



BENZODIAZEPINE

Rapid Test (BZO) strip

Introduction

The BZO Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Benzodiazepine in human urine at a cut-off concentration of 300 ng/mL which is only utilized for in vitro diagnostics (IVD). It merely provides a preliminary result, therefore, an alternative method with more specificity is recommended to be used to confirm the initial results, in which case, the most preferred method is gas chromatography-mass spectrometry (GC-MS). For substance abuse cases, clinical considerations must be taken into account, especially when test results are positive.

Summary

Benzodiazepines are drugs used for cases such as treating anxiety and insomnia. These drugs act through chemical nerve receptors called gamma-aminobutyric acids (GABA) and compared to barbiturates, are safer and more effective in treating the above-mentioned conditions. Benzodiazepine is used as a painkiller before surgery and medical procedures and for the treatment of epilepsy disorders and alcohol withdrawal. If taken continuously, for example, on a daily basis for several months and with a higher dosage than normal, the risk of addiction increases. Abrupt discontinuation causes withdrawal symptoms such as stomach problems, sleep disorders, unpleasant feelings, nausea, sweat, tremors, weakness, anxiety and changes in consciousness. This drug could be traced in urine in less than a month after use. Most of its concentration in urine is in the form of conjugated drug.

Typical detection windows for Benzodiazepine in urine are 3 to 7 days. BZO Rapid Urine Test strip is a rapid urine screening test which could be performed without laboratory equipment. In this test, monoclonal

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antibody is used for specific detection of high levels of Benzodiazepine in urine. Reaching 300 ng/ml, BZO rapid test becomes positive.

Function Method Basis

Rapid Benzodiazepine urine test employs an immunochromatographic method based on competitive binding. Substances that may be found in urine specimen compete with ones metabolized by the liver to bind to antibodies. During the test, the urine specimen flows upward via capillary action. If the amount of Benzodiazepine in the urine is below 300 ng/ml, it does not bind to the binding sites of the antibodies coated with gold nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the Benzodiazepine on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 300 ng/ml, no colored line will appear in the T area, since all the binding sites of the Benzodiazepine antibodies are occupied. So, if the presence of Benzodiazepine in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains Benzodiazepine amounts lower than the desired level in the test, a colored line will appear in this area. In order to have a procedural control, a colored line always appears in the area of the control line (C), indicating the appropriate volume of the specimen added to the membrane.

Compounds

The test strip contains anti-Benzodiazepine monoclonal antibody particles and a combination of conjugated Protein-Benzodiazepine. The secondary antibody prepared in goat species is also employed for the control line.

+ **Caution:** for in vitro diagnostic use only.

Do not use after the expiration date. The test must remain in its package until use. All specimens must be considered infectious and all precautions and safety measures for handling and use must be taken.

Storage

The package should be stored at room temperature or in refrigerator (2-30 °C). The test is valid only until the expiration date printed on the package. Keep from freezing.

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Specimen collection and preparation

+ **Urine collection:** The urine specimen must be collected in a clean and dry container. It could be collected at any time of the day.

+ **Specimen storage:** The urine specimen can be stored at 2-8 °C for more than 48 hours, and frozen for long-term storage at -20 °C. Frozen specimens must be thawed and mixed well before testing.

Contents and Required Supplies

Items found in the kit: + Brochure + Test strip

Required items not included in the kit: + Specimen collection container + Timer

User Manual

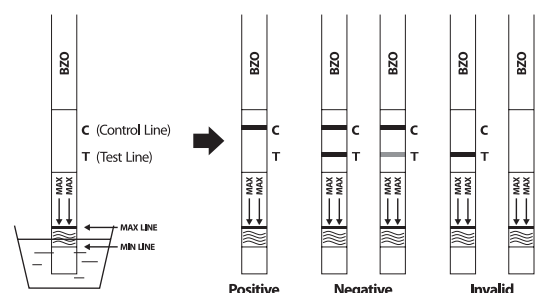
Before testing, let the test strip and the urine specimen reach room temperature (18-30 °C).

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

- Dip the test strip vertically into the urine specimen in the direction indicated by the arrows up to the MAX line, and hold it for at least 10-15 seconds (See illustration).

