

Rapid Methadone Urine Test

Ref. No. 1118

INTENDED USE

Acro Rapid Methadone Urine Test is a lateral flow, rapid immunoassay for the qualitative detection of Methadone in human urine at a cutoff of 300 ng/mL. The test is used to obtain a visual qualitative result.

This assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to a drug test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/ Mass Spectroscopy (GC/MS) analysis is preferred.

SUMMARY AND EXPLANATION

Methadone is a synthetic opioid, used medically as an analgesic and anti-addictive. Although chemically unlike morphine or heroin, methadone also acts on the opioid receptors and thus produces many of the same effects. Methadone is also used in managing chronic pain due to its long duration of action and very low cost.

TEST PRINCIPLE

The Acro Rapid Methadone Urine Test is based on the principle of competitive immunochemical reaction between an immobilized drug-protein conjugate and the drug or drug metabolites, which may be present in the urine sample for limited binding sites for the drug/drug metabolites of a labeled drug antibody. The test device contains a nitrocellulose membrane strip pre-coated with drug-protein conjugate in the test region and a wicking pad containing colored antibody-colloidal gold conjugate. During the test, the urine sample is allowed to migrate upward and hydrate the antibody-colloidal gold conjugate. The mixture then migrates along the membrane chromatographically by capillary action to the immobilized drug-protein band in 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 line in the test region (test line). When methadone is present in the urine sample, it will compete with the drug-protein conjugate for the limited antibody binding sites. When the drug is present in sufficient concentration in the urine sample, it will fill the limited antibody binding sites, which will inhibit attachment of the colored antibody-colloidal gold conjugate to the drug-protein conjugate in the test region. Therefore the presence of the test line indicates a **negative** result for methadone and the absence of the test line indicates a preliminary **positive** result for methadone.

Another visible line, control line, generated by a different antigen/antibody reaction is also present at a control region (C) next to the test region of the test strip. The control line should always appear, regardless of the presence of the drug or drug metabolites in the urine sample. This means that a **negative** urine sample will produce **two** lines (test line and control line), and a **positive** urine sample will generate only **one** line (control line). The presence of control line serves as a built-in control, which demonstrates that the test is performed properly.

REAGENTS & MATERIALS SUPPLIED

1. Test Cassette contains membrane-embedded reagents in a protein matrix containing sodium azide as a preservative.
2. Anti-methadone monoclonal antibody is from murine ascites.
3. Dropper. A transfer pipette is included with each test device inside the foil pouch.
4. Test Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Timer
2. Sample container

WARNINGS AND PRECAUTIONS

1. For laboratory *in vitro* diagnostic use only
2. Urine specimens may be potentially infectious. Proper handling and disposal procedures should be followed.
3. Avoid cross-contamination of urine samples. Use a new dropper or transfer pipette for adding each test sample.
4. Test device should remain sealed until ready for use.
5. Do not use the test kit after the expiration date.

STORAGE AND STABILITY

Store at 2-30°C (36-86°F) in the original sealed pouch. Do not freeze.

SPECIMEN COLLECTION AND HANDLING

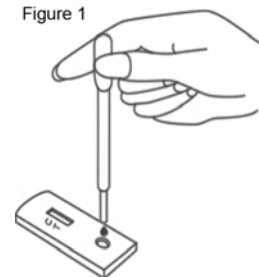
Fresh urine does not require any special handling or pretreatment. A clean dry plastic or glass container may be used for specimen collection. If the specimen is not immediately tested after the specimen collection, the specimen may be refrigerated at 2-8°C for up to 3 days or frozen at -20°C for longer period of time. Specimens that have been refrigerated must be equilibrated to room temperature prior to testing. Frozen specimens must be thawed and mixed thoroughly prior to testing.

Note: Urine specimens and all materials coming in contact with the specimens should be handled and disposed as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

ASSAY PROCEDURE

1. Bring the test components and urine sample to room temperature (15° - 28° C) before testing. Do not open the foil pouch until ready to begin testing.
2. Open the foil pouch at the notch and remove the test device and dropper prior to testing. Place the device on a clean, level surface.
3. Hold the dropper vertically and dispense 2 drops (80 µl) of urine sample without air bubbles into the sample well "S" of the test device (figure 1).
4. Read the result between 5-10 minutes (figure 2).

