

Rapid Buprenorphine Urine Test For *in vitro* diagnostic use only BUPDS10

INTENDED USE

Acro Rapid Buprenorphine Urine Test is a lateral flow, rapid immunoassay for the qualitative detection of Buprenorphine and Buprenorphine metabolites in human urine at a cutoff of 10ng/mL. The test is used to obtain a visual qualitative result and is intended for laboratory use only.

The rapid buprenorphine assay provides only a preliminary test 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

Buprenorphine, also colloquially referred to as bupe, is an opioid drug with partial agonist and antagonist actions. Buprenorphine is a semisynthetic opioid analgesic derived from thebain, a component of opium. Buprenorphine is indicated for the treatment of moderate to severe pain, peri-operative analgesia, and opioid dependence. It has a longer duration of action than morphine, and sublingual tablets offer an analgesic effect for 6 to 8 hours. (Joint Formulary Committee, 2004) The structure of Buprenorphine is similar as morphine but has antagonist and agonist properties. As an analgesic, Buprenorphine is believed to be 25 to 40 times more potent than morphine. When used as an antagonist, it is equivalent in potency to naltrexone. Both Subutex, a higher dose of Buprenorphine, and Suboxone, contains buprenorphine as active drug have been widely used in Europe and US as a substitution treatment for opiate addiction or dependence. It has also been shown that Buprenorphine has abuse potential and may itself cause dependency.

Buprenorphine exerts its analgesic effect via high affinity binding CNS opiate receptors. It has high affinity for the micro-receptors and dissociates from them slowly, which may contribute to its long duration of action and low physics dependence. Onset of analgesic effect occurs 15 minutes after IM injection, peak in approximately 1 hour, and persists up to 6 hours. When given IV, the time to onset and peak is shortened.

Buprenorphine and its metabolites may be present in urine after buprenorphine use. Major Buprenorphine metabolites include norbuprenorphine, norbuprenorphine, 3-β-D glucuronide, and buprenorphine 3-β-D-glucuronide.

The length of time following drug use for 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, the drug user's age, weight, activity and diet.

TEST PRINCIPLE

The Acro Rapid Buprenorphine 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 Buprenorphine 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 Buprenorphine and the absence of the test line indicates a preliminary **positive** result for Buprenorphine.

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 strip contains membrane-embedded reagents in a protein matrix containing sodium azide as a preservative.
2. Anti-Buprenorphine 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

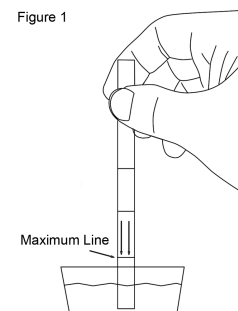
Fresh urine does not require any special handling or pretreatment. A clean dry plastic or glass container may be used for specimen collection. If the specimen is not immediately tested after the specimen collection, the specimen may be refrigerated at 2-8°C for up to 3 days or frozen at -20°C for longer 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 prior to testing.
3. Hold the test strip vertically and dip the arrow pointed end of the strip into the test specimen for about 10 seconds. Do NOT let urine level exceeds the maximum sample line (Figure 1).
4. Leave the test strip in the specimen container if the specimen level is below the maximum line of the test strip or remove the test strip from the container and lay it on a flat surface.
4. Read the result between 5-10 minutes.

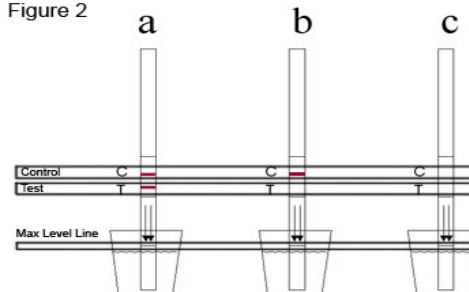
Figure 1



IMPORTANT: The result must be read at five - eight minutes. Waiting more than eight minutes may cause the test result to be inaccurate.

INTERPRETATION OF RESULTS

Figure 2



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 Buprenorphine level in the urine sample is at or above the detection sensitivity of 10 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 Buprenorphine level in the urine sample is below the detection sensitivity of 10 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.

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 rapid buprenorphine assay is designed for use with human urine only.
2. A preliminary positive result indicates only the presence of Buprenorphine or buprenorphine metabolites 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 Buprenorphine Urine Test detects Buprenorphine and its metabolites in urine at concentrations equal to or greater than 10 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-Buprenorphine 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 Buprenorphine related substances and concentrations that produced results approximately equivalent to the cutoff level for Buprenorphine.
 - c. Varying sample pH within the range of 4 and 9 has no significant effect on the assay results.
 - d. Varying sample specific gravity within the range of 1.003 and 1.040 has no significant effect on the assay results.

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.

