



Atlas One Step Benzodiazepines Test Cassette (Urine)

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

For *in vitro* diagnostic use only.

INTENDED USE

Atlas One Step Benzodiazepines Test Cassette (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 test will detect other Benzodiazepines, please refer to Analytical Specificity table in this package insert.

This assay provides only a qualitative, 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.

Atlas One Step Benzodiazepines Test Cassette (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. Atlas One Step Benzodiazepines Test Cassette (Urine) yields a positive result

when the Benzodiazepines in urine exceeds the cut-off level.

PRINCIPLE

Atlas One Step Benzodiazepines Test Cassette (Urine) is an immunoassay based on the principle of competitive binding. Drugs which 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 the cut-off level, will not saturate the binding sites of the antibody in the test Cassette. 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 cut-off level, 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 or a specimen containing a drug concentration less than the cut-off 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 Cassette contains mouse 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 Cassette 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 used test Cassette 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 Cassette is stable through the expiration date printed on the sealed pouch. The test Cassette 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 prolonged storage, specimens may be

frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS

Materials Provided

- Test Cassettes
- Package insert

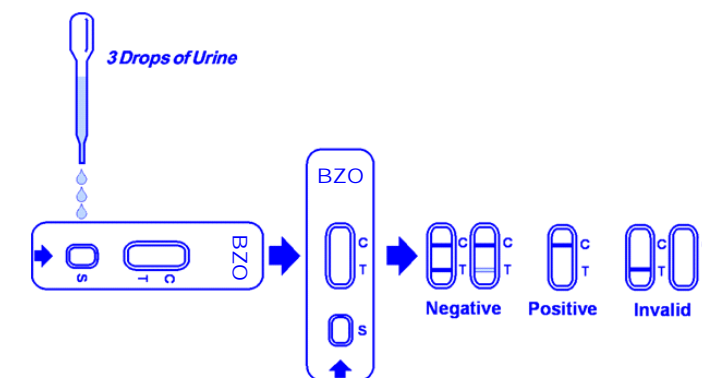
Materials Required But Not Provided

- Specimen collection container
- Timer

DIRECTIONS FOR USE

Allow the test Cassette, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

- Bring the pouch to room temperature before opening it. Remove the test Cassette from the sealed pouch and use it as soon as possible.
- 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.
- Wait for the red line(s) to appear. The result should be **read at 5 minutes**. It is important that the background is clear before the result is read. Do not interpret the result after 10 minutes.



INTERPRETATION OF RESULTS (Please refer to the 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 line 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 cut-off 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 Cassette. 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 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. Atlas One Step Benzodiazepines Test Cassette (Urine) 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) is the preferred confirmatory method. 12
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 indicates presence of the drug or its metabolites but does not indicate level of 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 cut-off level of the test.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using Atlas One Step Benzodiazepines Test Cassette (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 | Results | Other BZO Rapid Test | | Total Results |
|--------------------------------------|----------|----------------------|----------|---------------|
| | | Positive | Negative | |
| Atlas One Step BZO Test | Positive | 131 | 4 | 135 |
| | Negative | 14 | 149 | 163 |
| Total Results | | 145 | 153 | 298 |
| % Agreement with this commercial kit | | 90% | 97% | 94% |

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

| Method | Results | GC/MS | | Total Results |
|----------------------------------|----------|----------|----------|---------------|
| | | Positive | Negative | |
| Atlas One Step BZO Test Cassette | Positive | 131 | 8 | 139 |
| | Negative | 4 | 157 | 161 |
| Total Results | | 135 | 165 | 300 |
| % Agreement with GC/MS Analysis | | 97% | 95% | 96% |

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 | 75% | 30 | 27 | 3 |
| 300 | Cut-off | 30 | 11 | 19 |
| 375 | 125% | 30 | 5 | 25 |
| 450 | 150% | 30 | 0 | 30 |

Analytical Specificity

The following table lists compounds that are positively detected in urine by Atlas One Step Benzodiazepines Test Cassette (Urine) at 5 Minutes:

| Compound | Concentration (ng/mL) |
|--------------------------|-----------------------|
| Alprazolam | 196 |
| a-hydroxyalprazolam | 1,262 |
| Bromazepam | 1,562 |
| Chlordiazepoxide | 1,562 |
| 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 |

BIBLIOGRAPHY

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

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