BUP

One Step
Buprenorphine Single Drug Test (Urine)
Package Insert
(for single test card and single test strip formats)

A rapid, one step test for the qualitative detection of Buprenorphine in human urine.

For healthcare professionals including professionals at point of care sites.

For in vitro diagnostic use only.

INTENDED USE

The Buprenorphine Single Drug Test is a lateral flow chromatographic immunoassay for the qualitative detection of Buprenorphine in human urine at a level relative to the Buprenorphine cut-off concentration of 10 ng/mL. This assay is intended for use by professionals to assist in the determination of drug compliance. **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) or Liquid chromatography/mass spectrometry (LC/MS) are the preferred confirmatory methods. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are indicated.**

SUMMARY

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.[1] The plasma half life of Buprenorphine is 2-4 hours.[1] While complete elimination of a single dose of the drug can take as long as 6 days, the window of detection for the parent drug in urine is thought to be approximately 3 days.

Substantial abuse of Buprenorphine has also been reported in many countries where various forms of the drug are available. The drug has been diverted from legitimate channels through theft, doctor shopping, and fraudulent prescriptions, and been abused via intravenous, sublingual, intranasal and inhalation routes.

The Buprenorphine Single Drug Test 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 Buprenorphine Single Drug Test yields a positive result when the Buprenorphine in urine exceeds 10 ng/mL.

PRINCIPLE

The Buprenorphine Single Drug Test is an immunoassay based on the principle of competitive binding. Drugs that may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.

During testing, a urine specimen migrates upward by capillary action. Buprenorphine, if present in the urine specimen below 10 ng/mL, will not saturate the binding sites of antibody-coated particles in the test strip. The antibody-coated particles will then be captured by immobilized Buprenorphine conjugate and a visible colored line will show up in the test region. The colored line will not form in the test region if the Buprenorphine level exceeds 10 ng/mL because it will saturate all the binding sites of anti-Buprenorphine antibodies.

A drug-positive urine specimen will not generate a colored line in the test region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration lower than the cut-off will generate a line in the test region.

To serve as a procedural control, a colored line will always appear in the control region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test card and/or test strip contains mouse monoclonal anti-Buprenorphine antibody-coupled particles and Buprenorphine-protein conjugate. A goat antibody is employed in the control line system.

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 card and/or test strip 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.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C (36-86°F). The test card and/or test strip is stable through the expiration date printed on the sealed pouch. The test card and/or test strip 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 particles should be centrifuged, filtered, or allowed to settle obtain a clear specimen for testing.

Specimen Storage

Urine specimens may be stored at 2-8°C for up to 96 hours prior to assay. For long-term storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS

Materials Provided

- Test card and/or test strip
- Package insert

Materials Required But Not Provided

- Specimen collection container
- Timer
- External controls

DIRECTIONS FOR USE

Allow the test card and/or test strip, urine specimen, and/or controls to reach room temperature (15-30ºC) prior to testing.

Directions for Single Test Card

1. Bring the pouch to room temperature before opening it. Remove the test card from the sealed pouch and use it as soon as possible.
2. Remove the cap.
3. With the arrow pointing toward the urine specimen, immerse the test card vertically in the urine specimen for at least 10 to 15 seconds. **Immerse the strip to at least the level of the wavy lines, but not above the arrow on the test card. [See image (1).]**
4. Replace the cap and place the test card on a non-absorbent flat surface.
5. Start the timer and wait for the colored line(s) to appear.
6. The result should be read at 5 minutes. Results may be stable up to 1 hour after test initiation.

Directions for Single Test Strip

1. Bring the pouch to room temperature before opening it. Remove the test strip from the sealed pouch and use it as soon as possible.
2. With arrows pointing toward the urine specimen, immerse the test strip vertically in the urine specimen for at least 10 to 15 seconds. **Immerse the strip to at least the level of the wavy lines, but do not pass the maximum line (MAX) on the test strip. [See image (2).]**
3. Place the test strip on a non-absorbent flat surface.
4. Start the timer and wait for the colored line(s) to appear.
5. The result should be read at 5 minutes. Results may be stable up to 1 hour after test initiation.