- Place the test strip on a flat, non-absorbent surface, set the timer and wait for the colored line to appear. Interpret the results within 5 minutes.

+ Do not interpret the results after 10 minutes.



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Result Interpretation

Negative: Two lines appear, one line should be in the control area (C) and another colored line should appear in the test area (T). This negative result indicates that the Benzodiazepine concentration is below the detectable level of 300 ng/ml.

+ **Attention:** The color shade in the testing area may vary, but it should be considered negative whenever there is even a faint colored line.

Positive: A colored line should appear in the control area (C) and no line should be seen in the test area (T). This positive result shows that the concentration of Benzodiazepine is above the detectable level of 300 ng/ml.

Invalid: The control line (C) is not formed. 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. If the problem is not solved, stop using the strip and contact the after-sales service immediately.

Quality Control

The quality control of the method is included in the test, and the colored line appearing in the control area (C) is considered as an inner controller which confirms the sufficient volume of the specimen and the correct execution of the test. The test strip does not come with control standards. It is recommended to do the test with positive and negative controls in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Benzodiazepine Rapid Test strip provides only preliminary analytical and qualitative results, and therefore, a secondary analytical method must be employed to confirm the results. For the confirmation of the results of this test, gas chromatography-mass spectrometry (GC-MS) is preferred.

2. There is a possibility of error in this technique. As some interfering substances in the urine specimen may lead to wrong results.

3. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, and should the substances remain, the test should be performed with another urine specimen.

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4. A positive result indicates the presence of substances or its metabolites. A negative result, on the other hand, may not necessarily indicate the absence of substances in the urine. The drug level may be lower than the level detectable by the test.

5. The test is not able to detect the abuse of certain therapeutic cases.

+ **Expected values:** if Benzodiazepine concentration is less than 300 ng/ml, the result will be negative and if the concentration is greater than 300 ng/ml, the result will be positive. The cut-off value of the test is 300 ng/ml.

Executive Feature

Accuracy: The results obtained from Hana Gene's Benzodiazepine Rapid Test strip were compared with those of an available commercial kit. In a study on 95 urine specimens, including 43 positive and 52 negative specimens, Hana Gene's kit detected 43 of the specimens positive and 52 negative. The results are presented in table below:

Method		other BZO Rapid Test		Total Result
The BZO Rapid Test Dipstick	Results	Positive	Negative	
	Positive	43	0	43
	Negative	0	52	52
Total Result		43	52	95
% Agreement with this Rapid Test		> 99.9%	> 99.9%	> 99.9%

In order to compare the results of Hana Gene's Benzodiazepine Rapid test kit, using GC/MS method, 250 clinical specimens (123 positive; 127 negative) were collected, and Hana Gene's kit detected 121 positive and 126 of the specimens negative. The results are shown in the table below:

Method		GC/MS		Total Result
The BZO Rapid Test Dipstick	Results	Positive	Negative	
	Positive	121	1	122
	Negative	2	126	128
Total Result		123	127	250
% Agreement with GC/MS		98.4%	99.2%	98.8%

Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations of Oxazepam:

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0 ng/ml, 150 ng/ml, 225 ng/ml, 300 ng/ml, 375 ng/ml, 450 ng/ml and 900 ng/ml. The results show more than 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below cut-off value. This information is summarized in the table below:

Oxazepam Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	27	3
300	Cut-off	30	15	15
375	+25%	30	3	27
450	+50%	30	0	30
900	3X	30	0	30

Analytical Features:

The following table shows the compounds positively identified by the Benzodiazepine test strip in the urine specimen within 5 minutes.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Alprazolam	300	Flunitrazepam	200
a-hydroxyalprazolam	1,500	(±) Lorazepam	3,000
Bromazepam	900	RS-Lorazepam glucuronide	200
Chlordiazepoxide	900	Midazolam	6,000
Clobazam	200	Nitrazepam	200
Clonazepam	500	Norchlordiazepoxide	100
Clorazepate dipotassium	500	Nordiazepam	900
Delorazepam	900	Oxazepam	300
Desalkylflurazepam	200	Temazepam	100
Diazepam	300	Triazolam	3,000
Estazolam	6,000		

Precision

A study was carried out in three different centers by untrained people making use of three different lots of the product in order to evaluate the intra-rater, inter-rater, and inter-individual reliability.