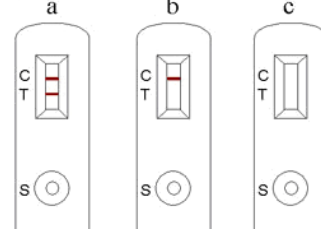
Figure 1



INTERPRETATION OF RESULTS

1. **Preliminary Positive:** a *rose-pink* color band appears in the Control Zone "C" but not in the Test Zone "T". A preliminary positive result indicates methadone level in the urine sample is at or above the detection sensitivity of 300 ng/mL. The sample should be confirmed.
2. **Negative:** two horizontal *rose-pink* color bands appear, one in the Control zone "C" and one in the Test Zone "T". A negative result indicates methadone level in the urine sample is below the detection sensitivity of 300 ng/mL.
3. **Invalid:** no *rose-pink* bands appear, or a band appears in the Test Zone "T", but not in the Control Zone "C". An invalid result may be due to improper testing procedures or deterioration of the kit components.

Figure 2



Note: There is no meaning attributed to line color intensity or width.

QUALITY CONTROL

An internal procedure control is included in the test device. A control line must form regardless of the presence or absence of drugs or metabolites. The presence of the line in the Control region indicates that a proper sample volume has been used. If the line in the Control region does not form, the test is considered invalid.

To ensure proper kit performance, it is recommended that the test devices be tested once a week or prior to use with external controls. External controls are available from commercial sources. It is important to make sure that the control values are within established limits. If the values of external controls do not fall within established limits, the test results are invalid. Additional controls may be tested according to guidelines or requirement of local state, and/or federal regulations or accrediting organizations.

LIMITATIONS OF PROCEDURE

1. The assay is designed for use with human urine only.
2. A preliminary positive result indicates only the presence of methadone and does not indicate or measure intoxication
3. There is a possibility that technical or procedural error as well as other substances as factors not listed may interfere with the test and cause false results. See SPECIFICITY section for substances that will produce positive results, or that do not interfere with the test performance.
4. If adulteration is suspected, the test should be repeated with a new sample.
5. Certain over the counter or prescription medications (or certain foods) may cause false results.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity:** The Acro Rapid Methadone Urine Test detects methadone and its metabolites in urine at concentrations equal to or greater than 300 ng/mL.
2. **Specificity:** Interference of substances that may be present in urine specimens, as well as sample effect of sample pH and specificity were studied.
 - a. Cross-reactivity of non-methadone related compounds at concentrations much higher than normally found in the urine of people using or abusing them were tested using the assay devices. No cross-reactivity was detected with the substances listed in Table I.
 - b. Table II lists Methadone related substances and concentrations that produced results approximately equivalent to the cutoff level for methadone.

Table-I: Compounds tested and found not to cross-react with the test at the concentrations of 10 µg/mL and 100 µg/mL in urine.

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

Table-II: Concentration of methadone-related compounds showing a positive response approximately equivalent to the methadone cut-off set for the test.

Compound /	Concentration in ng/ml
Methadone	300

3. Accuracy

Comparison with GC/MS and predicate device: The accuracy study was performed by testing urine samples with GC/MS analysis results and predicate device results of the same samples.

Comparison with GC/MS

Acro BAR	GC/MS, Cutoff 300ng/ml			
	Negative	Near Cutoff Negative (-50% to cutoff)	Near Cutoff Positive (cutoff to +50%)	Positive (>+50%)
Positive	0	2	12	45
Negative	72	10	1	0

Agreement of positive specimens: 57/59 = 98%

Agreement of negative specimens: 82/84 = 98%

4. Reproducibility. The reproducibility was evaluated at four different sites. The Acro Rapid Methadone Urine Test was tested against blind-labeled urine controls containing 0, 150, 225, 375, 450 and 600 ng/mL methadone at each site. The results are summarized as follows:

Test Sites	0 ng/mL		150ng/mL		225ng/mL		375ng/mL		450ng/mL		600ng/mL	
	#	Result	#	Result	#	Result	#	Result	#	Result	#	Result
1	20	20-	20	20-	20	5+, 15-	20	14+, 6-	20	20+	20	20+
2	16	16-	16	16-	16	5+, 11-	16	12+, 4-	16	16+	16	16+
3	16	16-	16	16-	16	4+, 12-	16	13+, 3-	16	16+	16	16+
4	16	16-	16	16-	16	4+, 12-	16	12+, 4-	16	16+	16	16+
Total	68	68-	68	68-	68	18+, 50-	68	51+, 17-	68	68+	68	68+

5. Effects of Urinary Specific Gravity

Ten urine specimens of normal high and low specific and low specific gravity ranges were spiked with 150ng/ml and 450ng/ml of secobarbital. The device was tested in duplicates using the ten urine specimens. The results demonstrate that varying ranges of urine specific gravity do not affect the test results.

6. Effect of 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 secobarbital to 150ng/ml and 450ng/ml. The spiked, pH-adjusted urine was tested with the test device in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.