



One Step Benzodiazepines Test Strip (Urine) Package Insert

REF 1183-S

A rapid, one step test for the qualitative detection of Benzodiazepines in human urine. For professional *in vitro* diagnostic use only.

INTENDED USE

The BZO One Step Benzodiazepines Test Strip (Urine) is a lateral flow chromatographic immunoassay for the detection of Oxazepam (major metabolite) in urine at a cut-off concentration of 300 ng/mL. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.

This assay provides only a qualitative, preliminary analytical test 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 drug of abuse test result, particularly when preliminary positive results are used.

SUMMARY

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 sedatives before some surgical and 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 BZO One Step Benzodiazepines Test Strip (Urine) is a rapid urine-screening test that can be performed without the use of an instrument. The test utilizes the antibody to selectively detect elevated levels of Benzodiazepines in urine. The BZO One Step Benzodiazepines Test Strip (Urine) yields a positive result when the Benzodiazepines in urine exceeds the cut-off level.

PRINCIPLE

The BZO One Step Benzodiazepines Test Strip (Urine) is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.

During testing, a urine specimen migrates upward by capillary action. Benzodiazepines, if present in the urine specimen below the cut-off level, will not saturate the binding sites of the antibody in the test strip. The antibody coated particles will then be captured by immobilized Benzodiazepines-protein conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the Benzodiazepines level exceeds the cut-off level, because it will saturate all the binding sites of anti-Benzodiazepines antibody.

A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test strip contains mouse monoclonal anti-Benzodiazepines antibody coupled particles and Benzodiazepines-protein conjugate. A goat antibody is employed in the control line system.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test strip should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test strip should be discarded according to local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test strip is stable through the expiration date printed on the sealed pouch. The test strip must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Urine Assay

The urine specimen must be collected in a clean and dry container. Urine collected at any time of day may be used. Urine specimens exhibiting visible particles should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.

Specimen Storage

Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS

Materials Provided

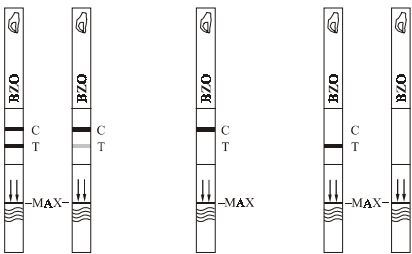
- Test strips
 - Package insert
- Specimen collection container
 - Timer

Materials Required But Not Provided

DIRECTIONS FOR USE

Allow the test strip, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

- Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
- With arrows pointing toward the urine specimen, immerse the test strip vertically in the urine specimen for at least 10-15 seconds. Do not pass the maximum line (MAX) on the test strip when immersing the strip. See the illustration below.
- Place the test strip on a non-absorbent flat surface, start the timer and wait for the colored line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes.



Negative Positive Invalid

INTERPRETATION OF RESULTS

(Please refer to the illustration above)

NEGATIVE: * **Two lines appear.** One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the Benzodiazepine concentration is below the detectable cut-off level.

*NOTE: The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

POSITIVE: **One colored line appears in the control line region (C).** No line appears in the test line region (T). This positive result indicates that the Benzodiazepine concentration exceeds the detectable cut-off level.

INVALID: **Control line fails to appear.** Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test strip. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

- The BZO One Step Benzodiazepines Test Strip (Urine) provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.^{1,2}
- It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
- A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
- 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.
- Test does not distinguish between drugs of abuse and certain medications.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the BZO One Step Benzodiazepines Test Strip (Urine) and a leading commercially available BZO rapid test. Testing was performed on 298 clinical specimens previously collected from subjects present for Drug Screen Testing. Ten percent of the specimens employed were either at -25% or +25% level of the cut-off concentration of 300 ng/mL Oxazepam. Presumptive positive results were confirmed by GC/MS. The following results were tabulated:

Method	Other BZO Rapid Test		Total Results
	Positive	Negative	
BZO One Step Test Strip	Positive	131	135
	Negative	14	163
Total Results	145	153	298
% Agreement	90%	97%	94%

When compared to GC/MS at the cut-off of 300 ng/mL, the following results were tabulated:

Method	GC/MS		Total Results
	Positive	Negative	
BZO One Step Test Strip	Positive	131	139
	Negative	4	161
Total Results	135	165	300
% Agreement	97%	95%	96%

Analytical Sensitivity

A drug-free urine pool was spiked with Oxazepam at the following concentrations: 0 ng/mL, 150 ng/mL, 225 ng/mL, 300 ng/mL, 375 ng/mL and 450 ng/mL. The result demonstrates > 99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Oxazepam Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	0	0
150	-50%	30	30	0
225	-25%	30	27	3
300	Cut-off	30	11	19
375	+25%	30	5	25
450	+50%	30	0	30

Analytical Specificity

The following table lists compounds that are positively detected in urine by the BZO One Step Benzodiazepines Test Strip (Urine) at 5 minutes.