INTERPRETATION OF RESULTS

(Please refer to illustration below)

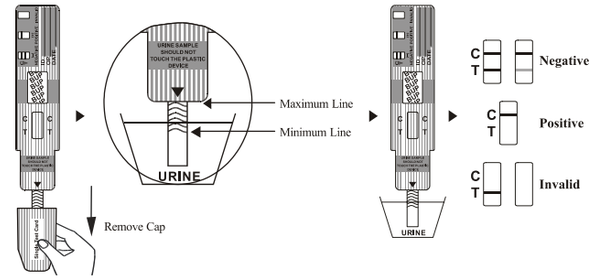
NEGATIVE:* Two lines appear. One colored line should be in the control region (C), and another apparent colored line should be in the test region (T). A negative result indicates that the Buprenorphine concentration is below the detectable level of 10 ng/mL.

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

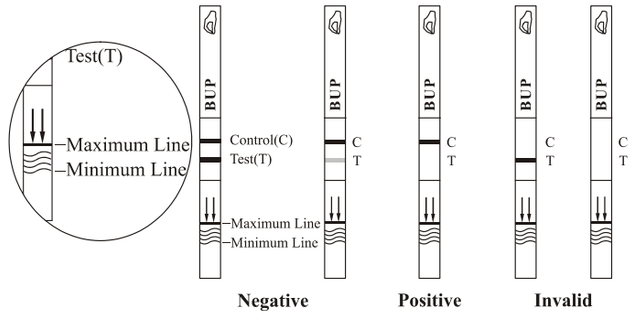
POSITIVE: One colored line appears in the control region (C). No line appears in the test region (T). A positive result indicates that the Buprenorphine concentration exceeds the detectable level (10 ng/mL).

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 using a new test strip. If the problem persists, discontinue using the lot immediately and contact the manufacturer.

Buprenorphine Single Test Card
IMAGE 1



Buprenorphine Single Test Strip
IMAGE 2



QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal positive 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 testing practices to confirm the test procedure and to verify proper test performance. Users should follow local, state, and federal guidelines for testing QC materials.

LIMITATIONS

1. The Buprenorphine Single Drug Test 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) or Liquid chromatography/mass spectrometry (LC/MS) are the preferred methods.[2]
2. It is possible 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 indicates presence of the drug but 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.

PERFORMANCE CHARACTERISTICS

Accuracy

226 urine samples were obtained and tested with the Buprenorphine Single Drug Test and by LC/MS the results are summarized below:

Specimen Cut-off Range by LC/MS							
		Negative*	<-25% of Cut-off	-25% Cut-off to Cut-off	Cut-off to +25% of Cut-off	>+25% of Cut-off	% Agreement
The Buprenorphine Single Drug Test	Positive	0	0	0	5	50	98% (55/56) (90%-99%)**
	Negative	150	15	5	1	0	>99% (170/170) (98%-100%)**

Total agreement with LC/MS: 225/226 = 99% (98%-99%)**

* Negative samples were confirmed negative using LC/MS by pooling these samples into groups of 15.

** Denotes 95% confidence interval

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 and 20 ng/mL. The assay cut-off level of 10 ng/mL was selected to correlate with the LC/MS analysis cut-off for Buprenorphine. 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	75	15
10	Cut-off	90	60	30
12.5	+25%	90	31	59
15	+50%	90	0	90
20	+100%	90	0	90

Specificity

The following table lists compounds that are positively detected in urine by the Buprenorphine Single Drug Test at 5 minutes.

Compound	Concentration (ng/mL)	Cross-Reactivity (%)
Buprenorphine	10	100
Norbuprenorphine	20	50
Buprenorphine 3-D-glucuronide	15	67
Norbuprenorphine 3-D-glucuronide	200	5

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 cut-off and 50% Buprenorphine above and below the 10 ng/mL cut-off 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 (15) urine samples with specific gravity ranging from 1.004 to 1.034 were spiked with Buprenorphine to the concentrations of 5 ng/mL, 15 ng/mL and 20 ng/mL. The Buprenorphine Single Drug Test was tested in duplicate using the fifteen neat and spiked urine samples. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

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 Buprenorphine to the concentrations of 5 ng/mL, 15 ng/mL and 20 ng/mL. The spiked, pH-adjusted urine was tested with the Buprenorphine Single Drug Test 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 Buprenorphine Single Drug Test at a concentration of 100 µg/mL.

