DRG

DRG® Benzodiazepines Screen Test (RAP-2865)

As of 22 Nov. 2006 (Vers. 1.1) **IVD**

INTENDED USE

The Benzodiazepines Screen Test Card and Test Strip devices are in vitro diagnostic (IVD), lateral flow, immunochromatographic, qualitative urinary assays for the rapid detection of benzodiazepine and its metabolites in human urine at the Substance Abuse Mental Health Services Administration (SAMHSA) cut-off level of 300 ng/ml. The tests are designed to obtain a visual, qualitative result and are intended for professional use only. They are not intended for quantitative results, or for over-the-counter sale. The Benzodiazepines Screen Test provides only preliminary analytical data. A more specific, alternative method is required to obtain a confirmed analytical result. SAMHSA has established gas chromatography / mass spectrometry (GC/MS) as the preferred confirmatory method. Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF TEST

Benzodiazepine is the most widely prescribed anxiolytic drug and is used extensively as an anti-anxiety agent, a hypnotic, a muscle relaxant and an anti-convulsant. Benzodiazepine is usually taken orally, or by intravenous or intramuscular injection. High dose abuse of benzodiazepine can result in drowsiness and confusion, and potentiates alcohol and other CNS depressant drugs. Benzodiazepine is metabolized in the liver and excreted in urine. Benzodiazepine and its metabolites are the primary urinary markers for detecting benzodiazepine use. The Benzodiazepines Screen Tests are a rapid, visual, lateral flow, competitive immunochromatographic assay for the qualitative detection of benzodiazepine and its metabolites in human urine. These in vitro diagnostic screening tests are based immunoassay principles designed specifically for the assay and identification of benzodiazepine and its metabolites at the cut-off level of 300 ng/ml or higher, as set by SAMHSA.

PRINCIPLE OF THE PROCEDURE

The Benzodiazepines Screen Test is a competitive immunoassay in which chemically modified and bound benzodiazepine conjugate competes with urinary benzodiazepine and its metabolites for limited, specific benzodiazepine antibody binding sites. Each test device contains a sample reaction unit, a pink-colored, antibody-colloidal gold conjugate unit pre-labeled with specific antibody, and a chromatographic membrane pre-coated with drug conjugate in the device test region. When free drug is absent from urine or at concentrations lower than the urinary detection cut-off level, the pink-colored, antibody-colloidal gold conjugate is free to bind to the immobilized drug conjugate pre-coated on the chromatographic membrane test region. Consequently, a pink-colored band will form in the test region indicating a negative result. When drug is present in urine at concentrations at or exceeding the detection cut-off level, it will bind to limited epitopes on the pink-colored, antibody-colloidal gold conjugate, completely competing with drug conjugate in the test region. In such cases, no band forms in the test region, indicating a positive result. The Benzodiazepines Screen Test also provides a built-in process control. A pink-colored band should always appear in the control region, regardless of the presence of any urinary benzodiazepine and its metabolites. This pink-colored control band verifies that: 1) sufficient urine volume was added, and 2) proper flow was obtained. If the control band is missing, the test was not performed correctly or failed to function correctly. In summary, negative urine will produce two pink-colored bands, one in the control region and one in the test region. Positive urine will produce one pink-colored band in the control region only.

REAGENTS AND MATERIALS SUPPLIED

1. Test device with the following components: a sample reaction unit, a pink-colored, colloidal gold conjugate unit, and a chromatographic membrane unit. A disposable sample pipette comes with the test card.
2. Test instructions

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MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine sample collection containers
2. Timer or clock

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Avoid cross contamination of urine samples by using a new sample collection container and pipette for each urine sample.
3. Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container.
4. Do not use a test device beyond the expiration date imprinted on the outside of the foil pouch.

STORAGE AND STABILITY

The test devices supplied can be stored at room temperature or refrigerated (2-28°C) and will remain stable until the expiration date. Do Not Freeze.

SAMPLE COLLECTION AND PREPARATION

The fresh urine samples should be collected in a clean, dry container such that same-day testing may be performed. Urine specimens may be refrigerated at 2-8°C for 48hours, or frozen at -20°C for assaying at a later date. Specimens that have been refrigerated or frozen must be equilibrated to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged or allowed to settle so that clear aliquots can be obtained for testing.

ASSAY PROCEDURE

1. Do not open foil pouch until ready to begin testing. Refrigerated test device should be allowed to equilibrate to room temperature (15-28°C) before pouch is opened.
2. Remove the test device from the sealed foil pouch by tearing along the notch.
3. **For Test Strip:** Immerse the strips in urine with the arrow end pointing towards the urine. Do not introduce urine above the maximum level, as indicated by the arrows.
4. **For Test Card:** Draw the urine sample into the pipette and dispense four drops (approximately 0.2ml) into the sample well of test device.
5. Read the test result at five minutes.

IMPORTANT

In order to prevent an incorrect reading, do not read the test results after more than 10 minutes. After 10 minutes, the intensity of the colored lines may change or a new line may appear. To avoid confusion, discard the test device after interpreting the result.

