


**ADVANCED QUALITY™ ONE STEP Benzodiazepines( BZO) TEST**
**( Saliva )**FOR *IN VITRO* DIAGNOSTIC USE ONLY**INTENDED USE**

THE ADVANCED QUALITY™ ONE STEP BENZODIAZEPINES TEST IS A RAPID, IMMUNOCHROMATOGRAPHIC ASSAY FOR THE DETECTION OF BENZODIAZEPINES IN HUMAN SALIVA. THE TEST IS USED TO SCREEN SALIVA FOR THE PRESENCE OF OXAZEPAM, THE MAIN METABOLITE OF BENZODIAZEPINES, AT A CUTOFF CONCENTRATION OF 50 NG/ML. THE TEST IS INTENDED FOR USE BY HEALTHCARE PROFESSIONALS ONLY.

*This test 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 spectrophotometry (GC/MS) is 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 THE TEST**

Benzodiazepines are therapeutically used for anxiolytic, hypnotic, anticonvulsant, and muscle relaxant effects. Acute higher doses lead to drowsiness, dizziness, muscle relaxation, lethargy and even coma.

Many of the benzodiazepines share a common metabolic route, and are excreted as oxazepam and its glucuronide in saliva. The presence of the oxazepam in the saliva indicates benzodiazepines use in the past 24 to 48 hours. Salivary concentrations are dependent on the time of sample collection and frequency of drug use.

The Advanced Quality One Step Benzodiazepines Test detects oxazepam, the main metabolite of benzodiazepines in saliva at a cutoff concentration of 50 ng/ml and greater as recommended by SAMSHA(NIDA). The test is a qualitative, visual screening immunoassay. The method employs unique antibodies to selectively identify the drug in the test saliva with a high degree of sensitivity and specificity.

**PRINCIPLE OF THE PROCEDURE**

The test device consists of a chromatographic absorbent device in which the drug or drug metabolites in the sample compete with a drug conjugate immobilized on a porous membrane support for the limited antibody sites. As the test sample flows up through the absorbent device, the labeled antibody-dye conjugate binds to the free drug in the specimen forming an antibody:antigen complex. This complex competes with immobilized antigen conjugate in the positive reaction zone and will not produce a magenta color band when the drug is above the detection level suggested for the immunoassay method. Unbound dye conjugate binds to the reagent in the negative control zone, producing a magenta color band, demonstrating that the reagents and device are functioning correctly.

A **negative** specimen produces two (2) distinct color bands, one in the test area and one in the control region.

A **positive** specimen produces only one (1) color band in the control region.

**REAGENTS AND MATERIALS SUPPLIED***FOR STRIP TEST*

1. Test strips individually foil pouched with a desiccant
2. Saliva cups
3. Package insert

*FOR CARD TEST*

1. Test cards individually foil pouched with a desiccant
2. Plastic dropper
3. Package insert

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Saliva collection containers (for card test)
2. Clock or Timer
3. Positive and negative saliva controls available from commercial distributors.

**WARNINGS AND PRECAUTIONS**

1. For *in vitro* diagnostic use only.
2. Avoid cross contamination of saliva samples by using a new saliva specimen cup for each sample.
3. Do not use the kit beyond the expiration date printed on the outside of the foil pouch.
4. Do not open the foil pouch until saliva specimen is collected and ready to be tested.
5. Saliva specimens may be infectious. Handle and dispose of all used specimens and devices in an approved biohazard container.

**STORAGE AND STABILITY**

The test device can be stored under refrigeration and at room temperature (2 - 30° C) and will be stable until the expiration date. **Do not** open foil pouch until ready to test.

**SAMPLE COLLECTION AND PREPARATION**

0.5 ml of saliva must be collected in a clean, dry, plastic or glass container, that does not contain preservative. Some plastics may adsorb drugs. If not tested immediately, saliva specimens may be stored refrigerated at 2-8° C for up to 7 days and then frozen(-20° C or colder) prior to assaying. Refrigerated or frozen samples must be warmed to room temperature and gently mixed before testing. Saliva samples exhibiting visible precipitates or turbidity should be centrifuged or allowed to settle so a clear aliquot may be sampled for this assay. Collection of samples may require mandatory procedures and custody and control records. Poppy seed ingestion has been associated with positive test results in some samples.

**ASSAY PROCEDURES***FOR STRIP TEST:*

1. Bring all materials and specimens to room temperature.
2. Remove test strip from the sealed foil pouch.
3. Dip the test strip into the saliva sample with the arrows pointing toward the specimen.
4. The saliva level should reach the maximum line marked on the strip, but must not exceed the maximum line.
5. Hold the strip in the saliva until a reddish color appears at the lower edge of the test membrane (approximately 10 seconds).
6. Withdraw the strip and place it face up on a clean, dry surface.
7. Read the result between 3 - 8 minutes after adding the sample.