Compound Concentration (ng/mL)	Compound Concentration (ng/mL)
Alprazolam 196	Flunitrazepam 390
a-hydroxyalprazolam 1,262	(±) Lorazepam 1,562
Bromazepam 1,562	RS-Lorazepam glucuronide 156
Chlordiazepoxide 1,562	Midazolam 12,500
Clobazam 98	Nitrazepam 98
Clonazepam 781	Norchlordiazepoxide 195
Clorazepate dipotassium 195	Nordiazepam 390
Delorazepam 1,562	Oxazepam 300
Desalkylflurazepam 390	Temazepam 98
Diazepam 195	Triazolam 2,500
Estazolam 2,500	

Precision

A study was conducted at three physicians' offices by untrained operators using three different lots of product to demonstrate the within run and between operator precision. An identical panel of coded specimens containing, according to GC/MS, no Oxazepam, 25% Oxazepam above and below the cut-off and 50% Oxazepam above and below the 300 ng/mL cut-off was provided to each site. The following results were tabulated:

Oxazepam Concentration (ng/mL)	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	15	15	0	15	0	15	0
150	15	15	0	13	2	13	2
225	15	6	9	7	8	13	2
375	15	0	15	1	14	3	12
450	15	0	15	0	15	0	15

Effect of Urinary Specific Gravity

Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 150 µg/L and 450 ng/mL of Oxazepam. The BZO One Step Benzodiazepines Test Strip (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

Effect of Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with Oxazepam to 150 ng/mL and 450 ng/mL. The spiked, pH-adjusted urine was tested with the BZO One Step Benzodiazepines Test Strip (Urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or Oxazepam positive urine. The following compounds show no cross-reactivity when tested with the BZO One Step Benzodiazepines Test Strip (Urine) at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

Acetaminophen	Deoxycorticosterone	MDE	β-Phenylethylamine
Acetophenetidin	Dextromethorphan	Meperidine	Phenylpropionamide
N-Acetylprocainamide	Diclofenac	Meprobamate	Prindisone
Acetylsalicylic acid	Diffunisal	Methadone	Prednisone
Aminopyrine	Digoxin	L-Methamphetamine	Procaine
Amitriptyline	Diphenhydramine	Methoxyphenamine	Promazine
Amobarbital	Doxylamine	(±) - 3,4-Methylenedioxyamphetamine	Promethazine
Amoxicillin	Ecgonine hydrochloride	Nalidixic acid	D,L-Propranolol
Ampicillin	Ecgonine methylester	(±) - 3,4-Methylenedioxy-methamphetamine	D-Propoxyphene
L-Ascorbic acid	(-) - ψ-Ephedrine	Morphine-3-β-D glucuronide	D-Pseudoephedrine
D,L-Amphetamine sulfate	[1R,2S] (-) Ephedrine	Morphine Sulfate	Quinacrine
Apomorphine	(L) - Epinephrine	Nalidixic acid	Quinidine
Aspartame	Erythromycin	Naloxone	Quinine
Atropine	β-Estradiol	Naltrexone	Ranitidine
Benzilic acid	Estrone-3-sulfate	Naproxen	Salicylic acid
Benzoic acid	Ethyl-p-aminobenzoate	Nicotinamide	Secobarbital
Benzoylcegonine	Fenpropion	Nifedipine	Serotonin
Benzphetamine	Furosemide	Nifedipine	Sulfamethazine
Bilirubin	Genistic acid	Norethindrone	Sulindac
(±) - Brompheniramine	Hemoglobin	D-Norpropoxyphene	Tetracycline
Caffeine	Hydralazine	Noscapine	Tetrahydrocortisone, 3-Acetate
Cannabidiol	Hydrochlorothiazide	D,L-Octopamine	Tetrahydrocortisone 3-(β-D-glucuronide)
Cannabinol	Hydrocodone	Oxalic acid	Tetrahydrozoline
Chloralhydrate	Hydrocortisone	Oxolinic acid	Thiamine
Chloramphenicol	O-Hydroxyhippuric acid	Oxycodone	Thioridazine
Chlorothiazide	p-Hydroxyamphetamine	Oxymetazoline	D,L-Tyrosine
(±) - Chlorpheniramine	p-Hydroxy-methamphetamine	Papaverine	Tolbutamide
Chlorpromazine	3-Hydroxytyramine	Penicillin-G	Triamterene
Chlorzine	Ibuprofen	Perphenazine	Trifluoperazine
Cholesterol	Imipramine	Phencyclidine	Trimethoprim
Clomipramine	Iproniazid	(±) - Isoproterenol	Trimipramine
Clonidine	Isoxsuprine	Phenelzine	Tryptamine
Cocacethylene	Ketamine	Phenobarbital	D,L-Tryptophan
Cocaine hydrochloride	Ketoprofen	Phentermine	Tyramine
Codine	Labeltal	Trans-2-phenylcyclopropylamine hydrochloride	Uric acid
Cortisone	Labetalol	L-Phenylephrine	Verapamil
(-) Cotinine	Loperamide	Maprotiline	Zemepirac
Creatinine	Maropitilone		

BIBLIOGRAPHY

- Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man*. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488
- Hawks RL, CN Chiang. *Urine Testing for Drugs of Abuse*. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

Index of Symbols

	Storage Temperature		Manufacturer		Do not reuse
	Lot Code		Authorized Representative		For <i>in vitro</i> diagnostic use
	Expiration		Caution, see instructions		Catalog No.

BIOMERICA, Inc.
1533 Norovnia Ave.
Newport Beach, CA 92663 USA

CE acc to IVDD 98/79/EC
MDSS
Burckhardtstr. 1
30163 Hannover, Germany