OneStep Buprenorphine Test

19094

Instructions

INTENDED USE

The OneStep Buprenorphine assay is a rapid, qualitative, competive binding immunoassay for the determination of Buprenorphine (at or above the cutoff level of 10 ng/ml) and its metabolites of Buprenorphine-3-β-D-glucuronide (at or above the cutoff level of 2.5 ng/ml) in urine. The *OneStep* Buprenorphine Test is not intended to monitor drug levels, but only to screen urines for the presence of Buprenorphine and its metabolites. This test is an *in vitro* diagnostic medical device for healthcare professional use only.

Note: The test provides only preliminary data which should be confirmed by other methods such as gas chromatography/mass spectrometry (GC/MS). 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

The *OneStep* Buprenorphine Test is an easy, fast, and visually read screening method without the need for instrumentation to arrive at a determination. The method employs a unique set of antibodies to selectively identify Buprenorphine and its metabolites in test samples with a high degree of sensitivity.

Buprenorphine (Buprenex) is a synthetic thebaine derivative that has both analgesic and opiod antagonist properties. As an analgesic, it is 25 to 40 times more potent than morphine. when used as an antagonist, it is equilalent in potency to naltrexone.[2]

Buprenorphine and its metabolites may be detected in urine as a result of Norbuprenorphine, Norbuprenorphine-3-β-D Glucuronide, Buprenorphine-3-β-D Glucuronide.[5] Immunoassay testing has been developed for the determination of Buprenorphine in urine.[6]

PRINCIPLE OF THE TEST

The *OneStep* Buprenorphine Test consists of a immuno-chromatographic absorbent device in which the drug or drug metabolites in the sample compete with a drug conjugate immobilized on a porous membrane for limited antibody sites. As the test sample flows up through the absorbent device, the free drug in the specimen competes with immobilized antigen conjugate in the test zone by binding to the antibody-dye conjugate forming an antibody-antigen complex and preventing the formation of a rose-pink color band when the drug is at or above the detection level.

In the case where free drug in the sample is below the detection level, antibody-dye conjugate is free to bind to the immobilized antigen in the test zone, producing a rose-pink color band. Furthermore, unbound dye conjugate binds to the reagent in the control zone, producing a rose-pink color band, demonstrating that the reagents and device are functioning correctly.

A **NEGATIVE** specimen produces two distinct color bands in both the test zone and control zone. A **POSITIVE** specimen produces only one color band in the control zone.

REAGENTS AND MATERIALS PROVIDED

1. Test Cassette — Contains membrane-immobilized reagents in a protein matrix containing sodium azide. REF *19094*
2. Dropper. — A transfer pipette is included with each test device inside the foil pouch. REF *PIP-003*
3. Urine Cups (optional) — REF *UCP-001*
4. Test Instructions — REF *PI-19094*

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Clock or timer

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic and professional use only.
2. Do not use the test device beyond the expiration date.
3. Use a new specimen container and dropper for each test to avoid cross contamination of urine samples.
4. Urine specimens may be infectious; properly handle and dispose of all used reaction devices in a biohazard container.
5. Visually inspect the foil package to insure it is intact. If the package is not intact, discard the device.

STORAGE AND STABILITY

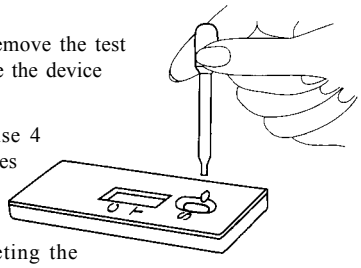
Store the test kit below 28°C; **do not freeze**. Refer to the expiration date for stability.

SAMPLE COLLECTION AND PREPARATION

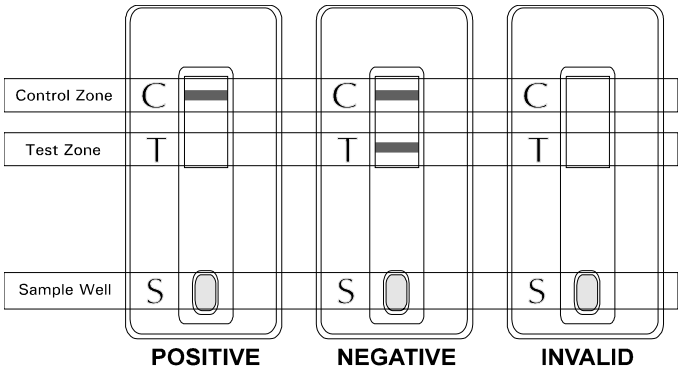
The sample must be collected in a clean, dry container, either plastic or glass, without preservatives. Urine specimens may be refrigerated (2° - 8° C) and stored up to forty-eight hours, or frozen (-20ºC or below) prior to assaying. If samples are refrigerated or frozen, they should be allowed to come to room temperature before testing. Urine samples exhibiting visible precipitates should be filtered, centrifuged, or allow to settle so that clear aliquots can be obtained for testing.

