

# 7-AC

## One Step Clonazepam Test Dip Card (Urine)

### Package Insert

This Instruction Sheet is for testing of 7-Aminoclonazepam (7-AC).  
A rapid, one step test for the qualitative detection of single drug and its metabolites in human urine.  
For forensic use only.

#### INTENDED USE

The One Step Clonazepam Test Dip Card (Urine) is a lateral flow chromatographic immunoassay for the detection of single drug and its metabolites in human urine.

Test	Calibrator	Cut-off
Clonazepam (7-AC)	7-Aminoclonazepam	100 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

Clonazepam (CLZ), sold under the brand name Klonopin among others, is a medication used to prevent and treat seizures, panic disorder, and for the movement disorder known as akathisia. It is a tranquilizer of the benzodiazepine class. It is taken by mouth.<sup>1</sup> It begins having an effect within an hour and lasts between 6 and 12 hours.<sup>2</sup> Common side effects include sleepiness, poor coordination, and agitation.<sup>1</sup> Long-term use may result in tolerance, dependence, and withdrawal symptoms if stopped abruptly.<sup>1</sup> Dependence occurs in one-third of people who take clonazepam for longer than four weeks.<sup>3</sup> It may increase risk of suicide in people who are depressed.<sup>1,4</sup> If used during pregnancy it may result in harm to the baby. It binds to GABAA receptors and increases the effect of the neurotransmitter gamma-Aminobutyric acid (GABA).<sup>3</sup> As a major metabolite, 7-aminoclonazepam be used to monitor use of the parent drug, clonazepam. Clonazepam, marketed as Klonopin and Rivotril, is a long-acting benzodiazepine with anxiolytic, anticonvulsant, muscle relaxant, and hypnotic properties.

The One Step Clonazepam Test Dip Card (Urine) yields a positive result when the concentration of 7-Aminoclonazepam exceeds the 100 ng/mL cut-off level.

#### PRINCIPLE

The One Step Clonazepam Test Dip Card (Urine) is an immunoassay based on the principle of competitive binding. Drug which may be present in the urine specimen compete against their respective drug conjugate for binding sites on their specific antibody.

During testing, a urine specimen migrates upward by capillary action. A drug, if present in the urine specimen below its cut-off concentration, will not saturate the binding sites of its specific antibody. The antibody will then react with the drug-protein conjugate and a visible colored line will show up in the test line region of the specific drug Dipcard. The presence of drug above the cut-off concentration will saturate all the binding sites of the antibody. Therefore, the colored line will not form in the test line region.

A drug-positive urine specimen will not generate a colored line in the specific test line region of the Dip card because of drug competition, while a drug-negative urine specimen will generate a line in the test line region because of the absence of drug competition.

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 contains a membrane Dipcard coated with drug-protein conjugate (purified bovine albumin) on the test line, a goat polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with mouse monoclonal 7-AC antibody.

#### PRECAUTIONS

- For Forensic Use Only. Do not use after the expiration date.
- The test dip card 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 dip card 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 dip card is stable through the expiration date printed on the sealed pouch. The test dip card 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 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 dip card
- Desiccant
- Package insert

##### Materials Required But Not Provided

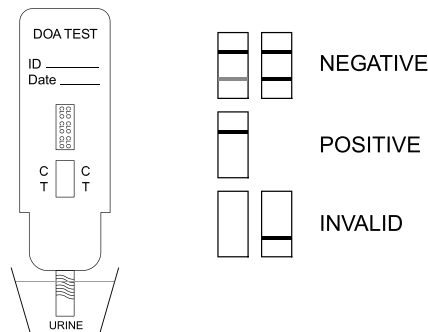
- Specimen collection container
- Timer

#### DIRECTIONS FOR USE

Allow the test dip card and urine specimen to come to room temperature [15-30°C (59-86°F)] prior to testing.

- Remove the test dip card from the foil pouch.
- Remove the cap from the test device. Label the device with patient or control identifications.
- Immerse the absorbent tip into the urine sample for 10-15 seconds. Urine sample should not touch the plastic device.
- Replace the cap over the absorbent tip and lay the device flatly on a non-absorptive clean surface.
- Read results at 5 minutes.

**DO NOT INTERPRET RESULT AFTER 10 MINUTES.**



#### INTERPRETATION OF RESULTS

(Please refer to the illustration above)

**NEGATIVE:** Two lines appear. \* One color line should be in the control region (C), and another apparent color line adjacent should be in the test region (T). This negative result indicates that the drug concentration is below the detectable level. \*NOTE: The shade of color in the test line region (T) will vary, but it should be considered negative whenever there is even a faint distinguishable color line.

**POSITIVE:** One color line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the drug concentration is above 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 using a new test dip card. If the problem persists, discontinue using the lot immediately and contact your supplier.

#### 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

- The One Step Clonazepam Test Dip Card (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.
- It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
- 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.
- A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
- 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.
- Test does not distinguish between drugs of abuse and certain medications.

#### PERFORMANCE CHARACTERISTICS

##### Reproducibility

Reproducibility studies were carried out using commercially available stork solutions of the drug analytes listed. Dilutions were made from the stork solution of each drug to the concentrations specified in the following tables. The results are listed in the following tables.

7-Aminoclonazepam conc. (ng/mL)	Total number of Determinations	Result	Precision
No drug present	40	40 negative	>99%
50	40	40 negative	>99%
150	40	40 positive	>99%
200	40	40 positive	>99%

##### Analytical Sensitivity

A drug-free urine pool was spiked with Clonazepam at the following concentrations: 0 ng/mL, -50% cutoff, -25% cutoff, +25% cutoff and +50% cutoff. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Clonazepam (7-AC) Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
50	-50%	30	30	0
75	-25%	30	28	2
100	Cut-off	30	20	10
125	+25%	30	3	27
150	+50%	30	0	30

##### Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that were detected positive in urine by The One Step Clonazepam Test Dip Card (Urine) at a read time of 5 minutes.

