One Step
Benzodiazepines Test Device (Urine)
Package Insert

(Catalog Number: 1183-C)

BIOMERICA

A rapid, one step test for the qualitative detection of Benzodiazepines in human urine.

For in vitro diagnostic use only.

INTENDED USE

The One Step Benzodiazepines Test Device (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 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 One Step Benzodiazepines Test Device (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 One Step Benzodiazepines Test Device (Urine) yields a positive result when the Benzodiazepines in urine exceeds cut-off concentration.

PRINCIPLE

The One Step Benzodiazepines Test Device (Urine) 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 antibody in the test. 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 detectable 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 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 device contains monoclonal anti-Benzodiazepines antibody coupled particles and Benzodiazepines-protein conjugate. A goat antibody is employed in the control line system.

PRECAUTIONS

- 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

The kit can be stored at room temperature or refrigerated (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 droppers
- Package insert

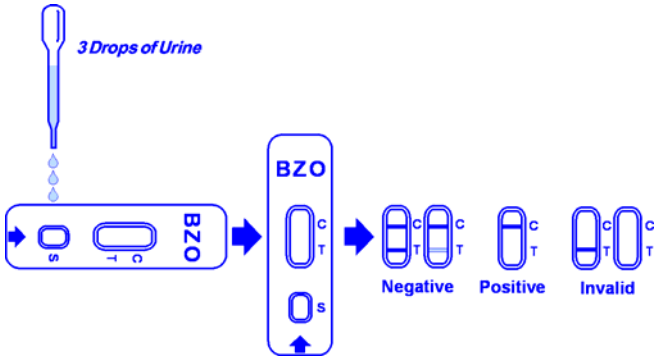
Materials Required But Not Provided

- Specimen collection container
- Timer

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 cut-off 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 One Step Benzodiazepines Test Device (Urine) provides only a preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography and mass spectrometry (GC/MS) are the preferred confirmatory methods. [1,2,3]
2. The One Step Benzodiazepines Test Device (Urine) is a qualitative screening assay and cannot determine either the drug concentration in the urine or the level of intoxication.
3. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
4. 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.
5. A positive result indicates presence of the drug or its metabolites but does not indicate level or intoxication, administration route or concentration in urine.
6. 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.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the One Step Benzodiazepines Test Device (Urine) 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
Biomerica One Step Test Device	Results	Positive	Negative	
	Positive	130	5	135
	Negative	14	149	163
Total Results		144	154	298
% Agreement with this Rapid Test Kit		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
Biomerica One Step Test Device	Results	Positive	Negative	
	Positive	130	6	136
	Negative	5	159	164
Total Results		135	165	300
% Agreement with GC/MS Analysis		96%	96%	96%

Eighty (84) of these specimens were also run using the One Step Benzodiazepines Test Device (Urine) 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 Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
150	-50%	30	30	0
225	-25%	30	26	4
300	Cut-off	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 One Step Benzodiazepines Test Device (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
Alprazolam	196
a-hydroxyalprazolam	1,262
Bromazepam	1,562
Chlordiazepoxide	1,562
Chlordiazepoxide HCI	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 specimens of normal, high, and low specific gravity ranges were spiked with 150 ng/mL and 450 ng/mL of Oxazepam respectively. The One Step Benzodiazepines Test Device (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. 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 One Step Benzodiazepines Test Device (Urine) 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 One Step Benzodiazepines Test Device (Urine) at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

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

BIBLIOGRAPHY

1. Tietz NW. *Textbook of Clinical Chemistry.* W.B. Saunders Company. 1986; 1735
2. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 2nd Ed. Biomedical Publ., Davis, CA. 1982; 488
3. Hawks RL, CN Chiang. *Urine Testing for Drugs of Abuse.* National Institute for Drug Abuse (NIDA), Research Monograph 73, 1986

	Storage Temperature	EC REP	Authorized Representative
LOT	Lot Code		Caution, See Instructions
	Expiration	IVD	For in vitro diagnostic use
	Manufacturer	REF	Catalog No.

Biomerica, Inc., 1533 Monrovia Avenue
Newport Beach, CA 92663 USA

EC REP

according to IVDD 98/79/ EC
MDSS
Burckhardtstrasse 1
30163 Hannover, Germany