TEST PROCEDURE

1. Bring the test components and urine sample to room temperature (15° - 28º C). before testing. Do not open 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. Holding the dropper vertically, dispense 4 drops (~ 120 µl) of urine without air bubbles into the sample well "S" of the test device.
4. Read the result at 5 minutes. Interpreting the results up to 10 minutes is acceptable.



INTERPRETATION OF RESULTS



1. **Positive.** A *rose-pink* color band appears in the Control Zone "C" but not in the Test Zone "T". This is a positive result and indicates the Buprenorphine level is at or above the detection sensitivity of 10 ng/ml or its metabolite Buprenorphine-3-β-D-glucuronide level is at or above the detection sensitivity of 2.5 ng/ml.
2. **Negative.** Two horizontal *rose-pink* color bands appear, one in the Control zone "C" and one in the Test Zone "T". This is a negative result and indicates the Buprenorphine level is below the detection sensitivity of 10 ng/ml or its metabolite Buprenorphine-3-β-D-glucuronide level is below the detection sensitivity of 2.5 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 has been incorporated into the test to ensure proper kit performance and reliability.

LIMITATIONS OF THE TEST

1 This product is designed for use with human urine only.

2 Although the test is very accurate, there is a possibility false results will occur due to the presence of interfering substances in the urine.

3 The test is a qualitative screening assay and is not for determining quantitative concentration levels or the level of intoxication.

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 and retest.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity**. The*OneStep* Buprenorphine Test detects Buprenorphine at concentration equal to or greater than 10 ng/ml and the major metabolites, Buprenorphine-3-β-D–Glucuronide, at concentration eqaul to or greater than 2.5 ng/ml.

2. **Specificity**. A study was conducted with the *OneStep* Buprenorphine Test to determine the cross-reactivity of non-Buprenorphine related compounds with the test at concentrations much higher than normally found in the urine of people using or abusing them. No cross-reactivity was detected with the substances listed in **Table I**.

   A separate study was conducted to determine the cross-reactivity of Buprenorphine related compounds with the test. Substances listed in **Table II** produced results approximately equivalent to the cutoff level for Buprenorphine.

Table-I: Compounds tested and found not to cross-react with the test at a 100µg/ml concentration in urine:

Acetaminophen	*Heroin*	*Oxazepam*
Acetylsalicylic Acid	*Histamine*	*Oxymorphine*
Amikacin	*Hydromorphone*	*Oxycodone*
Amitriptyline	*Hydrochlorothiazide*	*Phendimetrazine*
Ampicillin	*Imipramine*	*Penicillin G*
Arterenol	*Indomethacin*	*Pentobarbital*
Aspartame	*Levorphanol*	*d-Propoxyphene*
Benzoic Acid	*Ketoprofen*	*1-Propanol*
Benzoylecgonine ·HCl	*Δ^9-THC*	*Phencyclidine*
Caffeine	*11-Nor-Δ^9-THC-9-COOH*	*Phenobarbital*
Chlorpheniramine	*Methylphenidate*	*Phentermine*
Chlorpromazine ·HCl	*Methadone*	*Phenylpropanolamine*
Cimetidine	*Methaqualone*	*l-Phenylephrine*
Codeine	*Morhpine*	*Quinine*
Deoxyephedrine	*Morhpine 3-Glucuronide*	*Sodium Salicylate*
Dextromethorphan	*6-Monoacetylmorphine*	*Tryptophan*
Diazepam	*Nalorphine*	*Tetracycline*
Diethylpropion	*Naloxone*	*Tetrahydrozoline*
5,5Dihydrocodeine	*Naltrexone*	*Theophylline*
Doxylamine	*Noroxycodeine*	*Thioridazine*
Ecgonine ·HCl	*Noroxymorphine*	*Trifluoperazine*
Ecgonine Methyl Ester		
*EDDP**		

**2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolioline.*

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

The following Buprenorphine-related substances yield a positive result for Buprenorphine at 10 ng/ml Cut-Off Level:

Buprenorphine-3-β-D-Glucuronide	2.5 ng/ml
Buprenorphine	10 ng.ml
Nalorphine	1000 ng/ml
Norbuprenorphine	30000 ng/ml
Norbuprenorphine-3-β-D-Glucuronide	30000 ng/ml

Table-III: Interference with Endogenous Substance

The potential interference of endogenous physiological substances on recovery of buprenorphine using the one step rapid test was assessed by spiking known amounts of potentially interfering substances to into urine specimens having a known bubrenorphine-3-β-D –Glucuronide for each specimen. The buprenorphine concentration for each specimen (substance and final concentration listed in the table below) was determined . The observed and expected result are shown in the following.

