



**ToxCup® Drug Screen Cup with Adulteration Tests**

**One-Step AMP•COC•MET•OPI•PCP•THC Test with adulteration test pads for Creatinine, Nitrite, pH and Oxidizing Agents**

This package insert a combination of tests for amphetamine, cocaine, methamphetamine, opiates, phencyclidine and THC in ToxCup® devices with adulteration pads.

**Intended Use**

The ToxCup® Drug Screen Cup is an *in vitro* screen test for the rapid detection of amphetamine, cocaine, methamphetamine, opiates, phencyclidine and marijuana in human urine at or above the following concentrations:

AMP	Amphetamine	1000 ng/ml †
COC	Benzoylcegonine	300 ng/ml †
MET	Methamphetamine	500 ng/ml
OPI	Morphine	2000 ng/ml *†
OPI	Morphine	300 ng/ml *
PCP	Phencyclidine	25 ng/ml †
THC	11-nor-Δ9-Tetrahydrocannabinol-9-carboxylic acid	50 ng/ml †

\* Opiates test can be provided at either 300 ng/ml or 2000 ng/ml

† SAMSHA mandated cut-off concentration

Additionally ToxCup® can assess the validity of the urine sample simultaneous to drug-of-abuse testing. The adulteration pads of the ToxCup® device are chemical indicator assays that detect creatinine, nitrite, pH and oxidizing agents in urine. The ToxCup® provides visual qualitative results and is intended for professional *in vitro* diagnostic use only. It is not intended for over-the-counter sale to non-professionals.

**This assay provides only preliminary screening results for drugs-of-abuse. For a quantitative analytical result or to confirm positive results obtained by ToxCup®, a more specific alternative method must be used. The Substance Abuse Mental Health Sources Administration (SAMHSA), formerly the National Institute on Drug Abuse (NIDA) has established Gas Chromatography/Mass Spectrometry (GC/MS) as the preferred confirmation method.**<sup>1</sup>

**Summary and Explanation**

One-step immunoassays are widely used for the analysis of specific substances in biological fluids. The sensitivity and speed of one-step immunoassay have made them the most widely accepted method of preliminary screening for drugs of abuse. The ToxCup® Drug Screen Cup is a simple, fast, and visually read one-step immunoassay for the qualitative detection of amphetamine, cocaine, methamphetamine, opiates, phencyclidine, and marijuana in human urine.

**AMP:** Amphetamine is chemically related to the human body's natural catecholamines, epinephrine, and norepinephrine. It has therapeutic applications and is a potent sympathomimetic agent. Amphetamine use in acute higher doses leads to enhanced stimulation of the central nervous system and induces euphoria, alertness, reduced appetite, and a sense of increased energy and power. Generally about 30% of amphetamine is excreted unchanged in 24-hour urine.

**COC:** Cocaine derived from the leaves of the coca plant, is a potent central nervous system stimulant, and has been used as a local anesthetic. Cocaine use induces euphoria, confidence, and a sense of increased energy; these psychological effects are accompanied by increased heart rate, pupil dilation, fever, tremors, and sweating. Cocaine is generally smoked or administered intravenously or orally. Cocaine base can be smoked in the form commonly known as "crack", which is likely to lead to dependence since the effect is more rapid and heightened. Cocaine is primarily excreted as benzoylcegonine and can generally be detected for 24–60 hours after cocaine use or exposure.<sup>2</sup>

**MET:** Methamphetamine is a potent sympathomimetic agent with therapeutic applications. Methamphetamine use in acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, and a sense of increased energy and power. Methamphetamine is excreted in the urine as amphetamine and oxidized as deaminated derivatives. However, 40% of methamphetamine is excreted unchanged. Thus the presence of the parent compound in the urine indicates methamphetamine use. Methamphetamine can be detected in the urine within 4–6 hours after use and for 3–5 days, depending on urine pH level.<sup>2,3</sup>

**OPI:** Heroin, morphine and codeine are opiates that are derived from the resin of the opium poppy. Heroin is quickly metabolized to morphine. Thus, morphine and morphine glucuronide may both be found in the urine of a person who has taken only heroin. The body also converts codeine to morphine. Thus, the presence of morphine (or morphine metabolite) in the urine indicates heroin, morphine and/or codeine use. Generally, morphine and other opiates can be detected in the urine within 2 to 6 hours after use and remains detectable up to 3 days.<sup>2,3</sup>

**PCP:** Phencyclidine is an arylohexylamine that is used as a veterinary anesthetic. It is used illegally as a hallucinogen, and is commonly referred to as PCP, angel dust, crystal cyclone, love boat, hog, or killer weed. PCP can produce lethargy, disorientation, and loss of coordination, visual distortion, euphoria, ataxia, and even coma. PCP can be taken orally, by nasal ingestion, smoking, or intravenous injection. It is metabolized in the liver and excreted through the kidneys. The half-life of phencyclidine is about three days.

