

# One Step Multi-Drug, Multi-Line Screen Test Device

## Instruction Sheet for testing of any combination of the following drugs: BAR/BZO/MTD/MDMA/MOP/TCA

*A rapid, one-step screening test for the simultaneous, qualitative detection of multiple drugs and drug metabolites in human urine.*

*For healthcare professionals including professionals at point of care sites use only.*

*For in vitro diagnostic use only.*

### INTENDED USE

The One Step Multi-Drug, Multi-line Screen Test Device is a lateral flow chromatographic immunoassay for the qualitative detection of multiple drugs and drug metabolites in urine at the following cut-off concentrations:

Test	Calibrator	Cut-off
Barbiturates (BAR)	Secobarbital	300 ng/mL
Benzodiazepines (BZO)	Oxazepam	300 ng/mL
Methadone (MTD)	Methadone	300 ng/mL
Methylenedioxymethamphetamine (MDMA)	DL-3,4-Methylenedioxymethamphetamine HCl	500 ng/mL
Morphine (MOP 300 or OPI 300)	Morphine	300 ng/mL
Tricyclic Antidepressant (TCA)	Nortriptyline	1,000 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

The One Step Multi-Drug, Multi-Line Screen Test Device is a rapid urine screening test that can be performed without the use of an instrument. The test utilizes a monoclonal antibody to selectively detect elevated levels of specific drugs in urine.

### BARBITURATES (BAR)

Barbiturates are central nervous system depressants. They are used therapeutically as sedatives, hypnotics, and anticonvulsants. Barbiturates are almost always taken orally as capsules or tablets. The effects resemble those of intoxication with alcohol. Chronic use of barbiturates leads to tolerance and physical dependence.

Short acting Barbiturates taken at 400 mg/day for 2-3 months produces a clinically significant degree of physical dependence. Withdrawal symptoms experienced during periods of drug abstinence can be severe enough to cause death.

Only a small amount (less than 5%) of most Barbiturates are excreted unaltered in the urine.

The approximate detection time limits for Barbiturates are:

Short acting (e.g. Secobarbital)	100 mg PO (oral)	4.5 days
Long acting (e.g. Phenobarbital)	400 mg PO (oral)	7 days <sup>1</sup>

The BAR One Step Barbiturates Test Device yields a positive result when the Barbiturates in urine exceed the cut-off level.

### BENZODIAZEPINES (BZO)

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.

The BZO One Step Benzodiazepines Test Device yields a positive result when the Benzodiazepines in urine exceeds cut-off concentration of 300 ng/mL.

### METHADONE (MTD)

Methadone is a narcotic pain reliever for medium to severe pain. It is also used in the treatment of heroin (opiate dependence: Vicodin, Percocet, Morphine, etc.) addiction. Oral Methadone is very different than IV Methadone. Oral Methadone is partially stored in the liver for later use. IV Methadone acts more like heroin. In most states you must go to a pain clinic or a Methadone maintenance clinic to be prescribed Methadone.

Methadone is a long acting pain reliever producing effects that last from twelve to forty-eight hours. Ideally, Methadone frees the client from the pressures of obtaining illegal heroin, from the dangers of injection, and from the emotional roller coaster that most opiates produce. Methadone, if taken for long periods and at large doses, can lead to a very long withdrawal period. The withdrawals from Methadone are more prolonged and troublesome than those provoked by heroin cessation, yet the substitution and phased removal of methadone is an acceptable method of detoxification for patients and therapists.<sup>1</sup>

The MTD One Step Methadone Test Device yields a positive result when the Methadone in urine exceeds 300 ng/mL.

