolites, which may be present in the urine sample for the limited antibody binding sites. The test contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a wicking pad containing colored antibody-colloidal gold conjugate. During the test, the urine sample is allowed to migrate upward and hydrate the antibody-colloidal gold conjugate. The mixture then migrates along the membrane chromatographically by capillary action to the immobilized drug-protein band in the test region. When drug is absent in the urine, the colored antibody-colloidal gold conjugate and immobilized drug-protein bind specifically to form a visible line in the test region (test line). When benzodiazepine is present in the urine sample, it will compete with the drug-protein conjugate for the limited antibody binding sites. The test line will be less intense with increasing drug concentration. When the drug presents in sufficient concentration in the urine sample, it will fill the limited antibody binding sites, which will inhibit attachment of the colored antibody-colloidal gold conjugate to the drug-protein conjugate in the test region. Therefore the presence of the test line indicates a **negative** result for benzodiazepine and the absence of the test line indicates a **preliminary positive** result for benzodiazepine.

Another visible line, control line, generated by a different antigen/antibody reaction is also present at a control region (C) next to the test region of the test strip. The control line should always appear, regardless of the presence of the drug or drug metabolites in the urine sample. This means that a **negative** urine sample will produce **two** lines (test line and control line), and a **positive** urine sample will generate only **one** line (control line). The presence of control line serves as a built-in control, which demonstrates that the test is performed properly.

## REAGENTS AND MATERIALS PROVIDED

1. Test Cassette contains membrane-immobilized reagents in a protein matrix containing sodium azide as a preservative.
2. Anti-benzodiazepine monoclonal antibody is from murine ascities.
3. Dropper. A transfer pipette is included with each test device inside the foil pouch.
4. Test Instructions

## MATERIALS REQUIRED, BUT NOT PROVIDED

1. Timer
2. Sample container

## WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only
2. Urine specimens may be potentially infectious. Proper handling and disposal procedures should be established.
3. Avoid cross-contamination of urine samples. Use a new dropper or transfer pipette for adding each test sample.
4. Test device should remain sealed until ready for use.
5. Do not use the test kit after the expiration date.

## STORAGE AND STABILITY

Store at 2-30°C (36-86°F) in the original sealed pouch. Do not freeze.

## SPECIMEN COLLECTION AND HANDLING

Fresh urine does not require any special handling or pretreatment. A fresh urine sample should be collected in the container provided. Alternatively, a clean dry plastic or glass container may

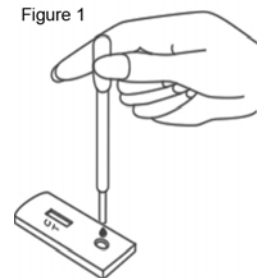
be used for specimen collection. If the specimen is not immediately tested after the specimen collection, the specimen may be refrigerated at 2-8°C for up to 2 days or frozen at -20°C for longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Frozen specimens must be thawed and mixed thoroughly prior to testing.

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

## ASSAY PROCEDURE

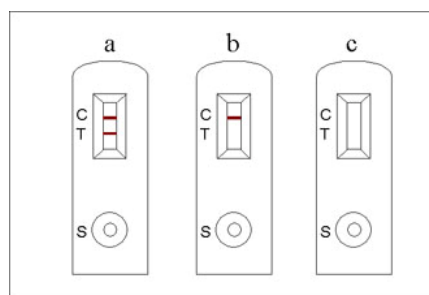
1. Bring the test components and urine sample to room temperature (15° - 28° C) before testing. Do not open the foil pouch until ready to begin testing.
2. Open the foil pouch at the notch and remove the test device and dropper prior to testing. Place the device on a clean, level surface.
3. Hold the dropper vertically and dispense 2 drops (~ 80 µl) of urine sample without air bubbles into the sample well "S" of the test device.
4. Read the result between 5 and 10 minutes.