**THC:** THC use may impair short-term memory and inhibit learning capacity. It may also alter mood and sensory perceptions, cause loss of coordination, induce anxiety, paranoia, hallucinations, depression, confusion, and increased heart rate. A tolerance to the cardiac and psychotropic effects can occur. Long-term THC use may be associated with behavioral disorders. Withdrawal from marijuana use may produce restlessness, insomnia, anorexia, and nausea.

The length of time following drug use of which a positive result may occur is dependent upon several factors, including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life, and the drug user's age, weight, activity and diet.

**Adulteration Tests**

The validity of Drugs-of-Abuse (DAU) screening depends on the integrity of the urine samples.<sup>4</sup> Contaminated or adulterated samples may cause erroneous results leading to significant consequences. Hence, it is important to ensure that the samples are intact and unadulterated prior to DAU testing.<sup>3,6</sup>

**Cr:** Creatinine is a normal urine constituent. Although the ranges are affected by age, sex, diet, muscle mass and local population distribution<sup>5</sup>, sample with creatinine level lower than 20 mg/dl should be considered diluted. The Department of Transportation (DOT) guideline<sup>7</sup> also states that urine specimens with creatinine levels less than 20 mg/dl may be indications of dilution or substitution.

**Ni:** Although nitrite is not a normal component of urine, nitrite levels of up to 10 mg/dl may be found in some urine specimens. Nitrite level above 50 mg/dl is above the clinical level and is considered abnormal.<sup>7</sup>

**Ox:** Normal urine specimen should be free of any oxidizing (Ox) agents. A positive 'Ox' detection in the urine suggests adulteration. Bleach and/or other oxidizing compounds are found in commercially available adulterant products.

**pH:** The normal urine pH ranges from 4–9. An abnormal 'pH' result (below pH 4 or above 10) indicates adulteration with acidic or alkaline adulterants added to the urine.

**Test Principle**

The ToxCup® Drug Screen Cup is based on the principle of competitive immunochemical reaction between a chemically labeled drug (drug-protein conjugate) and the drug or drug metabolites that may be present in the urine sample for the limited antibody binding sites. The test contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a pad containing colored antibody-colloidal gold conjugate. During the test, the urine sample is allowed to migrate upward and rehydrate the antibody-colloidal gold conjugate. The mixture then migrates along the membrane chromatographically by the capillary action to the immobilized drug-protein band on the test region. When drug is absent in the urine, the colored antibody-colloidal gold conjugate and immobilized drug-protein bind specifically to form a visible band at the test region as the antibody complexes with the drug-protein. When drug is present in the urine, it will compete with the drug-protein for limited antibody sites. The band at the test region will become less intense with increasing drug concentration. When a sufficient concentration of drug is present in the urine, it will fill the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate to the drug-protein at the test region. Therefore, the presence of a visible band at the test region indicates a **negative** result for the drug and the absence of the test band at the test region indicates a **positive** result for the drug.

A control band with a different antigen/antibody reaction is added to the immunochromatographic membrane strip at the control region (C) to indicate that the test performed properly. This control band should always appear regardless of the presence of drug or metabolite.

**Reagents & Materials Supplied**

- 25 individually wrapped test lids.
- 25 specimen cups
- One instruction sheet
- One Color Chart Card

Each drug test strip in the lid contains a colloidal gold pad coated with monoclonal anti-drug antibody and rabbit antibody. It also contains a membrane coated with drug-bovine protein conjugate in the test band and goat anti-rabbit antibody in the control band.

The adulteration test pads, creatinine(Cr)/Nitrite (Ni) and pH (pH)/Oxidizing Agent (Ox) contain the following:

- Cr:** 2.05% creatinine reactive indicator, 97.95% buffer and non-reactive ingredients.
- Ni:** 0.81% nitrite reactive indicators and 99.19% buffer and non-reactive ingredients.
- pH:** 0.10% reactive indicator and 99.9% non-reactive ingredients.
- Ox:** 0.22% indicator and 99.78% non-reactive ingredients.

**Warnings and Precautions**

- For professional *in vitro* diagnostic use only.
- Urine specimens may be potentially infectious. Proper handling and disposal methods should be established.
- The adulteration tests are not intended for use in the diagnosis of diseases or illness.
- Avoid cross-contamination of urine samples by using a new specimen collection container for each urine sample.
- Test device should remain sealed until ready for use.
- Do not use the test kit after the expiration date.

