



ADVANCED QUALITY™ ONE STEP Buprenorphine (BUP) TEST

(Urine)

FOR *IN VITRO* DIAGNOSTIC USE ONLY

INTENDED USE

THE ADVANCED QUALITY™ ONE STEP BUPRENORPHINE TEST IS A RAPID, IMMUNOCHROMATOGRAPHIC ASSAY FOR THE DETECTION OF BUPRENORPHINE AND ITS METABOLITES IN HUMAN URINE. THE TEST IS USED TO SCREEN URINE FOR THE PRESENCE OF BUPRENORPHINE AND ITS METABOLITES AT A CUTOFF CONCENTRATION OF 10NG/ML. THE TEST IS INTENDED FOR USE BY HEALTHCARE PROFESSIONALS ONLY.

This test 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 spectrophotometry (GC/MS) is the preferred confirmatory method. Clinical considerations and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.

SUMMARY AND EXPLANATION OF THE TEST

Buprenorphine is a potent analgesic often used in the treatment of opioid addiction. The drug is sold under the trade names Subutex™, Buprenex™, Temgesic™ and Suboxone™, which contain Buprenorphine HCl alone or in combination with Naloxone HCl. Therapeutically, Buprenorphine is used as a substitution treatment for opioid addicts. Substitution treatment is a form of medical care offered to opiate addicts (primarily heroin addicts) based on a similar or identical substance to the drug normally used. In substitution therapy, Buprenorphine is as effective as Methadone but demonstrates a lower level of physical dependence. Concentrations of free Buprenorphine and Norbuprenorphine in urine may be less than 1 ng/mL after therapeutic administration, but can range up to 20 ng/mL in abuse situations. The plasma half-life of Buprenorphine is 2-4 hours. While complete elimination of a single-dose of the drug can take as long as 6 days, the detection window for the parent drug in urine is thought to be approximately 3 days. The BUP One Step Buprenorphine Test Device (Urine) 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 Buprenorphine in urine. The BUP One Step Buprenorphine Test Device (Urine) yields a positive result when the Buprenorphine in urine exceed 10 ng/mL.

The test is a qualitative, visual screening immunoassay. The method employs unique antibodies to selectively identify the drug in the test urine with a high degree of sensitivity and specificity.

PRINCIPLE OF THE PROCEDURE

The test device consists of a chromatographic absorbent device in which the drug or drug metabolites in the sample compete with a drug conjugate immobilized on a porous membrane support for the limited antibody sites. As the test sample flows up through the absorbent device, the labeled antibody-dye conjugate binds to the free drug in the specimen forming an antibody:antigen complex. This complex competes with immobilized antigen conjugate in the positive reaction zone and will not produce a magenta color band when the drug is above the detection level suggested for the immunoassay method. Unbound dye conjugate binds to the reagent in the negative control zone, producing a magenta color band, demonstrating that the reagents and device are functioning correctly.

A **negative** specimen produces two (2) distinct color bands, one in the test area and one in the control region.

A **positive** specimen produces only one (1) color band in the control region.

REAGENTS AND MATERIALS SUPPLIED

FOR STRIP TEST

1. Test strips individually foil pouched with a desiccant
2. Package insert

FOR CARD TEST

1. Test cards individually foil pouched with a desiccant
2. Plastic dropper
3. Package insert

MATERIALS REQUIRED BUT NOT PROVIDED

1. Urine collection containers (for card test)
2. Clock or Timer
3. Positive and negative urine controls available from commercial distributors.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Avoid cross contamination of urine samples by using a new urine specimen cup for each sample.
3. Do not use the kit beyond the expiration date printed on the outside of the foil pouch.
4. Do not open the foil pouch until urine specimen is collected and ready to be tested.
5. Urine specimens may be infectious. Handle and dispose of all used specimens and devices in an approved biohazard container.

STORAGE AND STABILITY

The test device can be stored under refrigeration and at room temperature (2° - 30° C) and will be stable until the expiration date. **Do not** open foil pouch until ready to test.

