

One Step Benzodiazepines Test Strip (Urine) Package Insert

REF-R1110

English

A rapid, one step test for the qualitative detection of Benzodiazepines in human urine. For healthcare professionals including professionals at point of care sites For *in vitro* diagnostic use only.

INTENDED USE

The BZO One Step Benzodiazepines Test Strip 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 Benzodiazepines, please refer to Analytical Specificity table in this package insert.

This assay provides only a 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 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 yields a positive result when the Benzodiazepines in urine exceeds cut-off concentration.

PRINCIPLE

The BZO One Step Benzodiazepines Test Strip 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 cut-off, will not saturate the binding sites of the antibodies in the test strip. The antibody coated particles will then be captured by immobilized benzodiazepine 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 cut-off concentration, 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 lower 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 healthcare professionals including professionals at point of care sites.
- For *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 federal, state and local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C. The test strip is stable through the expiration date printed on the sealed pouch. The test strips 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 the 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

Materials Required But Not Provided

- Specimen collection container
- Timer
- External controls

DIRECTIONS FOR USE

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

1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
2. 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.

3. Place the test strip on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 5 minutes. Do not interpret the result after 10 minutes.

INTERPRETATION OF RESULTS

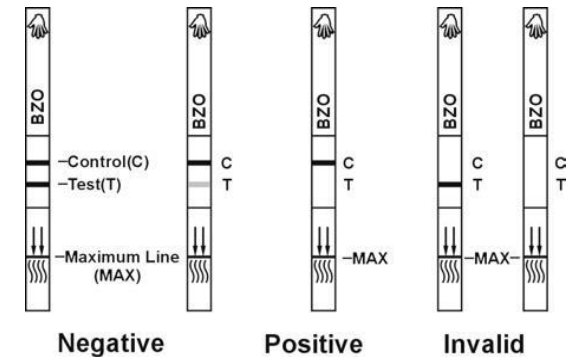
(Please refer to the illustration below)

NEGATIVE:* **Two lines appear.** One red line should be in the control region (C), and another apparent red or pink line should be in the test region (T). This negative result indicates that the Benzodiazepines concentration is below the detectable level.

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

POSITIVE: **One red line appears in the control region (C).** No line appears in the test region (T). This positive result indicates that the Benzodiazepines concentration exceeds the detectable 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 red line appearing in the control region (C) is considered an internal positive 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

1. The BZO One Step Benzodiazepines Test Strip 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 methods.^{1,2}
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. 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.
4. A Positive Result does not indicate level or intoxication, administration route or concentration in urine.
5. 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.

6. 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 and a leading commercially available BZO rapid test. Testing was performed on specimens previously collected from subjects presenting for Drug Screen Testing. Presumptive positive results were confirmed by GC/MS. The following results were tabulated:

Method	Other BZO Rapid Test		Total Result
	Positive	Negative	
BZO One step test strip	Positive	4	135
	Negative	149	163
Total result		145	298
%Agreement whit this commercial kit		%90	%94

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

Method	GC/MS		Total Result
	Positive	Negative	
BZO One step test strip	Positive	8	139
	Negative	157	161
Total result		135	300
%Agreement whit GC/MS Analysis		%97	%96

Eighty (84) of these samples were also run using the BZO One Step Benzodiazepines Test Strip by an untrained operator at a different site. Based on GC/MS data, the operator obtained a statistically similar Positive Agreement, Negative Agreement and Overall Agreement rate as the laboratory personnel.

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 100% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Oxazepam Conc. (ng/ml)	Percent of cutoff	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	27	3
300	Cutoff	30	11	19
375	25%	30	5	25
450	50%	30	0	30

Specificity

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

Compound	Concentration (ng/mL)
Alprazolam	196
a-Hydroxylalprazolam	1,262
Bromazepam	1,562
Chlordiazepoxide	1,562
Chlordiazepoxide HCl	781
Clonazepam	781
Clobazam	98

Clorazepate dipotassium	195
Delorazepam	1,562
Desalkylflurazepam	390
Diazepam	195
Estazolam	2,500
Flunitrazepam	390
(±) Lorazepam	1,562
RS-Lorazepam glucuronide	156
Midazolam	12,500
Nitrazepam	98
Norchlordiazepoxide	195
Nordiazepam	390
Oxazepam	300
Temazepam	98
Triazolam	2,500

Precision

A study was conducted at 3 physician's offices by untrained operators using 3 different lots of product to demonstrate the within run, between 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 Conc. (ng/ml)	n	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 (15) urine samples of normal, high, and low specific gravity ranges were spiked with 150 ng/mL and 450 ng/mL of Oxazepam respectively. The BZO One Step Benzodiazepines Test Strip was tested in duplicate using the fifteen neat and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity does not affect the test results.