## Storage

The ToxCup® Drug Screen Cup should be stored at room temperature 15°–30°C (59°–86°F) in the original sealed pouch. Do not open pouch until ready to perform the assay.

## Specimen Collection and Handling

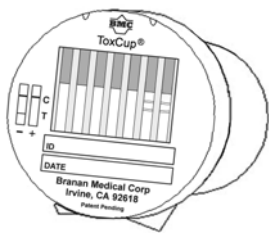
Fresh urine does not require any special handling or pretreatment. A fresh urine sample should be collected in the specimen container provided. Ensure that the sample volume meets the minimum level required as indicated on the side of the collection cup. Freshly voided, unadulterated specimens usually are in the temperature range of 90°–100°F. The temperature strip on the ToxCup® can be used as an aid in assessing sample integrity. Urine samples collected should be tested as soon as possible after collection, preferably within the same day. Specimens that have been refrigerated or frozen must be equilibrated to room temperature and mixed thoroughly prior to testing.

**Note:** All materials coming into contact with urine specimens should be handled and disposed of as if potentially infectious. Avoid direct contact and follow good laboratory practice.

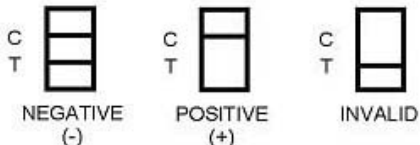
## Test Procedure

Do not open test lid pouch until ready to perform the test. Allow refrigerated or frozen specimens to warm to room temperature before testing.

1. Remove the test lid from the sealed pouch.
2. Twist the test lid securely onto the specimen cup after collection. Lay the cup on its side, as shown in the illustration on the right, to activate testing.
3. **Adulteration Pads:** Read results in 1 minute. Do not read after 2 minutes as reaction colors may fade. For ease of reading the adulteration pads, the cup may be tilted up-right, then returned to its side (as shown).
4. **Drugs-of-Abuse Tests:** Once the control bands (C) form (in 5 minutes or less) results are ready to interpret. Results are stable and may be interpreted up to 1 hour after the control bands (C) form.



## Interpretation of Results: Drugs-of-Abuse Tests



**Negative:** The presence of a colored band at the control region (C) and a colored band at a specific test region regardless of the intensity indicate that the result is negative for that particular test.

**Positive:** The presence of a colored band at the control region (C) and the absence of a colored band at the test region indicate a positive result for that particular test.

**Invalid:** No band appears at the control region (C). The test is inconclusive even if there is a band in the test region. If the test device does not produce a band at the control region, check testing procedures, samples, and/or control materials, and repeat the test using a new device.

**Important: Read each test independently. Do not compare color intensity of one test to another. Samples with faint test bands at the test regions should be considered negative. The ToxCup® Drug Screen Cup provides qualitative results for the presence of drug(s) at specified cut-off concentration(s). It is recommended that samples with questionable test band and positive result be confirmed with a more specific quantitative method (Gas Chromatography/Mass Spectrometry).**

## Interpretation of Results: Adulteration Tests

See enclosed color chart card. Qualitative results are obtained by visually comparing the color of each test pad with the corresponding color blocks on the chart.

## Quality Control

**Internal control:** The ToxCup® test device has built-in internal procedural controls. The appearance of the control band (C) is considered an internal procedural control. This band should always appear if adequate sample volume is used and the testing procedure is followed. Additionally, the background color should become clear and provide distinct test result. If the control band (C) does not appear then the test is invalid. The test should be repeated using a new device.

**External control:** It is recommended that negative and positive urine controls be used to initially test each new lot of product to ensure proper kit performance. The same assay procedure should be followed with external control materials as with a urine specimen. If external controls do not produce the expected results, do not run test specimens. Follow the proper federal, state and local guidelines when running external controls.

**Quality control testing at regular intervals is good laboratory practice and may be required by federal, state or local guidelines. Always check with the appropriate licensing or accrediting bodies to ensure that the quality program employed meets the established standards.**

## Limitations of Procedure

- The assay is designed for use with human urine only.
- Positive results only indicate the presence of drug/metabolites and do not indicate or measure intoxication.
- There is a possibility that technical or procedural errors as well as other substances in certain foods and medication may interfere with the test and cause false results. See Specificity section for the list of substances that will produce positive results, and interference section for list of compounds that do not interfere with test performance.
- If a drug/metabolite is found present in the urine specimen, the assay does not indicate frequency of drug use or distinguish between drugs of abuse and certain foods and/or medications.
- If it is suspected that the sample may have been mislabeled a new specimen should be collected.
- If it is suspected that the sample may have been tampered, the test should be repeated, and a new specimen should be collected.
- Abnormal adulteration test results do not indicate the use of a specific adulterant.
- If abnormal results are obtained with any adulteration test, the specimen should be retested and sent to a laboratory for confirmatory analysis.