Figure 1



**IMPORTANT:** Waiting more than ten minutes before reading the test result may cause the test result to be inaccurate.

## INTERPRETATION OF RESULTS



- a. **Preliminary Positive:** a *rose-pink* color band appears in the Control Zone "C" but not in the Test Zone "T". A preliminary positive result indicates benzodiazepine level in the urine sample is at or above the detection sensitivity of 200 ng/mL. The sample should be confirmed.
- b. **Negative:** two horizontal *rose-pink* color bands appear, one in the Control zone "C" and one in the Test Zone "T". A negative result indicates benzodiazepine level in the urine sample is below the detection sensitivity of 200 ng/mL.
- c. **Invalid:** no *rose-pink* bands appear, or a band appears in the Test Zone "T", but not in the Control Zone "C". An invalid result may be due to improper testing procedures or deterioration of the kit components.

**Note:** There is no meaning attributed to line color intensity or width.

## QUALITY CONTROL

An internal procedure control is included in the test device. A control line must form regardless of the presence or absence of drugs or metabolites. The presence of the line in the Control region indicates that a proper sample volume has been used. If the line in the Control region does not form, the test is considered invalid.

To ensure proper kit performance, it is recommended that the test devices be tested once a week or prior to use with external controls. External controls are available from commercial sources. It is important to make sure that the control values are within established limits. If the values of external controls do not fall within established limits, the test results are invalid. Additional controls may be tested according to guidelines or requirement of local state, and/or federal regulations or accrediting organizations.

## LIMITATIONS OF PROCEDURE

1. The assay is designed for use with human urine only.
2. A preliminary positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication
3. There is a possibility that technical or procedural error as well as other substances as factors not listed may interfere with the test and cause false results. See SPECIFICITY section for substances that will produce positive results, or that do not interfere with the test performance.
4. If adulteration is suspected, the test should be repeated with a new sample.
5. Certain over the counter or prescription medications (or certain foods) may cause false results.

## PERFORMANCE CHARACTERISTICS

1. **Sensitivity.** The Acro Rapid Benzodiazepine Urine Test detects benzodiazepine and its metabolites in urine at concentrations equal to or greater than 200 ng/mL.
2. **Specificity.** A study was conducted with the Acro Rapid Benzodiazepine Urine Test to determine the cross-reactivity of non-benzodiazepine related compounds with the test at concentrations much higher than normally found in the urine of people using or abusing them. No cross-reactivity was detected with the substances listed in Table I.

**Table-I: Compounds tested and found not to cross-react with the test at the concentrations of 10 µg/mL and 100 µg/mL in urine.**

Acetaminophen	Hemoglobin
Acetone	Ibuprofen
Albumin	(+/-)-Isoproterenol
Ampicillin	Ketamine
Ascorbic Acid	Levorphanol
Aspartame	Lidocaine
Aspirin	(+)-Naproxen
Atropine	Niacinamide
Benzocaine	Nicotine
Bilirubin	(+/-)-Norephedrine
Caffeine	Oxalic Acid
Chloroquine	Penicillin-G
(+)-Chlorpheniramine	Pheniramine
(+/-)-Chlorpheniramine	Phenothiazine
Creatine	1-Phenylephrine
Dexbrompheniramine	$\beta$ -Phenylethylamine
Dextromethorphan	Procaine
Diphenhydramine	Quinidine
Dopamine	Ranitidine
(+/-)-Epinephrine	Riboflavin
Erythromycin	Sodium Chloride
Ethanol	Sulindac
Furosemide	Theophylline
Glucose	Tyramine
Guaiacal Glyceryl Ether	4-Dimethylaminoantipyrine (1R, 2S)-(-)-N-Methyl-Ephedrine

Compound	Concentration	Relative to Cut-off Concentration
Oxazepam	200	1x
Alprazolam	140	0.7X
<i>a</i> -Hydroxyalprazolam	800	4x
Bromazepam	1000	5x
Chlordiazepoxide	1000	5.x
Chlorodiazepoxide HCl	500	2.5x
Clobazam	60	0.3x
Clonazepam	520	2.6x
Clorazepate dipotassium	140	0.7x
Delorazepam	1050	5.2x
Desalkylflurazepam	260	1.3x
Diazepam	140	0.7x
Estazolam	1600	8x
Flunitrazepam	260	1.3x
(+/-) Lorazepam	1050	5.2x
RS-Lorazepam glucuronide	100	0.5x
Midazolam	8400	42x
Nitrazepam	60	0.3x
Norchlordiazepoxide	140	0.7x
Nordiazepam	260	1.3x
Oxazepam	200	1x
Temazepam	60	0.3x
Triazolam	1600	8x

A separate study was conducted to determine the cross-reactivity of benzodiazepine related compounds with the test. Substances listed in **Table II** produced results approximately equivalent to the cutoff level for benzodiazepine.

