

BZO

One Step Benzodiazepines Test Device Package Insert

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 Device is a lateral flow chromatographic immunoassay for the detection of Oxazepam (major metabolite of Benzodiazepines) 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 Device 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 Device yields a positive result when the Benzodiazepines concentration in urine exceeds cut-off.

PRINCIPLE

The BZO One Step Benzodiazepines Test Device is an immunoassay based on the principle of competitive binding. Drugs that 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 concentration, will not saturate the binding sites of the antibodies in the test device. 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 antibodies.

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 because of the absence of drug competition.

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 device contains mouse monoclonal anti-Benzodiazepines antibody-coupled particles and benzodiazepines-protein conjugates. 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 device 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 test device 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 device is stable through the expiration date printed on the sealed pouch. The test device 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 long-term storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS

Materials Provided

- Test devices
- Disposable specimen droppers
- Package insert

Materials Required But Not Provided

- Specimen collection container
- Timer
- External controls

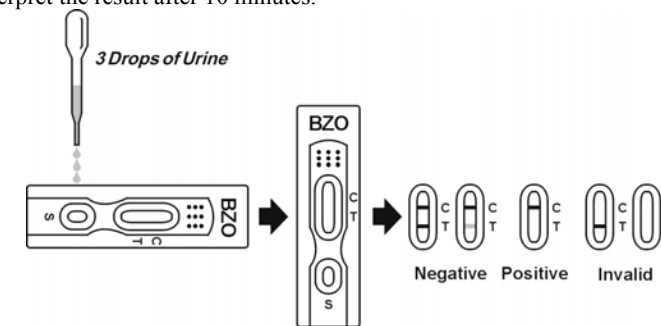
DIRECTIONS FOR USE

Allow the test device, 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 device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx. 100µl) to the specimen well (S) of the test

device, and then start the timer. Avoid trapping air bubbles in the specimen well (S). See the illustration below.

3. 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 illustration above)

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 region (T) may 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 device. 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 Device 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) are 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 cutoff 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 Device 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 Results	
	Results	Positive		Negative
	BZO One Step Test Device	Positive		130
	Negative	14	149	163
Total Results		144	154	298
% Agreement with this commercial kit		90%	97%	94%

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

Method	GC/MS		Total Results	
	Results	Positive		Negative
	BZO One Step Test Device	Positive		130
	Negative	5	159	164
Total Results		135	165	300
% Agreement with GC/MS Analysis		96%	96%	96%

Eighty (84) of these samples were also run using the BZO One Step Benzodiazepines Test Device 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 Concentration (ng/mL)	Percent of Cutoff	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	26	4
300	Cutoff	30	12	18
375	+25%	30	3	27
450	+50%	30	0	30

Analytical Specificity

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

Compound	Concentration (ng/mL)
Alprazolam	196
a-Hydroxyalprazolam	1,262
Bromazepam	1,562
Chlordiazepoxide	1,562
Chlordiazepoxide HCl	781
Clobazam	98
Clonazepam	781
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 300ng/mL cut-off was provided to each site. The following results were tabulated:

Oxazepam conc. (ng/mL)	n per site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	15	15	0	15	0	15	0
150	15	14	1	14	1	15	0
225	15	11	4	14	1	14	1
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 Device 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 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 Device 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 benzodiazepine. The following compounds show no cross-reactivity when tested with the BZO One Step Benzodiazepines Test Device 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
Amitriptyline	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-	

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

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- Hawks RL, CN Chiang. Urine Testing for Drugs of Abuse. National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

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