### METHYLENEDIOXYMETHAMPHETAMINE (MDMA)

Methylenedioxymethamphetamine (Ecstasy) is a designer drug first synthesized in 1914 by a German drug company for the treatment of obesity.<sup>3</sup> Those who take the drug frequently report adverse effects, such as increased muscle tension and sweating. MDMA is not clearly a stimulant, although it has, in common with amphetamine drugs, a capacity to increase blood pressure and heart rate. MDMA does produce some perceptual changes in the form of increased sensitivity to light, difficulty in focusing, and blurred vision in some users. Its mechanism of action is thought to be via release of the neurotransmitter serotonin. MDMA may also release dopamine, although the general opinion is that this is a secondary effect of the drug (Nichols and Oberlender, 1990). The most pervasive effect of MDMA, occurring in virtually all people who took a reasonable dose of the drug, was to produce a clenching of the jaws.

The MDMA One Step Ecstasy Test Device yields a positive result when the Methylenedioxymethamphetamine in urine exceeds 500 ng/mL.

### OPIATE (MOP 300 or OPI 300)

Opiate refers to any drug that is derived from the opium poppy, including the natural

products, morphine and codeine, and the semi-synthetic drugs such as heroin. Opioid is more general, referring to any drug that acts on the opioid receptor.

Opioid analgesics comprise a large group of substances which control pain by depressing the central nervous system. Large doses of morphine can produce higher tolerance levels, physiological dependency in users, and may lead to substance abuse. Morphine is excreted unmetabolized, and is also the major metabolic product of codeine and heroin. Morphine is detectable in the urine for several days after an opiate dose <sup>1</sup>.

The MOP One Step Opiate Test Device yields a positive result when the concentration of opiate exceeds the 300 ng/mL cut-off level.

### TRICYCLIC ANTIPRESSANT (TCA)

Tricyclic Antidepressants are commonly used for the treatment of depressive disorders. TCA overdoses can result in profound central nervous system depression, cardiotoxicity and anticholinergic effects. TCA overdose is the most common cause of death from prescription drugs. TCAs are taken orally or sometimes by injection. TCAs are metabolized in the liver. Both TCAs and their metabolites are excreted in urine mostly in the form of metabolites for up to ten days.

The TCA One Step Tricyclic Antidepressants Test Device yields a positive result when the Tricyclic Antidepressant in urine exceeds cut-off concentration 300 ng/mL.

### PRINCIPLE

The One Step Multi-Drug, Multi-line Screen Test Device is an immunoassay based on the principle of competitive binding. Drugs 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 strip. 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 strip 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

Each test line contains anti-drug mouse monoclonal antibody and corresponding drug-protein conjugates. Control line contains goat anti-rabbit IgG polyclonal antibodies and rabbit IgG.

### PRECAUTIONS

- For healthcare professionals including professionals at point of care sites.
- For *in vitro* diagnostic use only.
- Do not use after the expiration date.
- The test device 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 device should be discarded according to federal, state and local regulations.

## STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C. The test device is stable through the expiration date printed on the sealed pouch. The test device 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 precipitates should be centrifuged, filtered, or allowed to settle to obtain a clear supernatant 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 well before testing.

## MATERIALS

### Materials Provided

- Test devices
- Disposable droppers
- Package insert

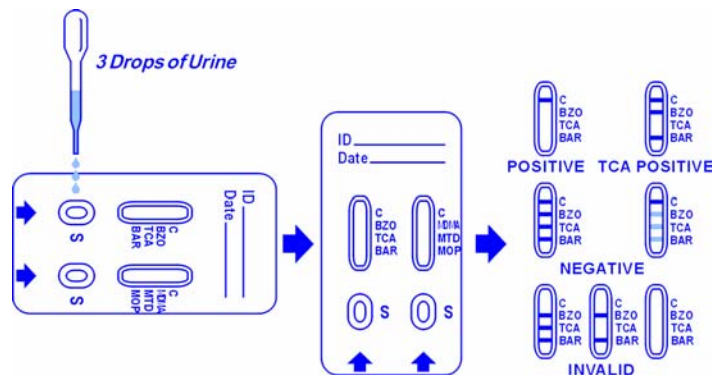
### Materials Required But Not Provided

- Specimen collection container
- External controls
- Timer

## DIRECTIONS FOR USE

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

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Hold the dropper vertically and transfer 3 full drops of urine (approx. 100 ul total volume) 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.
3. Wait for the colored lines(s) to appear. The results should be read at 5 minutes. Do not interpret results after 10 minutes.