**Table-II: Concentration of benzodiazepine-related compounds showing a positive response approximately equivalent to the benzodiazepine cut-off set for the test.**

Acro Benzodiazepine	GC/MS			
	Negative	Near Cutoff Negative (-50% to cutoff)	Near Cutoff Positive (cutoff to +50%)	Positive (> 50%)
Positive	0	1	16	25
Negative	60	15	3	0

Agreement among positive specimens is 39/42 = 93%

Agreement among negative specimens is 75/76 = 99%

4. **Cut-off study.** The cut-off of the test was determined by the repetitive assaying of six levels of benzodiazepine controls. The resultant data are summarized as follows:

Benzodiazepine conc.	# Tested	# Positive (+)	# Negative (-)	% Correct Results
0 ng/mL	50	0	60	100%
100 ng/mL	50	0	60	100%
150 ng/mL	50	9	41	82%
200 ng/mL	50	39	11	78%
250 ng/mL	50	48	2	96%
300 ng/mL	50	50	0	100%

5. **Reproducibility.** The reproducibility was evaluated at four different sites. The Acro Rapid Benzodiazepine Urine Test was tested against blind-labeled urine controls containing 0, 100, 150, 250, and 300 ng/mL benzodiazepine at each site. The results are summarized as follows:

Test Sites	0 ng/mL		100ng/mL		150ng/mL		250ng/mL		300ng/mL	
	#	Result	#	Result	#	Result	#	Result	#	Result
1	15	15-	15	15-	15	4+, 11-	15	15+	15	15+
2	15	15-	15	15-	15	1+, 14-	15	14+, 1-	15	15+
3	15	15-	15	15-	15	1+, 14-	15	12+, 3-	15	15+
4	15	15-	15	15-	15	0+, 14-	15	14+, 3-	15	15+
Total	60	60-	60	60-	60	6+, 54-	60	53+, 7-	60	60+

6. **Effect of Specimen pH.** Drug sample solutions with 50% below or 50% above cutoff concentration were adjusted to pH 4-9 and tested using Acro Rapid Benzodiazepine Urine Test. An unaltered sample was used as a control. The results demonstrate that varied specimen pH within the above range does not interfere with the performance of the test.

7. **Effect of Specimen Gravity.** Drug sample solutions with 50% below and above cutoff concentrations were adjusted to specific gravity 1.003-1.04 and tested using Acro Rapid Benzodiazepine Urine Test. An unaltered sample was used as a control. The results demonstrate that varied specimen specific gravity within the above range does not interfere with the performance of the test.

3. **Accuracy.** The accuracy study was performed by testing clinical benzodiazepine urine samples with the device and comparing the results with predicative device or quantitative GC/MS analysis results.

8. **Stability Study.** The stability study was performed by testing Acro Rapid Benzodiazepine Urine Test with drug urine samples with 50% below or above cutoff concentrations. The tests were undergone in room temperature every four months after the date of production, in the time frame of two years. The tests were stored at 2-30°C (36-86°F) in the original sealed pouch before testing. The results demonstrated the test is valid for two years.

#### BIBLIOGRAPHY

- Baselt, R. C., Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, Davis, CA, 1982.
- Urine testing for Drugs of Abuse. National Institute on Drug Abuse (NIDA), Research Monograph 73, 1986.
- Fed. Register, Department of Health and Human Services, Mandatory Guidelines for Federal Workplace Drug Testing Programs, 53, 69, 11970-11979, 1988
- Liu, Ray H. and Goldberger, Bruce A., Handbook of Workplace Drug Testing, AACC Press (1995)
- Gilman, A. G. and Goodman, L.S., The Pharmacological Basis of Therapeutics, eds. MacMillan Publishing, New York, NY, 1980.