



Acro Rapid Benzodiazepine Urine Test

For *in vitro* diagnostic use only
Ref No. 0518

INTENDED USE

The Acro Rapid Benzodiazepine Urine Test is a lateral flow, rapid immunoassay for the qualitative detection of oxazepam in human urine at 300 ng/mL cut-off concentration. This test is used to obtain a visual, qualitative result and is intended for laboratory 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.

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 is present 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 laboratory *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

1. Store at 2-30°C (36-86°F) in the original sealed pouch. Do not freeze.
2. The test is stable through the expiration date printed on the pouch.

3. The cassette must remain in the sealed pouch before use.
4. Do not use the test beyond the expiration date.

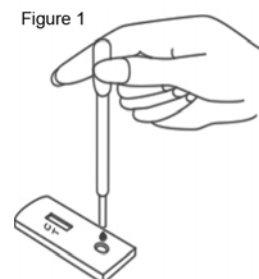
SPECIMEN COLLECTION AND HANDLING

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.

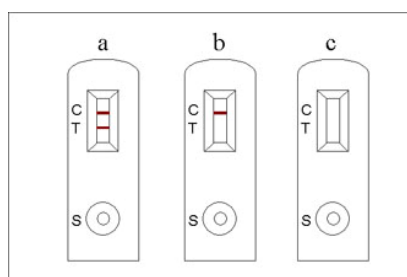
ASSAY PROCEDURE

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 3 drops (~ 100 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.



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

INTERPRETATION OF RESULTS



1. **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 oxazepam level in the urine sample is at or above the detection sensitivity of 300 ng/mL. The sample should be confirmed.
2. **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 oxazepam level in the urine sample is below the detection sensitivity of 300 ng/mL.
3. **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 indicates only the presence of benzodiazepine 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. Negative results may not necessarily indicate drug-free urine. For example, negative results can be obtained when drug is present but below the cutoff level of the test.
5. If adulteration is suspected, the test should be repeated with a new sample.
6. Certain over the counter or prescription medications (or certain foods) may cause false results. The test does not distinguish between drugs of abuse and certain medications.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity.** The Acro Rapid Benzodiazepine Urine Test detects oxazepam in urine at concentrations equal to or greater than 300 ng/mL.
2. **Specificity.** Interference of substances that may be present in urine specimens, as well as effect of sample pH and specific gravity was also studied.
 - a. Cross-reactivity of non-benzodiazepine related compounds at concentrations much higher than normally found in the urine of people using or abusing them were tested using the assay devices. No cross-reactivity was detected with the substances listed in Table I.
 - b. Table II lists Benzodiazepine related substances and concentrations that produced results approximately equivalent to the cutoff level for benzodiazepine.

- c. Varying sample pH within the range of 4 and 9 has no significant effect on the assay results.
d. Varying sample specific gravity within the range of 1.003 and 1.040 has no significant effect on the assay results.

Table-I: Compounds tested and found not to cross-react with the test at indicated concentrations in urine.