*FOR CARD TEST:*

1. Bring all materials and specimens to room temperature.
2. Remove test card from the sealed foil pouch.
3. Place the test card on a flat dry surface.
4. Using the provided plastic dropper, dispense 3 drops of saliva sample to the sample well of the test card. Start timing.
5. Read result between 3 - 8 minutes after adding the sample.

**READING THE TEST RESULTS**

*Read test results between 3 - 8 minutes.*

**Do not interpret results after 8 minutes.**

**NEGATIVE** Two (2) pink/purple bands form. In addition to the control band, a pink/purple band also appears in the test region.

*Note: This immunoassay is a screening test. A negative result indicates the drug level is below the detection sensitivity. It is important to understand that concentrations of the drug below cut off may cause a faint "ghost line" to form in the test region. This "ghost line" should be considered a negative result.*

**POSITIVE** One (1) pink/purple band appears in the control region. No band is found in the test region. This is an indication that the drug level is above the detection sensitivity level.

**INVALID** If there is no pink/purple band in the control area of the strip, the test result is invalid. Retest the sample using a new device.

*Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are positive. Positive results should be confirmed by an alternate method such as GC/MS.*

#### QUALITY CONTROL

1. Each test device has a control band to indicate that the sample volume and migration is adequate, and that the colloidal gold is dissolving as expected. An invalid result must be repeated using a new test device.
2. Positive and negative, drug free saliva controls can be used to validate reagent performance and establish test reliability. Commercial drug saliva controls are available, but not provided with this test. NIDA recommended guidelines for drugs of abuse screening indicate controls should contain the drug at a level at least 20% above the NIDA cutoff value. If control values do not fall within the established limits, assay results are invalid.

#### EXPECTED RESULTS

The Advanced Quality One Step Benzodiazepines Test identifies Oxazepam in human saliva at a cutoff concentration of 50ng/mL. The concentration of the drug can not be determined using this test. The test is intended to screen saliva to separate a negative result from a presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

#### PERFORMANCE CHARACTERISTICS

##### Sensitivity

The Advanced Quality One Step Benzodiazepines Test detects an average of 50 ng/ml of oxazepam in saliva.

##### Compounds detected:

Alprazolam	30050 ng/ml
Clorazepate-HCl	30050 ng/ml
Desmethyldiazepam	30050 ng/ml
Diazepam	30050 ng/ml
Estazolam	30050 ng/ml
Flurazepam	30050 ng/ml
Nitrazepam	30050 ng/ml
Oxazepam	30050 ng/ml
Temazepam	30050 ng/ml
Chlorradiazepoxide HC1	2 µg /ml
Clobazam	3 µg /ml
Clonazepam	2 µg /ml
Delorazepam	2 µg /ml
Flunitrazepam	1 µg /ml
Medazepam	1 µg /ml

##### Specificity and interfering substances

The following compounds were negative at concentration up to 100 µg/mL(unless otherwise noted):

acetaminophenol	isoproterenol
acetylsalicylic acid	ketamine
amikacin	lidocaine
amitriptyline	methadone
d,1-amphetamine	methamphetamine
arterenol	morphine
aspartame	naloxone
atropine sulfate	neomycin
benzoylcegonine	niacinamide
caffeine	11-nor- $\Delta$ 8-THC-9-COOH (10 µg/mL)
camphor	11-nor- $\Delta$ 9-THC-COOH (10 µg/mL)

chloroquine	perphenazine
chlorpheniramine	phencyclidine
cocaine	phenobarbital
cortisone	pheylethylamine- $\alpha$
deoxyepinephrine	phencyclidine
dextromethorphan	promethazine
digitoxin	pseudoephedrine
digoxin	rantidine
epinephrine ( $\pm$ )	salicylic acid
ephedrine	secobarbital
gentisic acid	tetracycline
glucose	tetrahydrozine
histamine	theophylline
guaiaicol glyceryl ether	thioridazine
homatropine	trifluoperazine
imipramine	

#### LIMITATIONS OF THE TEST

1. This product is designed to be used for the detection of oxazepam in human saliva only.
2. Although One Step Benzodiazepines Test is very accurate in detecting benzodiazepines metabolites in saliva, there is a possibility of false results due to the presence of interfering substances.
3. The test is a qualitative screening assay and is not suggested for determining the quantitative benzodiazepines level of saliva.
4. Adulterants, such as bleach or other strong oxidizing agents, when added to saliva specimens, may produce erroneous test results regardless of the analysis method used. If adulteration is suspected, obtain another saliva specimen.
5. There is the possibility that other substances and/or factors not listed may interfere with the test and cause false results, e.g. technical or procedural errors.
6. A positive result indicates the presence of oxazepam in saliva. This result does not indicate the level of intoxication nor is it intended to monitor drug levels.
7. Results should be confirmed using an alternate method. GC/MS is the preferred confirmatory method.

#### BIBLIOGRAPHY

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