DRG® Rapid BZO (RAP-3682)

Revised 12 Sept. 2006 **IVD**

INTENDED USE

The RapidBZO test is an immunochromatography based one step *in vitro* test. It is designed for qualitative determination of Benzodiazepines and their metabolites in human urine specimens above a cut-off level of 300-ng/ml oxazepam. This assay has not been evaluated in the point of care location and is for use by Healthcare Professionals only. This assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. The Substance Abuse Mental Health Services Administration (SAMHSA) has established gas chromatography/ mass spectrometry (GC/MS) as 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 indicated.

SUMMARY AND EXPLANATION

Benzodiazepines are a class of widely prescribed central nervous system depressants, which have anxiolytic, hypnotic, anticonvulsant and muscle relaxant effects. Chronic abuse can result in addiction and tardive dyskinnesia. Acute higher doses lead to drowsiness, dizziness, muscle relaxation, lethargy, and coma and possible death. The effects of benzodiazepines use last 4 – 8 hours. Many of the benzodiazepines share a common metabolic route, and are excreted as oxazepam and its glucuronide in urine. Oxazepam is detectable in the urine for up to 7 days after drug use. However, the length of time following drug use for which a positive result may occur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life, and the drug user's age, weight, activity and diet.

TEST PRINCIPLE

The RapidBZO test is based on the principle of specific immunochemical reaction between antibodies and antigens to analyze particular compounds in human urine specimen. The assay relies on the competition for binding antibody between drug conjugate and free drug, which may be present in the urine specimen being tested. When drug is present in the urine specimen, it competes with drug conjugate for the limited amount of antibody-dye conjugate. When the amount of drug is equal or more than the cut-off, 300 ng/ml of oxazepam, it will prevent the binding of drug conjugate to the antibody. Therefore, a positive urine specimen will not show a colored band on the test line zone, indicating a positive result, while the presence of a colored band indicates a negative result. A control line is present in the test window to work as procedural control. This colored band should always appear on the control line zone if the test device is stored in good condition and the test is performed appropriately.

MATERIALS PROVIDED

1. Instructions for use.
2. The RapidBZO test device. The amount of each coated antigen and/or antibody on the strip is less than 1.0 mg for antigen conjugate and is less than 1.0 mg for goat anti-mouse IgG antibody.
   Test zone: contains Oxazepam bovine protein antigen conjugates.
   Control zone: contains Goat anti-mouse IgG antibody.
   Conjugate pad: contains mice monoclonal anti-benzodiazepine antibody.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection container.
2. Timer or clock.

STORAGE AND STABILITY

The test device should be stored at 2 to 30°C and will be effective until the expiration date stated on the package. The product is humidity-sensitive and should be used immediately after being open. Any improperly sealed product should be discarded.

PRECAUTIONS

1. For in vitro diagnostic and forensic use only.
2. Do not use the product beyond the expiration date.
3. Handle all specimens as potentially infectious.
4. Humidity sensitive product, do not open foil pouch until it is ready to be tested.
5. Use a new urine specimen cup for each sample to avoid cross contamination.

SPECIMEN COLLECTION AND PREPARATION

It is required that approximately 150 µl of sample for each test. Fresh urine specimens do not need any special handling or treatment. Specimens should be collected in a clean, dry, plastic or glass container. If the assay is not performed immediately, urine specimen may be refrigerated at 2-8^{o}C or frozen up to 7 days. Specimens should be thawed and brought to room temperature before testing. Urine specimens exhibiting a large amount of precipitate or turbidity should be centrifuged or allowed to settle before testing. Avoid contact with skin by wearing gloves and proper laboratory attire.

QUALITY CONTROL

Good Laboratory practice recommends the daily use of control materials to validate the reliability of device. Control materials should be assayed as clinical specimen and challenging to the assay cutoff concentration, e.g., 25% above and below cutoff concentration. If control values do not fall within establish range, assay results are invalid. Control materials, which are not provided with this test kit, are commercially available. The Rapid Drugs of Abuse Test provides a built-in process control with a different antigen/antibody reaction at the control region (C). This control line should always appear regardless the presence of drug or metabolite. If the control line does not appear, the test device should be discarded and the obtained result is invalid. The presence of this control band in the control region serve as 1) verification that sufficient volume is added, 2) that proper flow is obtained.

PROCEDURE

1. Remove the test strip from the sealed foil pouch.
2. Dip the strip into the urine specimen with the arrow pointing toward the sample. The sample level should not be higher than the arrow pointed maximum line.
3. Hold the strip in the urine until a reddish color appears at the test area (approximately 20 seconds).
4. Withdraw the strip and place it face up on a clean, non-absorptive surface or leave the strip in urine if the urine level is not higher than arrow pointed maximum line.
5. Read the results at 5 minutes after adding the sample.

Do not interpret the result after 5 minutes.

INTERPRETATION OF RESULTS

NEGATIVE

Two colored bands form. The appearance of two colored bands, one in test line zone and the other in control line zone, indicates negative results. The negative result indicates that the Benzodiazepines concentration in the specimen is either zero or less than cut-off level.

