One Step Buprenorphine (BUP) Test

Package Insert for Buprenorphine Single Test Strip, Cassette, and Dipcard.

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

For Professional and In Vitro Diagnostic Use Only.

INTENDED USE

The One Buprenorphine Test is an easy-to-use, lateral flow chromatographic immunoassay for the qualitative detection of Buprenorphine in urine at the following cut-off concentration or greater:

Test	Calibrator	Cut-off
Buprenorphine (BUP)	Buprenorphine	10 ng/mL

This assay provides only a preliminary qualitative test result. Use a more specific alternate quantitative analytical method to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Apply clinical and professional judgment to any drug of abuse test result, particularly when preliminary positive results are obtained.

SUMMARY AND EXPLANATION OF THE TEST

The One Step Buprenorphine Test is a competitive immunoassay utilizing highly specific reactions between antibodies and antigens for the detection of drug and drug metabolite in human urine.
The One Step Buprenorphine Test is a rapid urine-screening test that utilizes monoclonal antibodies to selectively detect elevated levels of specific drug in urine without the use of an instrument.

BUPRENORPHINE (BUP)

Buprenorphine is a semisynthetic opioid analgesic derived from thebain, a component of opium. It has a longer duration of action than morphine when indicated for the treatment of moderate to severe pain, peri-operative analgesia, and opioid dependence. Low doses buprenorphine produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of opioids without experiencing withdrawal symptoms. Buprenorphine carries a lower risk of abuse, addiction, and side effects compared to full opioid agonists because of the "ceiling effect", which means no longer continue to increase with further increases in dose when reaching a plateau at moderate doses. However, it has also been shown that Buprenorphine has abuse potential and may itself cause dependency. Subutex®, and a Buprenorphine/Naloxone combination product, Suboxone®, are the only two forms of Buprenorphine that have been approved by FDA in 2002 for use in opioid addiction treatment. Buprenorphine was rescheduled from Schedule V to Schedule III drug just before FDA approval of Suboxone and Subutex.
The One Step Buprenorphine Test yields a positive result when the Buprenorphine in urine exceeds 10 ng/mL.

PRINCIPLE

The One Step Buprenorphine Test includes a chromatographic absorbent device in which the drug in the sample compete with a drug conjugate immobilized on a porous membrane for limited antibody sites.

Labeled antibody-dye conjugate mixes with sample specimen and binds to the free drug present forming an antibody-antigen complex. This complex competes with immobilized antigen conjugate in the test zone preventing the formation of a pink-rose color band when the drug concentration in the specimen is above 10ng/mL. Unbound dye conjugates bind to the reagent in the negative control zone and produces a pink-rose color band, demonstrating that the reagents and device are functioning correctly.

A negative specimen produces two distinct color bands in both the test region and the control region. A positive specimen produces only one color band in the control region.

REAGENTS

The test contains a membrane strip coated with drug-protein conjugates (purified bovine albumin) on the test line, a goat polyclonal antibody against gold-protein conjugate at the control line, and a dye pad which contains colloidal gold particles coated with mouse monoclonal antibody specific to Buprenorphine.

PRECAUTIONS

· For Professional Use Only.
· For *In Vitro* Diagnostic Use Only.
· Do not use after the expiration date.
· The test panel should remain in the sealed pouch until use.
· While urine is not classified by OSHA or the CDC as a biological hazard unless visibly contaminated with blood[8,9], the use of gloves is recommended to avoid unnecessary contact with the specimen.
· The used test card and urine specimen should be discarded according to federal, state and local regulations.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30°C (36-86°F). The test is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND HANDLING

Use fresh urine specimens. Urine specimens do not require any special handling or pretreatment. It is best to test urine specimens immediately after collection. However, if necessary, urine specimens may be refrigerated at 2 – 8°C (36 – 46°F) for two days or frozen at –20°C (-4°F) or colder for longer periods. The sample should be free from gross debris. Refrigerated samples should be allowed to warm to room temperature before testing.

MATERIALS

Materials Provided

· Test device · Desiccant · Package insert
· Disposable specimen dropper (for test cassette only)

Materials Required But Not Provided

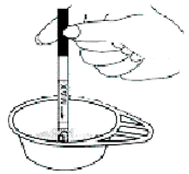
· Specimen collection container · Disposable gloves
· Timer

DIRECTIONS FOR USE

Allow the test card, and urine specimen to come to room temperature [15-30°C (59-86°F)] prior to testing. DO NOT INTERPRET RESULT AFTER 10 MINUTES.