Effect of the 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 in duplicate and interpreted according to the package insert. The results demonstrate that varying ranges of pH does 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 urine not associated with Benzodiazepines. The following compounds show no cross-reactivity when tested with the BZO One Step Benzodiazepines Test Strip at a concentration of 100 µg/mL.









Non Cross-Reacting Compounds

Acetaminophen	Estrone-3-sulfate	Oxymetazoline
Acetophenetidin	Ethyl-p-aminobenzoate	Papaverine
N-Acetylprocainamide	Fenoprofen	Penicillin-G
Acetylsalicylic acid	Furosemide	Pentazocine hydrochloride
Aminopyrine	Gentisic acid	Pentobarbital
Amitypyline	Hemoglobin	Perphenazine
Amobarbital	Hydralazine	Phencyclidine
Amoxicillin	Hydrochlorothiazide	Phenelzine
Ampicillin	Hydrocodone	Phenobarbital

L-Ascorbic acid	Hydrocortisone	Phentermine
DL-Amphetamine sulfate	O-Hydroxyhippuric acid	Trans-2-phenylcyclopropylamine hydrochloride
Apomorphine	p-Hydroxyamphetamine	L-Phenylephrine
Aspartame	p-Hydroxy-methamphetamine	β-Phenylethylamine
Atropine	3-Hydroxytyramine	Phenylpropanolamine
Benzilic acid	Ibuprofen	Prednisolone
Benzoic acid	Imipramine	Prednisone
Benzoylcegonine	Iproniazid	Procaine
Benzphetamine	(±) - Isoproterenol	Promazine
Bilirubin	Isoxsuprine	Promethazine
(±) - Brompheniramine	Ketamine	DL-Propranolol
Caffeine	Ketoprofen	D-Propoxyphene
Cannabidiol	Labetalol	D-Pseudoephedrine
Cannabinol	Loperamide	Quinacrine
Chloralhydrate	Maprotiline	Quinidine
Chloramphenicol	MDE	Quinine
Chlorothiazide	Meperidine	Ranitidine
(±) - Chlorpheniramine	Meprobamate	Salicylic acid
Chlorpromazine	Methadone	Secobarbital
Chlorquine	(L) Methamphetamine	Serotonin
Cholesterol	Methoxyphenamine	Sulfamethazine
Clomipramine	(±) - 3,4-Methylenedioxy-amphetamine hydrochloride	Sulindac
Clonidine	(±) - 3,4-Methylenedioxy-meth-	Tetracycline
Cocaethylene	amphetamine hydrochloride	Tetrahydrocortisone, 3- acetate
Cocaine hydrochloride	Morphine-3-β-D glucuronide	Tetrahydrocortisone 3-(β-D-glucuronide)
Codeine	Morphine Sulfate	Thiamine
Cortisone	Nalidixic acid	Tetrazolone
(-) Cotinine	Naloxone	Thioridazine
Creatinine	Naltrexone	DL-Tyrosine
Deoxycorticosterone	Naproxen	Tolbutamide
Dextromethorphan	Niacinamide	Triamterene
Diclofenac	Nifedipine	Trifluoperazine
Diffunisal	Norcodein	Trimethoprim
Digoxin	Norethindrone	Trimipramine
Diphenhydramine	D-Norpropoxyphene	Tryptamine
Doxylamine	Noscapine	DL-Tryptophan
Egonine hydrochloride	DL-Octopamine	Tyramine
Egonine methylester	Oxalic acid	Uric acid
(-) -V-Ephedrine	Oxolinic acid	Verapamil
[1R,2S] (-) Ephedrine	Oxycodone	Zomepirac
(L) - Epinephrine		
Erythromycin		
β-Estradiol		

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	
 Attention, see instructions for use	 Tests per kit
 For <i>in vitro</i> diagnostic use only	 Use by
 Store between 2-30°C	 Lot Number
 Authorized Representative	 Do not reuse



ISO



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