POSITIVE

One colored band forms. One colored band appears in control line zone. No colored band is found in test line zone. This is an indication that the Benzodiazepines level in the specimen is above the cut-off level.

INVALID
If there is no colored band in control line zone, the test result is invalid. Retest the sample with a new device. **Note:** A borderline (+/-) in test line zone should be considered negative result.

LIMITATION OF PROCEDURE
The assay is designed for use with human urine only. A positive result with any of the tests indicates only the presence of a drug/metabolite and does not indicate or measure intoxication. There is a possibility that technical or procedural error as well other substances in certain foods and medicines may interfere with the test and cause false results. Please refer to "SPECIFICITY" section for lists of substances that will produce either positive results, or that do not interfere with test performance. If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drug of abuse and certain foods and medicines.

EXPECTED RESULTS
The RapidBZO Test is a qualitative assay. It identifies Benzodiazepines in human urine at a concentration of 300 ng/ml or higher. The concentration of the Benzodiazepines cannot be determined by this assay. The test is intended to distinguish negative result from presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERFORMANCE CHARACTERISTICS
ACCURACY
The accuracy of the RapidBZO test was evaluated in comparison to GC/MS at a cut-off of 300 ng/ml of oxazepam. One hundred and fourteen urine specimens with GC/MS confirmed oxazepam concentration were evaluated in this study. The results are summarized and presented below:

	(-)		(+)		
RapidBZD Test	**Negative By GC/MS**	**Near cutoff negative (between –25% and c/o**	**Near cutoff positive (between c/o and +25%**	**GC/MS Positive (greater than +25%)**	**Percent agreement with GC/MS**
Positive	6	3	6	44	84.7
Negative	39	5	1	0	97.8
Total	45	8	7	44	

Positive % agreement: 84.7
Negative % agreement: 97.8.

Ten specimens were found discrepant between the RapidBZD and GC/MS method. When compared those data, 40% (4 out of 10) of the discrepancy specimens were found between –25% and +25% cut-off concentration (225 – 375 ng/ml).

SENSITIVITY
The cut-off concentration (sensitivity level) of RapidBZO test is determined to be 300ng/ml of oxazepam.

PRECISION

Three individuals observing the test result to determine the random error of visual interpretation performed the precision study. The test results were found to have no significant differences between the three observers.

Device	Control Con.	No. of	No. of positive			No. of borderline #			No. of negative		
	ng/ml	Tested	1*	2*	3*	1*	2*	3*	1*	2*	3*
	150	40							42	42	42
	225	40				21	21	21	19	19	19
BZD	300	40	33	33	33	7	7	7			
	375	40	40	40	40						
	450	40	40	40	40						

SPECIFICITY

Adding various drugs, drug metabolites, and other compounds that are likely to be present in urine tested the specificity for RapidBZO testy. All compounds were prepared in drug-free normal human urine.

INTERFERENCE TESTING

The RapidBZO test performance at cut-off level is not affected when pH and Specific Gravity ranges of urine specimen are at 4.0 to 9.0 and 1.005 to 1.035. The following substances were tested and confirmed not to interfere with RapidBZO test at the listed concentrations.

Glucose	2000mg/dl	**Human albumin**	2000 mg/dl
Human hemoglobin	10 mg/dl	**Urea**	4000 mg/dl
Uric acid	10 mg/dl		

SPECIFICITY

The following table lists compounds that are detected by RapidBZO test, which produced positive results when tested at levels equal or greater than the concentrations listed below:

Compounds	Concn. (ng/ml)	Compounds	Concn. (ng/ml)
Nitrazepam	100	Chloradiazepoxide HCl	300
Cobazam	300	Desmethyldiazepam	300
Oxazepam	300	Tenazepam	300
Alprazolam	1000	Bromazepam	1000
Diazepma	1000	Flunitrazepam	1000
Lorazepam	1000	Clonazepam	2000
Flurazepam	100		

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Each listed substance that commonly found in the urine was evaluated and indicated negative result at concentration of 100 µg/ml.

Acetaminophen	4-Acetamidophenol	Acetylsalicylic acid	Amikacin
Amitriptyline	Amobarbital	Arterenol	Aspartame
Ascorbic acid	Atrophine	Caffeine	Camphor
Chloroquine	Chlopheniramine	Cortisone	Deoxyephedrine
Dextromethorphan	Digitoxin	Digoxin	Diphenhydramine
Ecgonine	Ecgonine methyl ester	Ephedrine	Epinephrine
Gentisic	Guaiacol glycer ester	Histamine	Hydrochlorothiazide
Homatrophine	Imipramine	Ibuprofen	Isoproterenol
Ketamine	Lidocaine	Meperidine	Methadone
Methaqualone	Methylphenidate	Neomycin	Niacinamide
Perphenazine	Penicillin G	Phenylethylamine-α	Phenylpropanolamine
Promethazine	Pseudoephedrine	Quinine antidine	Salicyclic acid
Tetracycline	Tetrahydrozoline	Theophyline	Thioridazine
Trifluoperazine	Tryptophan	Tyramine	

REFERENCES

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