Non Cross-Reacting Compounds

4-Acetaminophenol	Acetone	Acetophenetidin
Acetylsalicylic acid	Albumin	Amitryptyline
Amobarbital	Amoxapine	Amoxicillin
d,l-Amphetamine	Ampicillin	Ascorbic acid
Aminopyrine	Apomorphine	Aspartame
Atropine	Benzilic acid	Benzoic acid
Benzphetamine	Bilirubin	Brompheniramine
Buspirone	Caffeine	Cannabidiol
Cannabinol	Cimetidine	Chloral hydrate
Chloramphenicol	Chlordiazepoxide	Chloroquine
Chlorothiazide	d-Chlorpheniramine	d,l-Chlorpheniramine
Chlorpromazine	Chlorprothixene	Cholesterol
Clomipramine	Clonidine	Cortisone
l-Cotinine	Creatinine	Cyclobarbital
Cyclobenzaprine	Deoxycorticosterone	l-Deoxyephedrine
l-Deprenyl	Dextromethorphan	Diazepam
Diclofenac	Diflunisal	Digoxin
4-Dimethylaminoantipyrine	Diphenhydramine	Dicyclomine
5,5-Diphenylhydantoin	Disopyramide	Doxylamine
Ecgonine	Ecgonine methylester	EDDP
EMDP	Efavirenz	Ephedrine
l-Ephedrine	l-Epinephrine	d,l-Epinephrine
Erythromycin	β-Estradiol	Estrone-3-sulfate
Ethanol	Ethyl-p-aminobenzoate	Etodolac
Famprofazone	Fenfluramine	Fenoprofen
Fentanyl	Fluoxetine	Furosemide
Gentisic acid	d-Glucose	Guaiacol glyceryl ether
Guaiacol glyceryl ether carbamate	Hemoglobin	Hydralazine
Hydrochlorothiazide	Hydrocodone	Hydrocortisone
Hydromorphone	o-Hydroxyhippuric acid	p-Hydroxymethamphetamine
p-Hydroxynorephedrine	3-Hydroxytyramine (Dopamine)	Hydroxyzine
Ibuprofen	Imipramine	Iproniazide
l-Isoproterenol	Isoxsuprine	Kanamycin
Ketamine	Ketoprofen	Labetalol
Levorphanol	Lidocaine	Lindane
Lithium carbonate	Loperamide	Maprotiline
Meperidine	Meprobamate	l-Methamphetamine
Methaqualone	Methadone	Methoxyphenamine
MDMA*	Methylphenidate	Mephentermine
Metoprolol	Morphine-3-β-D glucuronide	Morphine
Methyprylon	Nalidixic acid	Naloxone
Naltrexone	α-Naphthaleneacetic acid	Naproxen
Niacinamide	Nifedipine	Norcodeine
Normorphine	Nimesulide	Norethindrone
d-Norpropoxyphene	Noscapine	d,l-Octopamine
Orphenadrine	Oxalic acid	Oxazepam
Oxolinic acid	Oxycodone	Oxymetazoline
Oxymorphone	Papaverine	Pemoline
Penicillin-G	Pentazocine	Pentobarbital
Perphenazine	Phencyclidine	Phenelzine
Pheniramine	Phenobarbital	Phenothiazine
Phentermine	l-Phenylephrine	β-Phenylethylamine
d,l-Phenylpropanolamine	Prednisolone	Prednisone
5-β-Pregnane-3α,17α,21-triol-20-one	Procaine	Promazine
Promethazine	d-Propoxyphene	d,l-Propranolol
d-Pseudoephedrine	Quinacrine	Quinidine
Quinine	Ranitidine	Riboflavin
Salicylic acid	Secobarbital	Serotonin (5-Hydroxytryptamine)
Sodium chloride	Sulfamethazine	Sulindac
Temazepam	Tetracycline	Tetrahydrocortexolone
Tetrahydrocortisone, 3-acetate	Tetrahydrozoline	Thebaine
Theophylline	Thiamine	Thioridazine
l-Thyroxine	Tolbutamide	cis-Tramadol
Trans-2-phenylcyclopropylamine	Trazodone	Trimethobenzamide
Triamterene	Trimipramine	Trifluoperazine
Trimethoprim	Tryptamine	d,l-Tryptophan
Tyramine	d,l-Tyrosine	Uric acid
Verapamil	Zomepirac	

* (+) 3,4 Methylendioxymethamphetamine

BIBLIOGRAPHY

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