<i>Acetaminophen (100 ug/mL)</i>	<i>Estrone - 3 - sulfate (100 ug/mL)</i>	<i>Oxymetazoline (100 ug/mL)</i>
<i>Acetophenetidin (100 ug/mL)</i>	<i>Ethyl-p-aminobenzoate (100 ug/mL)</i>	<i>Papaverine (100 ug/mL)</i>
<i>N-Acetylprocainamide (100 ug/mL)</i>	<i>Fenoprofen (100 ug/mL)</i>	<i>Penicillin-G (100 ug/mL)</i>
<i>Acetylsalicylic acid (100 ug/mL)</i>	<i>Furosemide (100 ug/mL)</i>	<i>Pentazocine hydrochloride (100 ug/mL)</i>
<i>Aminopyrine (100 ug/mL)</i>	<i>Gentisic acid (100 ug/mL)</i>	<i>Pentobarbital (100 ug/mL)</i>
<i>Amitriptyline (100 ug/mL)</i>	<i>Glucose (500 ug/mL)</i>	<i>Perphenazine (100 ug/mL)</i>
<i>Amobarbital (100 ug/mL)</i>	<i>Hemoglobin (500 ug/mL)</i>	<i>Phencyclidine (100 ug/mL)</i>
<i>Amoxicillin (100 ug/mL)</i>	<i>Hydralazine (100 ug/mL)</i>	<i>Phenelzine (100 ug/mL)</i>
<i>Ampicillin (100 ug/mL)</i>	<i>Hydrochlorothiazide (100 ug/mL)</i>	<i>Phenobarbital (100 ug/mL)</i>
<i>L-Ascorbic acid (500 ug/mL)</i>	<i>Hydrocodone (100 ug/mL)</i>	<i>Phentermine (100 ug/mL)</i>
<i>DL-Amphetamine sulfate (100 ug/mL)</i>	<i>Hydrocortisone (100 ug/mL)</i>	<i>Trans-2-phenylcyclopropylamine hydrochloride (100 ug/mL)</i>
<i>Apomorphine (100 ug/mL)</i>	<i>O-Hydroxyhippuric acid (100 ug/mL)</i>	<i>L-Phenylephrine (100 ug/mL)</i>
<i>Aspartame (100 ug/mL)</i>	<i>p-Hydroxyamphetamine (100 ug/mL)</i>	<i>β-Phenylethylamine (100 ug/mL)</i>
<i>Atropine (100 ug/mL)</i>	<i>p-Hydroxymethamphetamine (100 ug/mL)</i>	<i>Phenylpropanolamine (100 ug/mL)</i>
<i>Benzilic acid (100 ug/mL)</i>	<i>3-Hydroxytyramine (100 ug/mL)</i>	<i>Prednisolone (100 ug/mL)</i>
<i>Benzoic acid (100 ug/mL)</i>	<i>Ibuprofen (200 ug/mL)</i>	<i>Prednisone (100 ug/mL)</i>
<i>Benzoylcegonine (100 ug/mL)</i>	<i>Imipramine (100 ug/mL)</i>	<i>Procaine (100 ug/mL)</i>
<i>Benzphetamine (100 ug/mL)</i>	<i>Iproniazid (100 ug/mL)</i>	<i>Promazine (100 ug/mL)</i>
<i>Bilirubin (100 ug/mL)</i>	<i>(±) - Isoproterenol (100 ug/mL)</i>	<i>Promethazine (100 ug/mL)</i>
<i>(±) - Brompheniramine (100 ug/mL)</i>	<i>Isosuprine (100 ug/mL)</i>	<i>DL-Propranolol (100 ug/mL)</i>
<i>Caffeine (100 ug/mL)</i>	<i>Ketamine (100 ug/mL)</i>	<i>D-Propoxyphene (100 ug/mL)</i>
<i>Cannabidiol (100 ug/mL)</i>	<i>Ketoprofen (100 ug/mL)</i>	<i>D-Pseudoephedrine (100 ug/mL)</i>
<i>Cannabinol (100 ug/mL)</i>	<i>Labetalol (100 ug/mL)</i>	<i>Quinacrine (100 ug/mL)</i>
<i>Chloralhydrate (100 ug/mL)</i>	<i>Loperamide (100 ug/mL)</i>	<i>Quinidine (100 ug/mL)</i>
<i>Chloramphenicol (100 ug/mL)</i>	<i>Maprotiline (100 ug/mL)</i>	<i>Quinine (100 ug/mL)</i>
<i>Chlorothiazide (100 ug/mL)</i>	<i>MDE (100 ug/mL)</i>	<i>Ranitidine (100 ug/mL)</i>
<i>(±) - Chlorpheniramine (100 ug/mL)</i>	<i>Meperidine (100 ug/mL)</i>	<i>Salicylic acid (100 ug/mL)</i>
<i>Chlorpromazine (100 ug/mL)</i>	<i>Meprobamate (100 ug/mL)</i>	<i>Secobarbital (100 ug/mL)</i>
<i>Chlorquine (100 ug/mL)</i>	<i>Methadone (100 ug/mL)</i>	<i>Serotonin (100 ug/mL)</i>
<i>Cholesterol (100 ug/mL)</i>	<i>(L) Methamphetamine (100 ug/mL)</i>	<i>Sodium Chloride (10,000 ug/mL)</i>
<i>Clomipramine (100 ug/mL)</i>	<i>Methoxyphenamine (100 ug/mL)</i>	<i>Sulfamethazine (100 ug/mL)</i>
<i>Clonidine (100 ug/mL)</i>	<i>(±) - 3,4-Methylenedioxyamphetamine-hydrochloride (100 ug/mL)</i>	<i>Sulindac (100 ug/mL)</i>
<i>Coccaethylene (100 ug/mL)</i>	<i>(±) - 3,4-Methylenedioxy-methamphetamine hydrochloride (100 ug/mL)</i>	<i>Tetracycline (100 ug/mL)</i>
<i>Cocaine hydrochloride (100 ug/mL)</i>	<i>Morphine-3- β-D glucuronide (100 ug/mL)</i>	<i>Tetrahydrocortisone, 3-acetate (100 ug/mL)</i>
<i>Codeine (100 ug/mL)</i>	<i>Morphine Sulfate (100 ug/mL)</i>	<i>Tetrahydrozoline (100 ug/mL)</i>
<i>Cortisone (100 ug/mL)</i>	<i>Nalidixic acid (100 ug/mL)</i>	<i>Thiamine (100 ug/mL)</i>
<i>(-) Cotinine (100 ug/mL)</i>	<i>Naloxone (100 ug/mL)</i>	<i>Thioridazine (100 ug/mL)</i>
<i>Creatinine (100 ug/mL)</i>	<i>Naltrexone (100 ug/mL)</i>	<i>DL-Tyrosine (100 ug/mL)</i>
<i>Deoxycorticosterone (100 ug/mL)</i>	<i>Naproxen (100 ug/mL)</i>	<i>Tolbutamide (100 ug/mL)</i>
<i>Dextromethorphan (100 ug/mL)</i>	<i>Niacinamide (100 ug/mL)</i>	<i>Triamterene (100 ug/mL)</i>
<i>Diclofenac (100 ug/mL)</i>	<i>Nifedipine (100 ug/mL)</i>	<i>Trifluoperazine (100 ug/mL)</i>
<i>Diflunisal (100 ug/mL)</i>	<i>Norcodein (100 ug/mL)</i>	<i>Trimethoprim (100 ug/mL)</i>
<i>Digoxin (100 ug/mL)</i>	<i>Norethindrone (100 ug/mL)</i>	<i>Trimipramine (100 ug/mL)</i>
<i>Diphenhydramine (100 ug/mL)</i>	<i>D-Norpropoxyphene (100 ug/mL)</i>	<i>Tryptamine (100 ug/mL)</i>
<i>Doxylamine (100 ug/mL)</i>	<i>Noscapine (100 ug/mL)</i>	<i>DL-Tryptophan (100 ug/mL)</i>
<i>Ecgonine hydrochloride (100 ug/mL)</i>	<i>DL-Octopamine (100 ug/mL)</i>	<i>Tyramine (100 ug/mL)</i>
<i>Ecgonine methylester (100 ug/mL)</i>	<i>Oxalic acid (100 ug/mL)</i>	<i>Uric acid (100 ug/mL)</i>
<i>(-) - Ψ - Ephedrine (100 ug/mL)</i>	<i>Oxolinic acid (100 ug/mL)</i>	<i>Verapamil (100 ug/mL)</i>
<i>[1R,2s] (-) Ephedrine (100 ug/mL)</i>	<i>Oxycodone (100 ug/mL)</i>	<i>Zomepirac (100 ug/mL)</i>
<i>(L) - Ephedrine (100 ug/mL)</i>		
<i>Erythromycin (100 ug/mL)</i>		
<i>β - Estradiol (100 ug/mL)</i>		

