

INSTANT-VIEW[®] Buprenorphine/Norbuprenorphine Urine Test (Cassette) C €

One Step Assay Rapid Visual Results For Qualitative In Vitro Diagnostic Use

INTENDED USE

This device is a qualitative immunoassay intended to detect buprenorphine (BUP) and its metabolite, norbuprenorphine (NBUP) in human urine. Results are preliminary positive when the combination of the concentrations of BUP and NBUP is greater than 10 ng/ml. It is for health care professional use only.

This test provides only a preliminary 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 High Performance Liquid Chromatography (HPLC) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

SUMMARY AND EXPLANATION OF THE TEST

Buprenorphine is an analgesic drug. It is also used in heroin substitution and detoxification treatment. With this increased medical use, it also occurs on the black market as an illicit drug; and fatalities have occurred when used in combination with other drugs.

Buprenorphine is administered clinically by intravenous, intramuscular or sublingual routes. Buprenorphine is metabolized by N-dealkylation to form the pharmacologically active Norbuprenorphine. Both buprenorphine and norbuprenorphine are also glucuronidated to the clinically inactive conjugates buprenorphine-3-beta-D-glucuronide and norbuprenorphine-3-beta-D-glucuronide.

Buprenorphine and its metabolite norbuprenorphine (along with the glucuronide forms) are both excreted in urine during the course of several days. Buprenorphine and its metabolites are eliminated mainly in the feces (68%), with a small proportion excreted in urine (27%). It was reported that urine samples taken from patients who had received treatment for 2 weeks with 4 mg of buprenorphine daily (sublingually) showed buprenorphine concentrations ranging from 54 to 260 ng/ml 24 hours after the dose. It was found in another study that the concentrations of the unconjugated buprenorphine and unconjugated norbuprenorphine in the urine samples collected 10 hours after a single dose intramuscular injection of 0.3 mg buprenorphine were 500 pg/ml and 2 ng/ml, respectively.

The concentration of the metabolite norbuprenorphine is usually higher than buprenorphine. The median ratio of buprenorphine to norbuprenorphine is dependent on the time between sampling and dose intake. It was reported that in suspected abusers, the ranges were 2.3 to 796 ng/ml for unconjugated buprenorphine and 5 to 2580 ng/mL for unconjugated norbuprenorphine. It was also found that the concentration of free buprenorphine and norbuprenorphine in urine may be relatively small (<1 ng/mL) if taken in clinically administered doses, but can reach up to 20 ng/mL if abused.

PRINCIPLE OF THE PROCEDURE

This assay is a one-step lateral flow chromatographic immunoassay. The test strip consists of 1) a burgundy-colored conjugate pad containing rabbit anti-buprenorphine antibodies and mouse IgG coupled to colloidal gold; and 2) a nitrocellulose membrane containing a Test (T) line and a Control (C) line. The T line is coated with buprenorphine-BSA, and the C line is coated with goat anti-mouse IgG antibody.

This test is a competitive binding immunoassay. The buprenorphine and norbuprenorphine in the urine specimen compete with the buprenorphine-BSA antigen coated on the nitrocellulose membrane for the limited binding sites of the conjugated anti-buprenorphine antibodies.

When an adequate amount of urine specimen is applied to the sample pad of the device, the urine specimen migrates by capillary action through the test strip. If the level of buprenorphine and / or norbuprenorphine in the urine specimen is below the cutoff concentration (10 ng/ml), the T line appears as a visible burgundy line. If the level of buprenorphine in the urine specimen is above the cutoff, no T line develops.

The C line will bind to the mouse IgG conjugate and form a burgundy color band regardless of the presence of buprenorphine. The C line serves as an internal qualitative control of the test system.

REAGENTS AND MATERIALS SUPPLIED

- 25 test devices, each sealed in a pouch with a dropper pipette.
- 1 package insert (Instructions for Use).

MATERIAL REQUIRED BUT NOT PROVIDED

- External Quality Controls: positive and negative
- Specimen collection containers
- Timer

STORAGE AND STABILITY

Store the product at room temperature 15-30°C (59-86°F). Each device may be used until the expiration date printed on the label if it remains sealed in its foil pouch.