A panel of coded specimens containing certain oxazepam concentrations with GC/MS method was used in each center, at 0 ng/ml, 25% above and below the minimum detection limit, and 50% above and below the minimum detection limit of 300 ng/ml.

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Oxazepam Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
150	10	10	0	10	0	10	0
225	10	9	1	9	1	9	1
375	10	1	9	1	9	1	9
450	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Oxazepam with concentrations of 150 ng/ml and 450 ng/ml was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain oxazepam concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

Effect of Urinary pH

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given oxazepam concentrations of 150 ng/ml and 450 ng/ml. The adjusted pH of the urine was done in a duplicate rapid Benzodiazepine test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the interferences with positive and negative oxazepam test results, a study was carried out. The following compounds did not show any interference in the test results with 100 ng/ml Benzodiazepine concentration:

Acetaminophen	Deoxycorticosterone	MDE	β-Phenylethylamine
Acetophenetidin	Dextromethorphan	Meperidine	Phenylpropanolamine
N-Acetylprocainamide	Diclofenac	Meprobamate	Prednisolone
Acetylsalicylic acid	Diflunisal	Metadone	Prednisone
Aminopyrine	Digoxin	L-Methamphetamine	Procaine
Amitriptyline	Diphenhydramine	Methoxyphenamine	Promazine
Amobarbital	Doxylamine	(±) - 3,4-Methylenedioxy-	Promethazine
Amoxicillin	Ecgonine	amphetamine	D,L-Propriolol
Ampicillin	Ecgonine methylester	(±) - 3,4-Methylenedioxy-	D-Propoxyphene
L-Ascorbic acid	(-)-ψ-Ephedrine	methamphetamine	D-Pseudoephedrine
D,L-Amphetamine sulfate	(1R,2S) (-) Ephedrine	Morphine-3-β-D glucuronide	Quinacrine
Apomorphine	(L) - Epinephrine	Morphine Sulfate	Quinidine
Aspartame	Erythromycin	Naproxen	Serotonin
β-Estradiol	Nalidixic acid	Quinine	Sulfamethazine

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Atropine	Naloxone	Ranitidine	Sulindac
Benizilic acid	Estrone-3-sulfate	Naltrexone	Salicylic acid
Benzoic acid	Ethyl-p-aminobenzoate	Naproxen	Secobarbital
Benzoylcegonine	Fenoprofen	Niacinamide	Serotonin
Benzphetamine	Furosemide	Nifedipine	Sulfamethazine
Bilirubin	Genticis acid	Norcodeine	Sulindac
(±) - Brompheniramine	Hemoglobin	Norethindrone	Tetracycline
Caffeine	Hydralazine	D-Norpropoxyphene	Tetrahydrocortisone,
Cannabidiol	Hydrochlorothiazide	Noscapine	3-Acetate
Cannabinal	Hydrocodone	D,L-Octopamine	Tetrahydrocortisone
Chloralhydrate	Hydrocortisone	Oxalic acid	3-(β-D-glucuronide)
Chloramphenicol	O-Hydroxyhippuric acid	Oxolinic acid	Tetrahydrozoline
Chlorothiazide	p-Hydroxyamphetamine	Oxycodone	Thiamine
(±) - Chlorpheniramine	p-Hydroxy-	Oxymetazoline	Thioridazine
Chlorpromazine	methamphetamine	Papaverine	D,L-Tyrosine
Chlorquine	3-Hydroxytyramine	Penicillin-G	Tolbutamide
Cholesterol	Ibuprofen	Pentazocine	Triamterene
Clomipramine	Imipramine	Pentobarbital	Trifluoperazine
Clonidine	Ipreniazid	Perphenazine	Trimethoprim
Cocaoethylene	(±) - Isoproterenol	Phencyclidine	Trimipramine
Cocaine	Isosuprine	Phenelzine	Tryptamine
Codeine	Ketamine	Phenobarbital	D,L-Tryptophan
Cortisone	Ketoprofen	Phentermine	Tyramine
(-) Cotinine	Labetalol	Trans-2-phenylcyclo-	Uric acid
Creatinine	Loperamide	propylamine hydrochloride	Verapamil
Maprotiline	L-Phenylephrine	Zomepirac	

References

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



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