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

Compound /	Concentration in ng/ml
<i>Oxazepam</i>	300
<i>Alprazolam</i>	196
<i>a-Hydroxyalprazolam</i>	1,262
<i>Bromazepam</i>	1,562
<i>Chlordiazepoxide</i>	1,562
<i>Chlorodiazepoxide HCl</i>	781
<i>Clobazam</i>	98
<i>Clonazepam</i>	781
<i>Clorazepate dipotassium</i>	195
<i>Delorazepam</i>	1,562
<i>Desalkylflurazepam</i>	390
<i>Diazepam</i>	195
<i>Estazolam</i>	2,500
<i>Flunitrazepam</i>	390
<i>(+/-) Lorazepam</i>	1,562
<i>RS-Lorazepam glucuronide</i>	156
<i>Midazolam</i>	12,500
<i>Nitrazepam</i>	98
<i>Norchlordiazepoxide</i>	195
<i>Nordiazepam</i>	390
<i>Oxazepam</i>	300

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

Acro Test	Less than half the cutoff concentration by GC/MS analysis or negative by the predicate device	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (greater than 50% above the cutoff concentration)
Positive	0	2	13	25
Negative	70	6	2	0

% Agreement among positives is 38/40 = 95%

% Agreement among negatives is 76/78 = 97%

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

Oxazepam conc.	# Tested	# Positive (+)	# Negative (-)	% Correct Results
0 ng/mL	60	0	60	100%
150 ng/mL	60	0	60	100%
225 ng/mL	60	7	53	88%
375 ng/mL	60	55	5	92%
450 ng/mL	60	60	0	100%
600 ng/mL	60	60	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, 150, 225, 375, 450 and 600 ng/mL oxazepam at each site. The results are summarized as follows:

Test Sites	0 ng/mL		150ng/mL		225ng/mL		375ng/mL		450ng/mL		600ng/mL	
	#	Result	#	Result	#	Result	#	Result	#	Result	#	Result
1	15	15-	15	15-	15	4+, 11-	15	15+	15	15+	15	15+
2	15	15-	15	15-	15	1+, 14-	15	14+, 1-	15	15+	15	15+
3	15	15-	15	15-	15	1+, 14-	15	12+, 3-	15	15+	15	15+
4	15	15-	15	15-	15	1+, 14-	15	14+, 1-	15	15+	15	15+
Total	60	60-	60	60-	60	7+, 53-	60	55+, 5-	60	60+	60	60+

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