## Performance Characteristics

### A. Sensitivity (Cutoff)

The sensitivity of the ToxCup® drug tests was evaluated using spiked drug samples. All sample concentrations were confirmed by GC/MS analysis. The cutoff concentrations (lowest concentration observed to produce a positive result) are as follows:

Drug	Tested Substance	Cutoff Concentration	Units
AMP	D-amphetamine	1000	ng/ml
COC	Benzoyllecgonine	300	ng/ml
OPI	Morphine	2000	ng/ml
OPI	Morphine	300	ng/ml*
MET	D-Methamphetamine	500	ng/ml
PCP	Phencyclidine	25	ng/ml
THC	11-nor-Δ9-Tetrahydrocannabinol-9-carboxylic acid	50	ng/ml

### B. Accuracy (Drugs-of-Abuse Tests)

The accuracy of the ToxCup® Drug Screen Cup was evaluated referencing GC/MS analyzed samples as well as comparing to the predicate method (other commercially available immunoassays). 40 presumed negative urine samples were collected from volunteer donors and tested with both the ToxCup® Drug Screen Cup and the predicate method. Of the 40 presumed negative urine samples tested, all were found negative by both methods (100% agreement).

Additionally, for each drug test on the ToxCup® device, a minimum of 40 clinical urine samples previously analyzed by GC/MS method with known concentration(s) of drug(s) values were blind labeled and evaluated. The results are summarized below:

Drug Test		GC/MS Near Neg. (below C/O)	GC/MS Near Pos. (+25% to C/O)	GC/MS Pos. (> +25%)	% Agreement w/ GC/MS	
					Neg (-)	Pos (+)
AMP (d-Amph.)	Pos. (+)	0	6	33	100%	98%
	Neg. (-)	5	1	0		
COC (Benzoyllecgonine)	Pos. (+)	0	7	32	100%	98%
	Neg. (-)	4	1	0		
MET (d-Methamp.)	Pos. (+)	0	5	34	100%	98%
	Neg. (-)	4	1	0		
OPI2000 (Morphine)	Pos. (+)	0	4	36	100%	100%
	Neg. (-)	5	0	0		
OPI300 (Morphine)	Pos. (+)	0	5	34	100%	98%
	Neg. (-)	4	1	0		
PCP (Phencyclidine)	Pos. (+)	0	5	35	100%	100%
	Neg. (-)	4	0	0		
THC (Δ9-THC-COOH)	Pos. (+)	0	9	30	100%	98%
	Neg. (-)	5	1	0		

\*Some Near Negative and Near Positive specimens were diluted from more concentrated samples.

### B1. Accuracy (Adulteration tests)

The accuracy of the adulterant tests was verified by an independent laboratory analyzing ten each of the normal and the various adulterated urine specimens. Low creatinine samples were obtained by diluting normal urine with water at a 1:4 ratio. HCl or NaOH was added to urine to obtain acidic or basic specimens. Ultra Bleach, pyridinium chlorochromate (PCC) and sodium nitrite were added to produce the respective adulterated samples.

Results were as follows:

Adulterant Test	Normal Samples			Adulterated Samples		
	# of Samples	Conc./ Level	Results	# of Samples	Conc./ Level	Results
Creatinine	10	50 mg/dl	All normal	10	<10 mg/dl	All abnormal
pH	10	pH = 7	All normal	10	pH = 2.5	All abnormal
				10	pH = 12	All abnormal
Nitrite	10	<5 mg/dl	All normal	10	>50 mg/dl	All abnormal
Oxidizing Agent	10	None	All normal	10	1% v/v Bleach	All abnormal
				10	1% v/v PCC	All abnormal

### Expected Results (Adulteration Pads)

**Cr:** Daily creatinine excretion, related to the muscle mass of the human body is usually constant. DOT guidelines state that creatinine levels of less than 20 mg/dL are indicative of dilution.<sup>7</sup>

**Ni:** Although nitrite is not a normal component of urine, nitrite levels of up to 10 mg/dl may be found in some urine specimens due to urinary tract infections, bacterial contamination or improper storage. Nitrite level above 50mg/dl exceeds the clinical level and is considered abnormal.