Do not freeze and/or expose the kit to temperatures over 30°C (86°F).

SPECIMEN COLLECTION

1. Each urine specimen must be collected in a clean container. Do not combine specimens.
2. Specimens may be kept at 15-30°C (59-86°F) for 8 hours, at 2-8°C for up to 3 days and at -20°C or lower for long term storage.

PRECAUTION

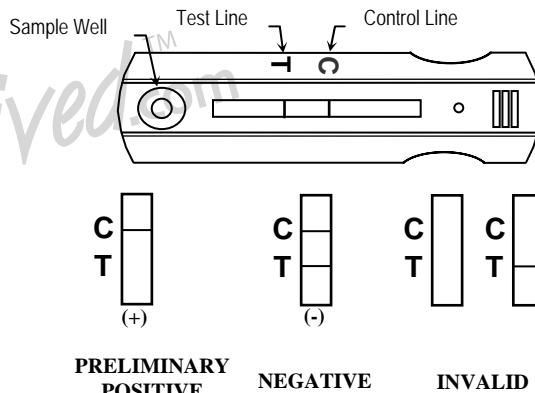
1. The instructions must be followed exactly to obtain accurate results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Dispose of all specimens and used assay materials as potentially biohazardous.

ASSAY PROCEDURE

1. Refrigerated specimens and other test materials, including devices, **must be equilibrated to room temperature before testing.**
2. Remove the test device from its pouch and place it on a flat surface. Label the device with specimen identification.
3. Holding the dropper vertically, add four drops of the urine specimen to the sample well.
4. Read the test result between four (4) to seven (7) minutes after adding the specimen.

INTERPRETATION OF RESULTS

IMPORTANT: Do not read test results after seven (7) minutes. The T Line should always be interpreted independently of the C Line.



Positive:

If only the C line appears, the result indicates that the buprenorphine and/or norbuprenorphine level in the sample is at a cutoff of 10 ng/ml or higher.

Samples with preliminary positive results should be confirmed with a more specific method before a positive conclusion is made.

A positive test result does not always mean an individual has taken the drug illegally as the drug can be administered legally.

Negative:

If both C line and T line appear, the result indicates that the buprenorphine and/or norbuprenorphine level is below 10 ng/ml.

Note: A faint T line should be considered negative.

Invalid:

If no C line develops within 4 minutes, the result is invalid. Repeat the assay with a new test device.

QUALITY CONTROL

• Built-in Control Features

This test contains a built-in control feature, the C line. The appearance of the burgundy C line indicates an adequate volume of specimen has been absorbed and the capillary flow through the test strip has occurred. The C line should always appear. If the Control line does not develop within 4 minutes, review the entire procedure and repeat test with a new device.

• External Quality Control

Users should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls. SAMHSA

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recommends that the concentration of drug(s) in positive and negative controls be approximately 25% above and below the cutoff concentration of the assay.

LIMITATIONS

1. This test is for *professional in-vitro* diagnostic use only.
2. Results obtained by this device provide only a preliminary qualitative result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result.
3. This product is designed to detect only buprenorphine and / or norbuprenorphine in human urine.
4. Adulterants such as bleach or other strong oxidizing agents may produce erroneous test results. When adulteration is suspected, collect a fresh specimen and repeat the test with a new device.
5. Samples in which bacterial contamination is suspected should not be used. These contaminants may interfere with the test and cause false results.

EXPECTED VALUES

This device is designed to detect the combination of buprenorphine and norbuprenorphine at a cutoff of 10 ng/ml.

PERFORMANCE CHARACTERISTICS

1. Accuracy

The accuracy of this device was evaluated using 54 clinical urine specimens and 40 drug free urine samples with varying un-hydrolyzed concentrations of both buprenorphine and norbuprenorphine in different ratios, each blind-labeled. The results from this test device agreed 100% with the HPLC/MS and GC/MS on the non-hydrolyzed specimens at levels below 75% of the cutoff (negative) and above 125% of the cutoff (positive). Three (3) discrepancies were observed on the specimens of buprenorphine/norbuprenorphine at the level between 75% and 125% of the cutoff. The overall agreement was 96.8% (3 discrepancies/94 specimens).