(Please refer to the illustration above)

**POSITIVE:** No line appears in the Test region (T) for a specific drug tested. **One reddish line appears in the control region (C).** The positive result indicates that the drug concentration in the urine sample exceeds the designated cut-off for a specific drug.

**NEGATIVE:\*** The appearance of a colored line in C region and a colored line in the T region for a specific drug indicate a negative test result. Up to four colored lines may appear: one line in the C region, and up to three lines in the T region. This negative result indicates that the drug concentrations in the urine sample are below the designated cut-off levels for a particular drug tested.

**\*NOTE:** The shade of reddish color in the test region (T) may vary, but it should be considered negative whenever there is even a faint color line.

**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 device. If the problem persists, contact the clinical coordinator.

## 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 practice to confirm the test procedure and to verify proper test performance.

## LIMITATIONS

1. The One Step Multi-Drug, Multi-line Screen Test Device 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.<sup>3,4,7</sup>
2. There is a possibility 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 does not indicate level or 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.
6. Test does not distinguish between drugs of abuse and certain medications.
7. A positive test result might be obtained from certain foods or food supplements.

## PERFORMANCE CHARACTERISTICS

### Accuracy

A side-by-side comparison was conducted using the One Step Multi-Drug, Multi-line Screen Test Device and commercially available drug rapid tests. Testing was performed on approximately 1,000 specimens previously collected from subjects presenting for Drug Screen Testing. Some specimens in the +/- 25% cut-off levels were prepared by diluting from the more concentrated clinical specimens with the neat urine. Presumptive positive results were confirmed by GC/MS. Negative urine samples were screened initially by Predicate test. Approximately 10% negative samples were confirmed by GC/MS. The following compounds were quantified by GC/MS and contributed to the total amount of drugs found in presumptive positive urine samples tested in the following clinical studies:

Test	Compounds Contributed to the Totals of GC/MS
BAR	Amobarbital, Secobarbital, Butalbital, Phenobarbital
BZO	Oxazepam, Nordiazepam, a-OH-Alprazolam, Desalkylflurazepam
MTD	Methadone
MDMA	Methylenedioxymethamphetamine, Methylenedioxyamphetamine
MOP	Morphine, Codeine
TCA	Amitriptyline, Desipramine, Doxepin, Imipramine, Nortriptyline, Desmethyldoxepin

The following results were tabulated:

Method	Multi-Drug Multi-Line	GC/MS					
		Neg.*	Neg. (< -25% cutoff)	Near cutoff neg. (-25% cutoff to cutoff)	Near cutoff pos. (cutoff to +25% cutoff)	Pos. (> +25% cutoff)	% agreement with GC/MS
BAR	Positive	0	0	2	4	118	92
	Negative	150	2	6	1	10	99
BZO	Positive	0	2	0	6	122	98
	Negative	150	9	2	2	1	98
MTD	Positive	0	0	0	2	118	93
	Negative	150	17	10	8	1	>99
MDMA	Positive	0	0	3	6	79	98
	Negative	150	0	2	0	2	98
MOP	Positive	0	1	4	4	115	98
	Negative	150	2	3	2	1	97
TCA**	Positive	0	9	2	14	20	>99
	Negative	150	24	7	0	0	94

\*Negative urine samples were screened by predicate tests.

\*\*Note: TCA concentration was based on HPLC data.

Method	Predicate Test Results		% Agreement with Predicate Test	
	Positive	Negative		
BZO	Positive	130	0	98
	Negative	2	162	>99
TCA	Positive	45	0	92
	Negative	4	177	>99
BAR	Positive	124	0	98
	Negative	2	167	>99
MTD	Positive	120	0	87
	Negative	18	168	>99
MDMA	Positive	88	0	97
	Negative	2	152	>99
MOP	Positive	124	0	94
	Negative	8	150	>99

### Analytical Sensitivity

A drug-free urine pool was spiked with drugs to the concentrations at  $\pm 50\%$  cut-off and  $\pm 25\%$  cut-off. The results are summarized below.