SAMPLE COLLECTION AND PREPARATION

10 ml of urine must be collected in a clean, dry, plastic or glass container, that does not contain preservative. Some plastics may adsorb drugs. If not tested immediately, urine specimens may be stored refrigerated at 2-8°C for up to 7 days and then frozen (-20° C or colder) prior to assaying. Refrigerated or frozen samples must be warmed to room temperature and gently mixed before testing. Urine samples exhibiting visible precipitates or turbidity should be centrifuged or allowed to settle so a clear aliquot may be sampled for this assay. Collection of samples may require mandatory procedures and custody and control records. Poppy seed ingestion has been associated with positive test results in some samples.

ASSAY PROCEDURES

FOR STRIP TEST:

1. Bring all materials and specimens to room temperature.
2. Remove test strip from the sealed foil pouch.
3. Dip the test strip into the urine sample with the arrows pointing toward the specimen.
4. The urine level should reach the maximum line marked on the strip, but must not exceed the maximum line.
5. Hold the strip in the urine until a reddish color appears at the lower edge of the test membrane (approximately 10 seconds).
6. Withdraw the strip and place it face up on a clean, dry surface.
7. Read the result between 3 - 8 minutes after adding the sample.

FOR CARD TEST:

1. Bring all materials and specimens to room temperature.
2. Remove test card from the sealed foil pouch.
3. Place the test card on a flat dry surface.
4. Using the provided plastic dropper, dispense 3 drops of urine sample to the sample well of the test card. Start timing.
5. Read result between 3 - 8 minutes after adding the sample.

READING THE TEST RESULTS

Read test results between 3 - 8 minutes.

Do not interpret results after 8 minutes.

NEGATIVE Two (2) pink/purple bands form. In addition to the control band, a pink/purple band also appears in the test region.

Note: This immunoassay is a screening test. A negative result indicates the drug level is below the detection sensitivity. It is important to understand that concentrations of the drug below cut off may cause a faint "ghost line" to form in the test region. This "ghost line" should be considered a negative result.

POSITIVE One (1) pink/purple band appears in the control region. No band is found in the test region. This is an indication that the drug level is above the detection sensitivity level.

INVALID If there is no pink/purple band in the control area of the strip, the test result is invalid. Retest the sample using a new device.

Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are positive. Positive results should be confirmed by an alternate method such as GC/MS.

QUALITY CONTROL

- Each test device has a control band to indicate that the sample volume and migration is adequate, and that the colloidal gold is dissolving as expected. An invalid result must be repeated using a new test device.
- Positive and negative, drug free urine controls can be used to validate reagent performance and establish test reliability. Commercial drug urine controls are available, but not provided with this test. NIDA recommended guidelines for drugs of abuse screening indicate controls should contain the drug at a level at least 20% above the NIDA cutoff value. If control values do not fall within the established limits, assay results are invalid.

EXPECTED RESULTS

The Advanced Quality Buprenorphine Test identifies Buprenorphine and its metabolites in human urine at a cutoff concentration of 500ng/mL. The concentration of the drug can not be determined using this test. The test is intended to screen urine to separate a negative result from a presumptive positive result. All positive results must be confirmed using an alternate method, preferably GC/MS.

PERFORMANCE CHARACTERISTICS Accuracy

A correlation study was conducted on fifty-eight (58) clinical specimens from patients reporting Buprenorphine use and one-hundred fifty (150) urine specimens collected from presumed non-drug users. Using the BUP One Step Buprenorphine Test Device (Urine), the specimens were tested and compared to the self-reported use of Buprenorphine. All specimens, including the ones showing negative results, were then confirmed by LC/MS. The following results were tabulated:

Method		Patient Self-Report		Total Results	
BUP One Step Test Device	Results	positive	negative		
		positive	51	0	51
		negative	7	150	157
Total Results		58	150	208	
%Agreement		88%	> 99%	97%	

When compared at 10 ng/mL with LC/MS, the following results were tabulated:

Method		Patient Self-Report		Total Results	
BUP One Step Test Device	Results	positive	negative		
		positive	55	2	57
		negative	1	168	169
Total Results		56	170	226	
%Agreement		98%	99%	99%	

Analytical Sensitivity

A drug-free urine pool was spiked with Buprenorphine at the following concentrations: 0 ng/mL, 5 ng/mL, 7.5 ng/mL, 10 ng/mL, 12.5 ng/mL and 15 ng/mL. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Buprenorphine Concentration (ng/ml)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0%	90	90	0
5	-50%	90	90	0
7.5	-25%	90	78	12
10	Cut-off	90	48	42
12.5	+25%	90	24	66
15	+50%	90	0	90

ANALYTICAL SPECIFICITY

The following table lists compounds that are positively detected in urine by the BUP One Step Buprenorphine Test Device (Urine) at 5 minutes.

Compound	Concentration (ng/mL)	Compound	Concentration (ng/mL)
Buprenorphine	10	Buprenorphine 3-D-Glucuronide	15
Norbuprenorphine	20	Norbuprenorphine 3-D-Glucuronide	200

PRECISION

A study was conducted at 3 physician's offices by untrained operators using 3 different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing no Buprenorphine, 25% Buprenorphine above and below the cutoff and 50% Buprenorphine above and below the 10 ng/mL cutoff were provided to each site. The following results were tabulated:

Buprenorphine Concentration (ng/mL)	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	15	15	0	15	0	15	0
5	15	15	0	15	0	15	0
7.5	15	8	7	10	5	9	6
12.5	15	0	15	1	14	0	15
15	15	0	15	0	15	0	15

EFFECT OF URINARY SPECIFIC GRAVITY

Fifteen urine samples with specific gravities ranging from 1.004 to 1.034 were spiked with Buprenorphine to the concentrations of 5 ng/mL, and 15 ng/mL. The BUP One Step Buprenorphine Test Device (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do 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 Buprenorphine to 5 ng/mL and 15 ng/mL. The spiked, pH-adjusted urine was tested with the BUP One Step Buprenorphine Test Device (Urine) in duplicate. 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 Buprenorphine positive urine. The following compounds show no cross-reactivity when tested with the BUP One Step Buprenorphine Test Device (Urine) at a concentration of 100 µg/mL.

NON CROSS-REACTING COMPOUNDS

4-Acetamidopheno l	5,5-Diphenylhydantoin	Lithium carbonate	Trans-2- phenyl
Acetone	Disopyramide	Loperamide	cyclopropylamine
Acetophenetidin	Doxylamine	Maprotiline	L-Phenylephrine
Acetylsalicylic acid	Ecgonine hydrochloride	Meperidine	B-Phenylethylamine
N-Acetylprocainamide	Ecgonine methylester	Mephentermine	Phenylpropanolamine
Albumin	EDDP	Meprobamate	(D,L-norephedrine)
Aminopyrine	Efavirenz (Sustiva)	Methadone	(±) Phenylpropanolamine
Amitriptyline	EMD P	D- Methamphetamine	Prednisolone
Amobarbital	Ephedrine	L- Methamphetamine	Prednisone
Amoxapine	(1r,2s)-(-)Ephedrine	Methaqualone	5 beta- pregnane3alpha17alpha- 21triol 21
Amoxicillin	(-)-ψ-Ephedrine	Methoxyphenamine	Procaine
L-Amphetamine	(±)-Epinephrine	(-) 3,4- Methylenedioxy- amphetamine (MDA)	Promazine
Ampicillin	Erythromycin	(+) 3,4 Methylenedioxy- methamphetamine	Promethazine
Apomorphine	β-Estradiol	Methylphenidate	D,L-Propranolol
Aspartame	Estrone-3-sulfate	Methyprylon	D-Propoxyphene
Atropine	Ethanol (Ethyl alcohol)	Methaqualone	D-Pseudoephedrine
Benzilic acid	Ethyl-p-aminobenzoate	Metoprolol	Quinacrine
Benzoic acid	Etodolac	Morphine sulfate	Quinidine
Benzoyllecgonine	Famprofazone	Morphine- 3-β-D- glucuronide	Quinine
Benzphetamine	Fenfluramine	Nalidixic acid	Ranitidine
Bilirubin	Fenoprofen	Nalorphine	Riboflavin
(±)-Brompheniramine	Fentanyl	Naloxone	Salicylic acid
Buspirone	Fluoxetine	Naltrexone	Secobarbital
Caffeine	Furosemide	Methyprylon	Serotonin