**HSA: Human Serum Albumin*

		Spike Dose (0 ng/ml)		Spike Dose (5 ng/ml)	
Interfering Substance	Final Concentration	Observed Results	Expected Result	Observed Results	Expected Result
Acetone	1000 mg/dl	Negative	Negative	Positive	Positive
Ascorbate	300 mg/dl	Negative	Negative	Positive	Positive
Creatine	500 mg/dl	Negative	Negative	Positive	Positive
Globulin	500 mg/dl	Negative	Negative	Positive	Positive
Glucose	1500 mg/dl	Negative	Negative	Positive	Positive
Hemoglobin	300 mg/dl	Negative	Negative	Positive	Positive
NaCl	6000 mg/dl	Negative	Negative	Positive	Positive
Oxalic acid	50 mg/dl	Negative	Negative	Positive	Positive
HSA*	500 mg/dl	Negative	Negative	Positive	Positive
Urea	2000 mg/dl	Negative	Negative	Positive	Positive
Ethanol	1000 mg/dl	Negative	Negative	Positive	Positive
DL-thyroxine	12 mg/dl	Negative	Negative	Positive	Positive
Digoxin	15 mg/dl	Negative	Negative	Positive	Positive
Apomorphine	10 mg/dl	Negative	Negative	Positive	Positive
Tetracycline	20 mg/dl	Negative	Negative	Positive	Positive
D-glucuronic Acid	20 mg/dl	Negative	Negative	Positive	Positive
Uric Acid	23 mg/dl	Negative	Negative	Positive	Positive
Ampicillin (sodium)	20 mg/dl	Negative	Negative	Positive	Positive

3. **Cutoff Characterization.** The expected results set at 50% below and 50% above the determined cut-off values of 10 ng/ml of Bupernorphine and 2.5 ng/ml of Buprenorphine-3-β-D–Glucuronide matched that of the observed results. See Table below.

	Buprenorphine			
	5.0 ng/ml		20.0 ng/ml	
Sample No.	Observed Results	Expected Result	Observed Results	Expected Result
1	Positive	Positive	Positive	Positive
2	Positive	Positive	Positive	Positive
3	Positive	Positive	Positive	Positive
4	Positive	Positive	Positive	Positive
5	Positive	Positive	Positive	Positive
6	Positive	Positive	Positive	Positive
7	Positive	Positive	Positive	Positive
8	Positive	Positive	Positive	Positive
9	Positive	Positive	Positive	Positive
10	Positive	Positive	Positive	Positive
11	Positive	Positive	Positive	Positive
12	Positive	Positive	Positive	Positive
13	Positive	Positive	Positive	Positive
14	Positive	Positive	Positive	Positive
15	Positive	Positive	Positive	Positive

	Buprenorphine-3-β-d Glucuronide			
	1.25ng/ml		5.0 ng/ml	
Sample No.	Observed Results	Expected Result	Observed Results	Expected Result
1	Negative	Negative	Positive	Positive
2	Negative	Negative	Positive	Positive
3	Negative	Negative	Positive	Positive
4	Negative	Negative	Positive	Positive
5	Negative	Negative	Positive	Positive
6	Negative	Negative	Positive	Positive
7	Negative	Negative	Positive	Positive
8	Negative	Negative	Positive	Positive
9	Negative	Negative	Positive	Positive
10	Negative	Negative	Positive	Positive
11	Negative	Negative	Positive	Positive
12	Negative	Negative	Positive	Positive
13	Negative	Negative	Positive	Positive
14	Negative	Negative	Positive	Positive
15	Negative	Negative	Positive	Positive

4. **Accuracy**. In order to show that the one-step rapid tests are able to detect the presence of Buprenorphine and its major metabolites Buprenorphine-3-β-D-glucuronide in urine samples at and above the determined cut-off levels, the following tests were performed. Seventy(70) Buprenorphine and Buprenorphine-3-β-D-glucuronide free urine were obtained from patients. The same urine sample from each patient is aliquoted into three groups: Group A (Negative Control), Group B (spiked with concentrations ranging from 20 to 1280 ng/ml Buprenorphine by GC/MASS) and Group C (spiked with concentrations ranging from 20 to 1280 Buprenorphine-3-β-D-glucuronide by GC/MASS). The results of using negative control and different GC/MS concentration levels of Buprenorphine and Buprenorphine-3-β-D-glucuronide are shown below.

	GC / mass>10 ng/ml of Buprenorphine	GC / mass<10 ng/ml of Buprenorphine
OneStep Positive	70	0
OneStep Negative	0	7 0
	GC / mass>2 . 5 ng/ml of Buprenorphine-3-β-D Glucuronide	GC / mass<2 . 5 ng/ml of Buprenorphine-3-β-D Glucuronide
OneStep Positive	70	0
OneStep Negative		0

When compared to GC/MS the relative sensitivity was 100%. The relative specificity was 100%.

5. **Precision**. The precision was determined by replicate assays of three different patient urine samples with kits from three different production lots. The resultant data indicated 100% precision for the duplicates within each lot and no appreciable inter-lot variation when testing both positive and negative spiked samples across three (3) different lots of devices.

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