Drug Conc. (Cut-off range)	n	BAR		BZO		MTD	
		-	+	-	+	-	+
0% Cut-off	30	30	0	30	0	30	0
-50% Cut-off	30	30	0	30	0	30	0
-25% Cut-off	30	23	7	24	6	26	4
Cut-off	30	14	16	15	15	13	17
+25% Cut-off	30	7	23	6	24	5	25
+50% Cut-off	30	0	30	0	30	0	30

Drug Conc. (Cut-off range)	n	MDMA		MOP		TCA	
		-	+	-	+	-	+
0% Cut-off	30	30	0	30	0	30	0
-50% Cut-off	30	30	0	30	0	30	0
-25% Cut-off	30	27	3	20	10	25	5
Cut-off	30	17	13	18	12	13	17
+25% Cut-off	30	6	24	7	23	7	23
+50% Cut-off	0	0	30	0	30	0	30

Eighty (80) of these samples for each drug test were also run using ACON's multi-drug test device by an untrained operator at a physician's office. Based on GC/MS data, the operator obtained a statistically similar positive agreement, negative agreement and overall agreement rate as the laboratory personnel.

### Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that are detected positive in urine by the One Step Multi-Drug, Multi-Line Screen Test Device at 5 minutes.

BARBITURATES	ng/mL
Secobarbital	300
Amobarbital	300
Alphenol	300
Aprobarbital	200
Butabarbital	75
Butalbital	2,500
Butethal	100
Cyclopentobarbital	600
Pentobarbital	300
Phenobarbital	100
BENZODIAZEPINES	
Oxazepam	300
Alprazolam	200
a-Hydroxyalprazolam	1,262
Bromazepam	1,560
Chlordiazepoxide	1,560
Chlordiazepoxide HCl	781
Clobazam	100
Clonazepam	780

Clorazepate dipotassium	200
Delorazepam	1,560
Desalkylflurazepam	390
Diazepam	195
Estazolam	2,500
Flunitrazepam	390
(±) Lorazepam	1,560
RS-Lorazepam glucuronide	160
Nitrazepam	100
Norchlordiazepoxide	200
Nordiazepam	390
Oxazepam	300
Temazepam	100
Triazolam	2,500
METHADONE	
Methadone	300
Doxylamine	50,000
MDMA	
DL-3,4-Methylenedioxyamphetamine HCl (MDMA)	500
3,4-Methylenedioxyamphetamine HCl (MDA)	3,000
3,4-Methylenedioxyethyl-amphetamine (MDE)	300
OPIATE 300 (MOP)	
Morphine	300
Codeine	300
Ethylmorphine	200
Hydrocodone	50,000
Hydromorphone	780
Levophanol	1500
6-Monoacetylmorphine	180
Morphine 3-β-D-glucuronide	1,000
Norcodeine	10,000
Oxycodone	30,000
Thebaine	6,250
TCA	
Nortriptyline	1,000
Nordoxepine	1,000
Trimipramine	2,000
Amitriptyline	1,000
Promazine	1,000
Desipramine	125
Imipramine	250
Clomipramine	50,000
Doxepin	2,000
Maprotiline	750
Promethazine	37,500

### Precision

A study was conducted at three physician offices by untrained operators using three different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens, containing drugs at the concentration of  $\pm 50\%$  and  $\pm 25\%$  cut-off level, was labeled as a blind and tested at each site. The results are given below:

Drug Conc.	n	Site A		Site B		Site C	
		-	+	-	+	-	+
Negative	90	90	0	90	0	90	0
-50% Cut-off	90	83	7	87	3	90	0
-25% Cut-off	90	67	23	75	15	80	10
+25% Cut-off	90	28	62	30	60	22	68
+50% Cut-off	90	1	89	0	90	2	88

### Effect of Urinary Specific Gravity

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.000-1.037) were spiked with drugs at 50% below and 50% above cut-off levels respectively. The One Step Multi-Drug, Multi-Line Screen Test Device was tested in duplicate using fifteen drug-free urine and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity does not affect the test results.