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INTERPRETATION OF RESULTS

Negative

Two pink-colored bands appear, one in the control region and one in the test region. A negative result indicates free drug is absent from urine or at concentrations lower than the detection cut-off level of the test.

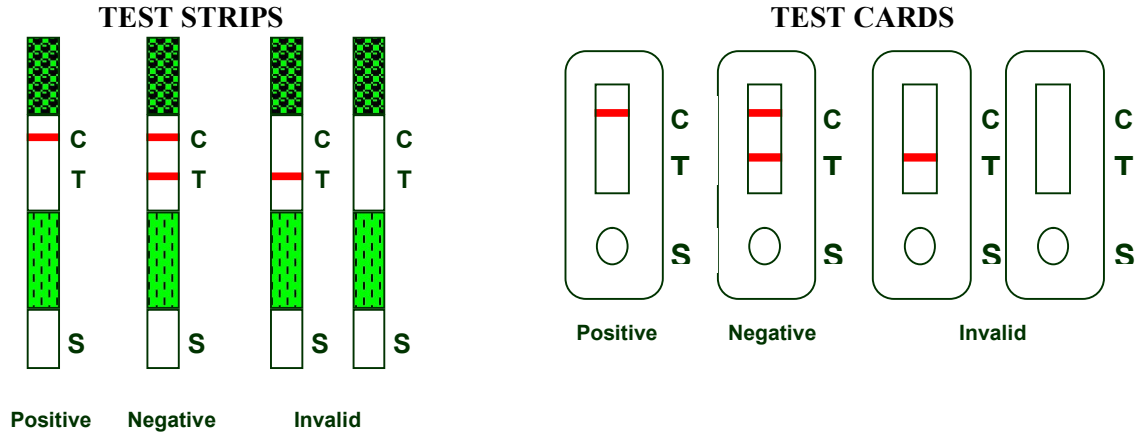
Positive

One colored band appears in the control region with no apparent band appearing in the test region. A positive result indicates free drug is present in urine at concentrations at or exceeding the detection cut-off level of the test.

Invalid

No band appears in the control region, or a pink-colored band appears in the test region only. An invalid test result may be due to improper assay procedures or damage to the device. With an invalid result, the assay is inconclusive and the specimen should be re-tested using a new test device.

Note: The test band intensity may be weaker or stronger than that of the control band, but a very faint band in the test region indicates that the free drug concentration is near the cut-off level in the urine. The specimen should be re-tested or confirmed with a more specific method before a positive determination is made.



PROCESS AND QUALITY CONTROL

Good laboratory practice requires use of control materials that ensure proper test device performance and reliability. Quality control standards are available for the validation of device functionality from commercial sources such as BioRad, Alltech and Biopool. To test the quality of control standards, use the assay procedure for testing urine samples. The SAMHSA recommended guidelines for drugs of abuse screening test devices indicate that controls should contain the drug of abuse analyte at levels at least 25% above cut-off values.

AFTER TESTING

Urine specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and dispose of all used test devices in an approved biohazard container. Residual urine should be disposed of in a medically approved manner after the completion of all testing, including the confirmatory assay.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The Benzodiazepines Screen Test has been designed for the detection of benzodiazepine and its metabolites in urine at the detection sensitivity of 300 ng/ml, as suggested for immunoassay methods by SAMHSA. In sensitivity studies performed, concentrations of benzodiazepine equal to or lower than 200 ng/ml were identified as negative for all samples. Concentrations of benzodiazepine equal to or higher than 300 ng/ml were identified as positive results for all samples. Thus, the cut-off level of the Benzodiazepines Screen Test was determined to be 300 ng/ml for both Test Card and Test Strip devices.

PRECISION

In order to determine the precision of both the Benzodiazepines Screen Test Card and Test Strip devices, both within-lot and inter-lot reproducibility studies were performed. Results of within-lot reproducibility studies clearly showed excellent repeatability for all positive and negative urine samples using one lot of Benzodiazepines Screen Test Card devices and one of Test Strip devices. The results of inter-lot reproducibility studies clearly demonstrated that there was no appreciable inter-lot variation when testing both positive and negative samples across three different lots of Benzodiazepines Screen Test Card and Test Strip devices.

SPECIFICITY STUDY

Device specificity was tested against compounds related to, or not associated with, benzodiazepine, prepared in drug-free, normal human urine. The following compounds produced positive results with the Benzodiazepines Screen Test at levels at or greater than the concentrations listed below.