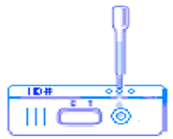
[For Strip]

1) Remove the strip from the foil wrapper or the desiccated container (bring the container to the room temperature before opening to avoid condensation of moisture in container). Label the strip with patient or control identifications.
2) Immerse the strip into the urine with the arrow end pointing toward the urine. Do not cover the urine over the MAX (maximum) line. You may leave the strip in the urine or you may take the strip out after a minimum of 15 seconds in the urine and lay the strip flatly on a non-absorptive clean surface.
3) Read results at 5 minutes.



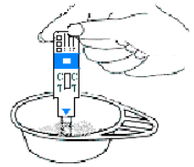
[For Cassette]

1) Remove the test device from its foil wrapper by tearing along the slice (bring the container to the room temperature before opening to avoid condensation of moisture in container). Label the device with patient or control identifications.
2) Using the specimen dropper, withdraw the urine sample from the specimen cup and slowly dispense 3 drops (approximately 120uL) into the circular sample well, being careful not to overfill the absorbent pad.
3) Read results at 5 minutes.



[For Dipcard]

1) Remove the test device from the foil pouch.
2) Remove the cap from the test device. Label the device with patient or control identifications.
3) Immerse the absorbent tip into the urine sample for 5 seconds. Urine sample should not touch the plastic device.
4) Replace the cap over the absorbent tip and lay the device flatly on a non-absorptive clean surface.
5) Read results at 5 minutes.



INTERPRETATION OF RESULTS

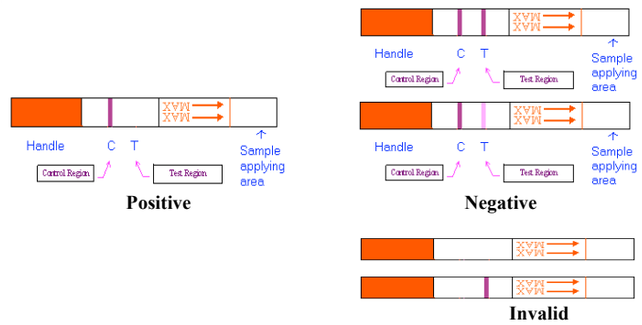
(Please refer to the previous illustration)
NEGATIVE: Two lines appear. * One color line should be in the control region (C), and another apparent red or pourple line adjacent should be in the test region (T). This negative result indicates that the drug concentration is below the detectable level.
***NOTE:** The shade of red in the test line region (T) will vary, but it should be considered negative whenever there is even a faint pink line.

POSITIVE: One color line appears in the control region (C). No line appears in the test region (T). This positive result indicates that the drug concentration is above the detectable level.

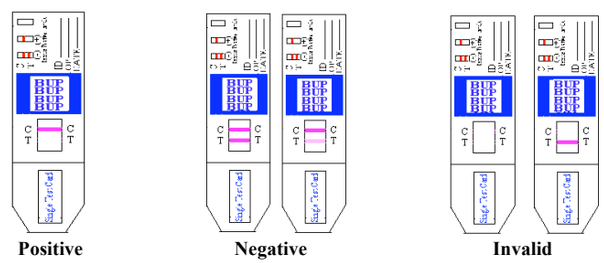
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test device. If the problem persists, discontinue using the lot immediately and contact your supplier.

[Cassette]

[Strip]



[Dipcard]



QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

LIMITATIONS

1. The One Step Buprenorphine 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) is the preferred confirmatory method. [3,4,7]
2. There is a possibility 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 and a new test device.
4. A Positive result does not indicate intoxication of the donor, the concentration of drug in the urine, or the route of drug administration.
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.
7. A positive test result may be obtained from certain foods or food supplements.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using the One Step Buprenorphine Test and other commercially available rapid drug tests. Testing was performed on 120 specimens per drug type previously collected from subjects presenting for drug screen testing. All the presumptive positive and negative results were confirmed by GC/MS. The following compounds were quantified by GC/MS and contributed to the total amount of drugs found in presumptive positive urine samples tested.

The following results are tabulated from these clinical studies:

%Agreement with Commercial Kit

	BUP
Positive Agreement	100%
Negative Agreement	100%
Total Results	100%

One Step Buprenorphine results compared to a GC/MS method (with a cutoff of 100 ng/mL) using clinical samples.