### Effect of the Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with drugs at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with the One Step Multi-Drug, Multi-Line Screen Test Device. The results demonstrate that varying ranges of pH does 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 Cocaine, Amphetamine, Methamphetamine, Marijuana, Opiate or Phencyclidine positive urine. The following compounds show no cross-reactivity when tested with the One Step Multi-Drug, Multi-Line Screen Test Device at a concentration of 100  $\mu\text{g/mL}$ .

### Non Cross-Reacting Compounds

Acetaminophen	β-Estradiol	Perphenazine
Acetophenetidin	Estrone-3-sulfate	Phencyclidine
N-Acetylprocainamide	Ethyl-p-aminobenzoate	Phenelzine
Acetylsalicylic acid	Fenoprofen	Trans-2-phenylcyclopropylamine hydrochloride
Aminopyrine	Furosemide	Phentermine
	Gentisic acid	L-phenylephrine
DL-Amphetamine	Hemoglobin	β-Phenylethylamine
Amoxicillin	Hydralazine	Phenylpropanolamine
Ampicillin	Hydrochlorothiazide	Prednisolone
L-Ascorbic acid	Hydrocortisone	Prednisone
Apomorphine	O-Hydroxyhippuric acid	DL-Propranolol
Aspartame	p-Hydroxyamphetamine	D-Propoxyphene
Atropine	3-Hydroxytyramine	D-Pseudoephedrine
Benzilic acid	p-Hydroxynorephedrine	Quinacrine
Benzoic acid	Ibuprofen	Quinidine
Benzoylcegonine		Quinine
Benzphetamine	Iproniazid	Ranitidine
	(±) - Isoproterenol	Salicylic acid

Bilirubin	Isoxsuprine	Serotonin
(±) - Brompheniramine	Ketamine	Sulfamethazine
Buspirone		Sulindac
Caffeine	Ketoprofen	Temazepam
Cannabidiol	Labetalol	Tetracycline
Canabinol	Loperamide	Tetrahydrocortisone, 3-acetate
Chloralhydrate		Tetrahydrocortisone
		3-(β-D-glucuronide)
Chloramphenicol	Meperidine	Theophylline
Chlorothiazide	Mephentermine	Tetrahydrozoline
(±) - Chlorpheniramine	Meprobamate	Thiamine
Chlorpromazine	D-Methamphetamine	Thioridazine
Chlorquine	Methoxyphenamine	DL-Tyrosine
Cholesterol	Methylphenidate	Tolbutamide
Clonidine	Nalidixic acid	Trans-2-
		phenylcyclopropylamine
Cocaine	Naloxone	Trazodone
Cortisone	Naltrexone	Triamterene
(-) Cotinine	Naproxen	Trifluoperazine
Creatinine		Trimethoprim
		Tryptamine
Deoxycorticosterone	Niacinamide	DL-Tryptophan
	Nifedipine	Tyramine
Dextromethorphan	Nimesulide	Uric acid
	Norethindrone	Verapamil
Diclofenac	D-Norpropoxyphene	Zomepirac
Dicyclomine	Noscapine	
Diflunisal	DL-Octopamine	
Digoxin	Oxalic acid	
5,5-Diphenylhydantoin	Oxolinic acid	
Diphenhydramine	Oxymetazoline	
Doxylamine	Papaverine	
Egonine	Penicillin-G	
(-) -ψ-Ephedrine	Pentazocine	
[1R,2S] (-) Ephedrine		
(-) -γ- Epinephrine		
Erythromycin		

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