Chlordiazepoxide	300ng/ml	**Bromazepam**	1000ng/ml
Estazolam	300ng/ml	**Flunitrazepam**	1000ng/ml
Flurazepam	300ng/ml	**Lormetazepam**	1000ng/ml
Oxazepam	300ng/ml	**Nitrozepam**	1000ng/ml
Alprazolam	150ng/ml	**Prazepam**	1000ng/ml
Clorazepam	150ng/ml	**Lorazepam**	1500ng/ml
Diazepam	150ng/ml	**Triazolam**	1500ng/ml
Nordiazepam	150ng/ml	**Medazepam**	2000ng/ml
Temazepam	150ng/ml	**Delorazepam**	5000ng/ml
Clobazam	200ng/ml	**Clonazepam**	25000ng/ml

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The following compounds were found not to have any impact on negative results when tested at levels of up to 100 µg drug/ml (100,000 ng drug/ml).

Acetaminophen (N-Acetyl-p- aminophenol)	Ethanol
Acetone	Furosemide
Albumin	Glucose
Amitriptyline	Guaiacol glyceryl ether
Ampicillin	Hemoglobin
Aspartame (Asp-Phe Methyl Ester)	Imipramine
Aspirin (Acetylsalicylic Acid)	(+/-)-Isoproterenol
Atropine	(1R,2S)-(-)-N-Methyl-Ephedrine
Benzocaine (Ethyl p-Aminobenzoate)	Lidocaine
Bilirubin	(+)-Naproxen
Caffeine	(+/-)-Norephedrine
Chloroquine	Penicillin-G (Benzylpenicillin)
(+)-Chlorpheniramine	Pheniramine
(+/-)-Chlorpheniramine	Phenothiazine (Thiodiphenylamine)
Creatine	L-Phenylephrine
Dexbrompheniramine ([+]- Brompheniramine)	-Phenylethylamine
4-Dimethylaminoantipyrine	Procaine
Dopamine (3-Hydroxytyramine)	Promethazine
Doxylamine	Quinidine
(+/-)-Ephedrine	Ranitidine
(-)-Ephedrine	Sulindac
(+)-Epinephrine	Tyramine
Erythromycin	Vitamin C (L-Ascorbic Acid)

ASSAY COMPARISONS & EQUIVALENCY

Accuracy and equivalency comparisons of both the Benzodiazepines Screen Test Card and Test Strip were evaluated against 174 individual in-house laboratory urine samples, as well as against 325 individual external SAMHSA-certified clinical laboratory urine samples. The results have been tabulated below.

Table 1. Benzodiazepines Card vs. Emit® II

	EMIT II (+)	EMIT II (-)	Row Totals
(+)	171	0	171
(-)	3	325	328
Col. Totals	174	325	499

When compared to Emit® II, the percent agreement with Benzodiazepines Screen Test Card positive samples was 171/174 or 98.3%. Negative samples recovered at 325/325 or 100%, while the overall relative accuracy obtained was 496/499 or 99.4%.

Table 2. Benzodiazepines Strip vs. Emit® II

	EMIT II (+)	EMIT II (-)	Row Totals
(+)	172	0	172
(-)	2	325	327
Col. Totals	174	325	499

When compared to Emit® II, the percent agreement with Benzodiazepines Screen Test Strip positive samples was 172/174 or 98.9%. Negative samples recovered at 325/325or 100%, while the overall relative accuracy obtained was 497/499 or 99.6%.

Table 3. Benzodiazepines Card vs. GC/MS

	GC/MS (+)	GC/MS (-)	Row Totals
(+)	110	2	112
(-)	0	275	275
Col. Totals	110	277	387

When compared to the GC/MS data, the relative sensitivity or percent agreement of Benzodiazepines Screen Test Card positive samples with the external clinical study was 110/110 or 100%. Negative samples recovered a relative specificity of agreement of 275/277 or 99.3%. Finally, the overall relative accuracy obtained was 385/387 or 99.5%.

Table 4. Benzodiazepines Strip vs. GC/MS

	GC/MS (+)	GC/MS (-)	Row Totals
(+)	110	2	**112**
(-)	0	275	**275**
Col. Totals	110	277	**387**

When compared to the GC/MS data, the relative sensitivity or percent agreement of Benzodiazepines Screen Test Strip positive samples with the external clinical study was 110/110 or 100%. Negative samples recovered a relative specificity of agreement of 275/277 or 99.3%. Finally, the overall relative accuracy obtained was 385/387 or 99.5%.

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LIMITATIONS OF PROCEDURE

1. This diagnostic test is designed for the medical or forensic in vitro detection of benzodiazepine and its metabolites in human urine.
2. This diagnostic test only provides a qualitative screening for benzodiazepine in urine. It is not to be used for the quantitative determination of benzodiazepine in urine. A positive result with this test indicates only the presence of benzodiazepine and its metabolites and should be confirmed by GC/MS. It does not indicate intoxication.
3. There is a possibility of false positive results due to user error or the presence of interfering factors in urine.
4. See "SPECIFICITY STUDY" for substances that produce positive results at high concentrations and those substances, which do not interfere with test performance.
5. Adulterants, such as bleach or other strong oxidizing agents, may produce erroneous test results when added to urine specimens, regardless of the analysis method used. If an adulteration is suspected, a fresh urine specimen should be used.

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