**pH:** Urine pH may range from 4 to 10. Values below pH 4.0 or above pH 10 are abnormal and indicative of adulteration.

**Ox:** The presence of oxidizing agents in the urine is abnormal and indicative of adulteration.

### C. Precision

For each drug test of the ToxCup® device, drug-free normal urine was spiked with the corresponding drug standard to various concentrations (-50%, -25%, +25% and +50%). For each concentration prepared, a total of 25 tests were performed to validate the test performance around the cut-off concentration. The results for each drug test in the ToxCup® Drug Screen Cup are summarized below:

Drug Test	Total # of Test per Conc.	Concentration											
		-50%			-25%			+25%			+50%		
		-	+/-	+	-	+/-	+	-	+/-	+	-	+/-	+
AMP	25	25	0	0	20	5	0	0	3	22	0	0	25
COC	25	25	0	0	19	6	0	0	4	21	0	0	25
MET500	25	25	0	0	19	5	1	0	5	20	0	1	24
MOR2000	25	25	0	0	12	13	0	0	5	20	0	0	25
MOR300	25	25	0	0	18	7	0	0	5	20	0	2	23
PCP	25	25	0	0	6	17	2	0	3	22	0	1	24
THC	25	25	0	0	12	11	2	0	4	21	0	0	25

### D. Specificity

The specificity for the ToxCup® Drug Screen Cup was determined by testing various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in drug-free normal human urine. The effect of specimens with various pH (4.5–8.5) and specific gravity (1.005–1.030) ranges was also evaluated and found not to interfere with the ToxCup® Drug Screen Cup.

The following compounds produced positive results when tested at or above the concentrations listed below.

Compound	Concentration (ng/ml)
<b>Amphetamine</b>	
d-Amphetamine	1,000
dl-Amphetamine	2,500
(+/-)3,4-methylenedioxyamphetamine(MDA)	1,250
d-Methamphetamine	50,000
(+/-)3,4-methylenedioxymethamphetamine(MDMA)	50,000
<b>Cocaine</b>	
Benzoylcegonine	300
Cocaine	300
<b>Methamphetamine (500 ng/ml cut-off)</b>	
d-Methamphetamine	500
(+/-)3,4-methylenedioxyamphetamine(MDMA)	500
l-Methamphetamine	10,000
d-Amphetamine	50,000
Ephedrine	50,000
Mephentermine	50,000
(+/-)3,4-methylenedioxyethylamphetamine(MDEA)	50,000
(+/-)3,4-methylenedioxyamphetamine(MDA)	100,000
l-Amphetamine	> 100,000
<b>Opiates (2000 ng/ml cut-off)</b>	
Morphine	2,000
Codeine	2,000
Ethylmorphine	5,000
Morphine-3-glucuronide	5,000
Nalorphine	5,000
Heroin	10,000
Hydrocodone	40,000
Hydromorphone	50,000
<b>Opiates (300ng/ml cut-off)</b>	
Morphine	300
Codein	300
Ethyl morphine	300
Hydromorphone	400

### PCP

Phencyclidine	25
Tenocyclidine	2,000

### THC

11-nor-Δ8-THC-9-carboxylic acid	50
11-hydroxy-Δ9-tetrahydrocannabinol	1,000
Δ8-tetrahydrocannabinol	5,000
Δ9-tetrahydrocannabinol	5,000
Cannabinol	10,000
Cannabidiol	> 100,000

### E. Interference

The following compounds were found not to cross-react when tested at concentrations up to 100 µg/ml.

Acetaminophen	4-Dimethylaminoantipyrine	Maprotiline
Acetone	Diphenhydramine	(+)-Naproxen
Albumin	Dopamine	Niacinamide
Amitriptyline	(+/-)-Epinephrine	Nicotine
Ampicillin	Erythromycin	(+/-)-Norephedrine
Ascorbic Acid	Ethanol	Oxalic Acid
Aspartame	Furosemide	Penicillin-G
Aspirin	Glucose	Pheniramine
Atropine	Guaiacol Glyceryl Ether	Phenothiazine
Benzocaine	Hemoglobin	l-Phenylephrine
Bilirubin	Ibuprofen	β-Phenylethylamine
Caffeine	Imipramine	Quinidine
Chloroquine	(+/-)-Isoproterenol	Riboflavin
(+)-Chlorpheniramine	Ketamine	Sodium Chloride
(+/-)-Chlorpheniramine	Levorphanol	Sulindac
Creatine	Lidocaine	Theophylline
Dexbrompheniramine	(1R,2S)-(-)-N-Methyl-	Trimipramine
Dextromethorphan	Ephedrine	Tyramine

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