Compound	Concentration (ng/mL)
7-Aminoclonazepam	100
Triazolam	10,000
Clonazepam	3000
Delorazepam	500
Flunitrazepam	80,000

Lorazepam glucuronide	80,000
Lorazepam-D4	>10,000
Chlordiazepoxide HCL	80,000
Desalkylflurazepam	5,000

#### Effect of Urinary Specific Gravity

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.005, 1.015, 1.030) were spiked with drugs at 50% below and 50% above cut-off levels respectively. The One Step Clonazepam Test Dip Card (Urine) was tested in duplicate using ten drug-free urine and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

#### Effect of Urinary pH

The pH of an aliquoted negative urine pool was adjusted to pH ranges of 4.0, 4.5, 5.0, 6.0 and 9.0, and spiked with drugs at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with The One Step Clonazepam Test Dip Card (Urine). The results demonstrate that varying ranges of pH do 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 either drug-free urine or 7-AC positive urine. The following compounds show no cross-reactivity when tested with The One Step Clonazepam Test Dip Card (Urine) at a concentration of 100 µg/mL.

#### Non Cross Reacting Compounds

4-Acetamidopenol	Doxepin HCl	RS-Lorazepam glucuronide
1-Adamantanamine hydrochloride	Doxylamine	Norchlordiazepoxide
Chlorpheniramine maleate	Ethylmorphine	Nordiazepam
Caffeine	Ethylone HCl	Temazepam
Cafacolor	Hydrocodone	Amobarbital
(±) Chlorpheniramine	Hydromorphone	Buprenorphine
Ibuprofen	Imipramine	-3-D-Glucuronide
Ranitidine	Levorphanol	Norbuprenorphine
Loratadine	Midazolam	Oxymorphone
Cefadroxil	N-Desmethyl-cis-tramadol	EMDP
Nordiazepam-D5	Nitrazepam	Disopyramide
Telmisartan	Normeperidine-D4	D-Amphetamine
Citicoline Sodium	Norpropoxyphene	Mephedrone
Gatifloxacin	O-desmethyl-tramadol	Amantadine Hydrochloride
Indapamide	Oxymorphone-D3	Phentermine
Amoxicillin	Pentobarbital	(+/-)-4-Hydroxyamphetamin e HCL
Cefixime	R(+)-Methcathinone	D, L-Amphetamine
Buspirone HCl	S(-)-Methcathinone	(+/-)-Methylenedioxyamphe tamine(MDA)
Diclofenac sodium	Trimipramine	I-Methamphetamine
Ciprofloxacin hydrochloride	Zolpidem-D6	Doxepine
Ephedrine Hydrochloride	Zolpidem-D7	Maprotiline
Papaverine Hydrochloride	Zopiclone	JWH-018 5-Pentanoic acid metabolite
Promethazine Hydrochloride	Desloratadine Citrate Disodium	JWH-073 4-butanoic acid metabolite
Bupropion Hydrochloride	Zomepirac	JWH-018 4-Hydroxypentyl metabolite
Scopolamine Hydrobromide	Methedrone	JWH-018
Procaine Hydrochloride	Naphyrone	5-Hydroxypentylmetabolite
Tramadol Hydrochloride	Compound Glycyrrhizin Capsules	JWH-073 4-Hydroxybutyl metabolite
Morphine	Cefuroxime Axetil	JWH-019
Codeine Phosphate	Cefaclor Dispersible Tablets	5-hydroxyhexylmetabolite
Diacetyl Morphine Hydrochloride	Azithromycin	JWH-019 6-Hydroxyhexyl
Naloxone hydrochloride	Dextromethorphan Hydrobromide	Ethyl-β-D-glucuronide
Codeine Phosphate	Aspirin	Ethyl-β-D-glucuronide-D5
		Ecgonine
		Ecgonine methylester
		(1R,2S)-(-)-Ephedrine

Methadone	Oyster calcium carbonate	β-Phenylethy lamine
Buprenorphine Hydrochloride	Fexofenadine Hydrochloride	Fentanyl
Naltrexone	Rabeprazole sodium	Nicotine
Metoprolol	Paracetamol Hydrochloride	6-acetylmorphine
Topiramate	Mosapride Citrate	Bilirubin
Trazodone Hydrochloride	Dexamethasone acetate	Nalorphine
Sertraline Hydrochloride	Desloratadine	Normorphine
(±) Lorazepam	Vitamin U belladonna aluminum	Norcodeine
Alpha-Hydroxyalprazolam	Metronidazole	Delta-9-Tetrahydrocannabi nol
Alprazolam	(+/-)3,4-Methylenedioxy-n-ethyl(-)-11-nor-9-carboxy-delta9-amphetamine(MDEA)	THC
Amitriptyline HCl	Trimethobenzamide	11-Hydroxy-Δ9-Tetrahydroc annabinol
Butabarbital	Chloroquine	Δ8-THC-COOH
Butalbital	p-Hydroxymethamphetamine	Alphenal (Phenobarbital)
Butylone HCl	Cocaethylene	LAAM HCl
Clenbuterol HCl	Cocaine HCl	Cotinine
Clomipramine	Codeine	Compound Tropicamide
Clorazepate Dipotassium	Heroin	Eye Drops
Cyclobenzaprine Hydrochloride	Bromazepam	Brompheniramine
Desipramine	Clobazam	Flephedrone(4-fluoromethc athinone)
Dihydrocodeine HCl	Diazepam	Methylone
Diphenhydramine HCl	Estazolam	Pyrovalerone

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