Cannabidiol	Gentisic acid	Metoprolol	(5-hydroxytyramine)
Cannabinol	D (+) Glucose	Nimesulide	Sodium chloride
Chloralhydrate	Guaiacol Glyceryl Ether	Norcodein	Sulfamethazine
Chloramphenicol	Guaiacol Glyceryl Ether	Morphine	Sulindac
Chlordiazepoxide	carbamate	Alpha- Naphthaleneacetic Acid	sulfate Temazepam
Chloroquine	Hemoglobin	Norethindrone	Tetracycline
Chlorothiazide	Hydralazine	Normorphine	Tetrahydrocortisone, 3- acetate
(+)-Chlorpheniramine	Hydrochlorothiazide	D- Norpropoxyphene	Tetrahydrozoline
(±)-Chlorpheniramine	Hydrocodone	Noscapine	Thebaine
Chlorpromazine	Hydrocortisone	D,L-Octopamine	Theophylline
Chlorprothixene	Hydromorphone	Orphenadrine	Thiamine
Cholesterol	p-Hydroxyamphetamine	Oxalic acid	Thioridazine
Cimetidine	O-Hydroxyhippuric acid	Oxazepam	(chlorpromazine)
Clomipramine	p- Hydroxymethamphetamine	Oxolinic acid	L-Thyroxine
Clonidine	p-Hydroxynorephedrine	Oxycodone	Tolbutamine
Cocaine HCl	Hydroxyzine	Oxymetazoline	Cis-Tramadol
Codeine	3-Hydroxytyramine	Oxymorphone	Trazodone
Cortisone	Ibuprofen	Papaverine	Triamterene
(-) Cotinine	Imipramine	Pemoline	Trifluoperazine
Creatinine	Iproniazid	Penicillin-G	Trimethobenzamide
Cyclobarbital	(-)-Isoproterenol	Pentazocine	Trimethoprim
Cyclobenzaprine	Isoxsuprine	Pentobarbital	Trimipramine
Deoxycorticosterone	Kanamycin	Perphenazine	Tryptamine
(-) Deoxyephedrine	Ketamine	Phencyclidine	D, L-Tryptophan
R (-) Deprenyl HCl	Ketoprofen	Phenelzine	Tyramine
Dextromethorphan	Labetalol	Pheniramine	D, L-Tyrosine
Diazepam	L-Ascorbic acid	Phenobarbital	Uric acid
Diclofenac	L-Ephedrine	Phenothiazine	Verapamil
Dicyclomine	L-Epinephrine	Phentermine	Zomepirac
Diflunisal	Levorphanol		

Digoxin	Lidocaine		
4-Dimethylaminoantipyrine	Lindane		
Diphenhydramine	(hexachlorocyclohexane)		

LIMITATIONS OF THE TEST

1. This product is designed to be used for the detection of Buprenorphine in human urine only.
2. Although The Advanced Quality One Step Buprenorphine Test is very accurate in detecting the level of Buprenorphine in urine, there is a possibility of false results due to the presence of interfering substances in the urine.
3. The test is a qualitative screening assay and is not suggested for determining the quantitative level of Buprenorphine in urine.
4. Adulterants, such as bleach or other strong oxidizing agents, when added to urine specimens, may produce erroneous test results regardless of the analysis method used. If adulteration is suspected, obtain another urine specimen.
5. There is the possibility that other substances and/or factors not listed above may interfere with the test and cause false results, e.g. technical or procedural errors.

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