		GC/MS		Total
		Positive	Negative	
One Step Buprenorphine Test	Positive	38	0	38
	Negative	2	40	42
Total		40	40	80
% Agreement		95%	>99%	97.5%

	Negative Samples	Near Cut-off Negative Samples [between 50% of cut-off and cut-off]	Near Cut-off Positive Samples [between cut-off and 150% of cut-off]	Positive Samples [>150% of cut-off]	Agreement with GC/MS
One Step Buprenorphine Test Positive	0	0	34	4	95%
One Step Buprenorphine Test Negative	4	36	2	0	>99%

Reproducibility

Reproducibility studies were carried out using commercially available standards. Each standard was diluted in normal, drug-free urine to give the appropriate concentration. Each specimen, at each concentration of analyte, was tested four times daily, in duplicate, for five consecutive days. A total of 40 determinations were made at each concentration. The results are given below:

Buprenorphine Conc. (ng/mL)	Total number of Determinations	Result	Precision
No drug present	40	40 negative	>99%
5	40	40 negative	>99%
7.5	40	40 negative	>99%
10	40	40 positive	>99%
15	40	40 positive	>99%

Analytical Sensitivity

A drug-free urine pool was spiked with drugs at concentrations listed. The results are summarized below.

Drug concentration Cut-off Range	n	BUP	
		-	+
0% Cut-off	10	10	0
-50% Cut-off	10	10	0
-25% Cut-off	10	10	0
Cut-off	10	0	10
+25% Cut-off	10	0	10
+50% Cut-off	10	0	10

Analytical Specificity

The following table lists the concentration of compounds (ng/mL) that were detected positive in urine by One Step Drug of Abuse Test at a read time of 5 minutes.

Drug	Concentration (ng/ml)
Buprenorphine (BUP)	
Buprenorphine	10
Norbuprenorphine	10
Codeine	No reaction at 10ug/mL
Morphine	No reaction at 100ug/mL

Effect of Urinary Specific Gravity

Fifteen (15) urine samples of normal, high, and low specific gravity ranges (1.005, 1.015, 1.03) were spiked with drugs at 50% below and 50% above cut-off levels respectively. The One Step Buprenorphine Test was tested in duplicate using ten drug-free urine and spiked urine samples. 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 pH ranges of 4.0, 4.5, 5.0, 6.0 and 9.0, and spiked with drugs at 50% below and 50% above cut-off levels. The spiked, pH-adjusted urine was tested with The One Step Buprenorphine Test. 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 drug positive urine containing Buprenorphine. The following compounds show no cross-reactivity when tested with The One Step Buprenorphine Test at concentrations of 100 µg/mL.

Non Cross-Reacting Compounds

Acetaminophen
N-Acetylprocainamide
Aminopyrine
Ampicillin
Apomorphine
Atropine
Benzoic acid
Bilirubin
Caffeine
Chloralhydrate
Chlorothiazide
Chlorpromazine
Cholesterol
Cortisone
Acetophenetidin
Acetylsalicylic acid
Amoxicillin
L-Ascorbic acid
Aspartame
Benzilic acid
Benzphetamine*
D/L-Brompheniramine
Cannabidol
Chloramphenicol
D/L-Chloropheniramine
Chloroquine
Clonidine
L-Cotinine
Creatinine
Dextromethorphan
Diflunisal
Diphenhydramine
L -Ψ -Ephedrine
Estrone-3-sulfate
[1R,2S] (-) Ephedrine
Erythromycin
Furosemide
Hemoglobin
Hydrochlorothiazide
O-Hydroxyhippuric acid
p-Hydroxytyramine
Iproniazid
Isoxsuprine
Ketoprofen
Loperamide
Meprobamate
Methylphenidate
Naloxone
Naproxen
Nifedipine
D-Norpropoxyphene
D/L-Octopamine
Oxolinic acid
Papaverine
Pentazocine hydrochloride
Phenelzine
L-Phenylephrine
Phenylpropanolamine
Prednisone
D-Propoxyphene
Quinacrine
Quindine
Salicylic acid
Sulfamethazine
Tetracycline
Tetrahydrocortisone 3 (b-D-glucuronide)
Thiamine
D/L-Tyrosine
Triamterene
Trimethoprim
D/L-Tryptophan
Uric acid
Zomepirac
Deoxycorticosterone
Diclofenac
Digoxin
Ecgonine methyl ester
b-Estradiol
Ethyl-p-aminobenzoate
L(–)-Epinephrine
Fenoprofen
Gentisic acid
Hydralazine
Hydrocortisone
p-Hydroxyamphetamine
Ibuprofen
D/L-Isoproterenol
Ketamine
Labetalol
Meperidine
Methoxyphenamine
Nalidixic acid
Naltrexone
Niacinamide
Norethindrone
Noscapine
Oxalic acid
Oxymetazoline
Penicillin-G
Perphenazine
Trans-2-phenylcyclo-propylamine hydrochloride
β-Phenylethylamine
Prednisolone
D/L-Propranolol
D-Pseudoephedrine
Quinine
Ranitidine
Serotonin
Sulindac
Tetrahydrocortisone 3-acetate
Tetrahydrozoline
Thioridazine
Tolbutamide
Trifluoperazine
Tryptamine
Tyramine
Verapamil

*Parent compound only; metabolizes into amphetamine and methamphetamine in the body.

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