<i>Acetaminophen</i>	<i>Magnesium Hydroxide</i>
<i>Aspirin</i>	<i>Manganese</i>
<i>Biotin</i>	<i>Meclizine HCl</i>
<i>Boron</i>	<i>Molybdenum</i>
<i>Caffeine</i>	<i>Naproxen Sodium</i>
<i>Calcium</i>	<i>Niacin</i>
<i>Calcium Carbonate</i>	<i>Nikel</i>
<i>chloride</i>	<i>Oxymetazoline HCl</i>
<i>Chlorpheniramine Maleate</i>	<i>Pantothenic Acid</i>
<i>Chromium</i>	<i>Phenylephrine HCl</i>
<i>Citric Acid</i>	<i>phosphorus</i>
<i>Copper</i>	<i>potassium</i>
<i>Dextromethorphan Hydrobromide</i>	<i>Pseudoephedrine HCl</i>
<i>Dimenhydrinate</i>	<i>Selenium</i>
<i>Diphenhydramine HCl</i>	<i>Silicon</i>
<i>Doxylamine Succinate</i>	<i>Simethicone</i>
<i>Famotidine</i>	<i>Sodium Bicarbonate</i>
<i>Folic Acid</i>	<i>Thiamin</i>
<i>Guafenesin</i>	<i>Tin</i>
<i>Ibuprofen</i>	<i>Vanadium</i>
<i>Iodine</i>	<i>Vitamin A</i>
<i>Iron</i>	<i>Vitamin B12</i>
<i>L-Lysine</i>	<i>Vitamin B6</i>
<i>Loperamide HCl</i>	<i>Vitamin C</i>
<i>Loratadine</i>	<i>Vitamin D</i>
<i>Lutein</i>	<i>Vitamin E</i>
<i>Lycopene</i>	<i>Vitamin K</i>
<i>Magnesium</i>	<i>Zinc</i>

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

Compound	Concentration in ng/ml
<i>Buprenorphine</i>	10ng/ml
<i>Norbuprenorphine</i>	10ng/ml
<i>Codeine</i>	No reaction when tested at 10ug/ml
<i>Morphine</i>	No reaction when tested at 100ug/ml

3. **Cut-off Study.** The cut-off of the test was determined by the repetitive assaying of six levels of Buprenorphine controls. The resultant data are summarized as follows:

Buprenorphine conc.	# Tested	# Positive (+)	# Negative (-)	% Correct Results
0 ng/mL	40	0	40	100%

5 ng/mL	40	0	40	100%
7.5 ng/mL	40	5	35	88%
10 ng/mL	40	30	10	75%
12.5 ng/mL	40	30	10	75%
15 ng/mL	40	40	0	100%

4. **Reproducibility.** The reproducibility was evaluated at 3 different sites. The Acro Rapid Buprenorphine Urine Test was tested against blind-labeled urine controls containing 0, 5, 7.5, 12.5, and 15 ng/mL Buprenorphine at each site. The results are summarized as follows:

Test Sites	0 ng/mL		5ng/mL		7.5ng/mL		12.5ng/mL		15ng/mL	
	#	Result	#	Result	#	Result	#	Result	#	Result
1	20	20-	20	20-	20	2+, 18-	20	16+, 4-	20	20+
2	20	20-	20	20-	20	20-	20	5+, 15-	20	20+
3	20	20-	20	20-	20	3+, 17-	20	20+	20	20+

5. **Accuracy.** 60 urine specimens from non-drug users and GC/MS confirmed containing no Buprenorphine and 60 urine specimens containing known (spiked) amount of Buprenorphine were tested with the rapid buprenorphine test device. The test results are listed in the table below.

Rapid BUP Test	GC/MS			
	Negative (<50%-cutoff)	Near Cutoff Negative (50%- to Cutoff)	Near Cutoff Positive (cutoff to 50%+)	Positive (>50%+ cutoff)
Positive	0	2	7	40
Negative	60	9	2	0

Agreement of negative specimens: 69/71 = 97%

Agreement of positive specimens: 47/49 = 96%

BIBLIOGRAPHY

1. Huang, W., Andollo, W., Hearn W.L. J. Anal. Toxicol., 16: 307-310 (1992).
2. Glare, P.A., Wlask, T.D., and Pippenger, C.E., Ther. Drug Monit., 13: 226-232 (1991).
3. Walsh, T.D., Cheater, F.M., Pharm. J., 10:525-527 (1983).
4. Mitchell, J.M., Paul, B.D., Welch, P., Cone, E.j. J. Anal. Toxicol., 15: 49-53 (1991).
5. Cone, E.J., Dickerson, S., Paul, B.D., Mitchell, J.M., J. Anal. Toxicol., 17:156-164 (1993).
6. Joint Formulary Committee. British National Formulary, 47th edition. London: British Medical Association and Royal Pharmaceutical Society of Great Britain; 2004. ISBN 0-85369-584-9

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ACRO BIOTECH, LLC



CEpartner4U
3951DB, 13. NL
Tel: +31(0)6-516.536.26

9500 Seventh Street, Unit M
Rancho Cucamonga
California 91730, USA
http://acrobiotech.com