Buprenorphine/ Norbuprenorphine	BUP and NBUP		Total I	Agreement	
	Positive	Negative			
HPLC/MS (ng/ml)	Negative (<75%)	0	49	49	100%
	75% - cutoff	1*	5	6	83.3%
	Cutoff - 125%	18	2*	20	90%
	Positive (>12.5)	19	0	19	100%
Total		38	56	94	

*Indicates discrepancy.

2. Reproducibility

Reproducibility was evaluated at three POL locations. Personnel with diverse educational backgrounds and work experience performed the tasks. Eighty-pooled drug-free human urine specimens were spiked with buprenorphine at different levels. All specimens were blind labeled and tested. Results are summarized in the table below.

Buprenorphine	Site I	Site II	Site III	Expected Result
0 ng/ml	10 -	10 -	10 -	10 -
5 ng/ml	15-	15-	15-	15-
7.5 ng/ml	14-, 1+	14-, 1+	14-, 1+	15-
12.5 ng/ml	14+, 1-	13+, 2-	14+, 1-	15+
15 ng/ml	15+	15+	15+	15+
30 ng/ml	10+	10+	10+	10+
Agreement	Site	96.3%	96.3%	97.5%
	Average	97.1%		

Norbuprenorphine	Site I	Site II	Site III	Expected Result
0 ng/ml	10 -	10 -	10 -	10 -
5 ng/ml	15-	15-	15-	15-
7.5 ng/ml	14-, 1+	13-, 2+	14-, 1+	15-
12.5 ng/ml	15+	15+	15+	15+
15 ng/ml	15+	15+	15+	15+
30 ng/ml	10+	10+	10+	10+
Agreement	Site	98.8%	97.5%	98.8%
	Average	98.4%		

The results indicated 97.1% and 98.4% separately concordance with the expected results.

3. Cross-Reactivity

To evaluate the cross-reactivity of the device, compounds structurally related to buprenorphine were studied. The lowest concentration of the drugs producing a positive response equivalent to the cutoff level of the device was listed in the table below.

Compounds Name	Concentration (ng/ml)
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Buprenorphine	10
Norbuprenorphine	10
Buprenorphine-3- -D-glucuronide	750
Norbuprenorphine-3- -D-glucuronide	30,000
Nalorphine	100,000

4. Interfering Substances

The following substances, often found in urine, were spiked and evaluated in urine pools containing 0, and 15 ng/ml buprenorphine and norbuprenorphine, separately, with the device. No interference was observed from those analytes at the concentration listed in the following tables.

Substances in this table were tested and found not to interfere with the test results; negative, (0 ng/ml) and positive, (15 ng/ml) for buprenorphine and norbuprenorphine at a concentration of 100 µg/ml.

Acetaminophen	Cortisone
Acetylsalicylic Acid	Dextromethorphan
Amikacin	Ethanol
Amitriptyline	Lidocaine
Ampicillin	Methadone
Arterenal	Methanol
Aspirin	Oxalic Acid
Benzoic Acid	Penicillin-G (Benzylpenicillin)
Benzoylcegonine	β-phenylthylamine
Caffeine	Phenylpropanalamine
(+)-Chlorpheniramine	Ranitidine
(+/-)-Chlorpheniramine	Salicylic Acid
Cocaine	Thioridazine













The following substances were tested and confirmed not to interfere with BUP/NBUP test device at the concentration listed below.

Biological Analytes	Concentration
Albumin(serum)	2,000 µg/ml
Bilirubin	1,000 µg/ml
Creatine	1,000 µg/ml
Hemoglobin	1,000 µg/ml
Glucose	2,000 µg/ml
Vitamin C (L-Ascorbic Acid)	1,000 µg/ml
Uric Acid	1,000 µg/ml
pH	5.0-9.0

There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results.

REFERENCES

- FDA Guidance for Labeling Urine Drugs of Abuse Screening Testing, Kshit Mohan, 7/21/87.
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 15°C - 30°C	Temperature limitation		Use by YYYY-MM
 LOT	Batch/Lot code		In vitro diagnostic medical device
	Manufacturer		Catalog number
	Contains sufficient for < n > tests		Consult instructions for use
	For IVD performance evaluation only		Do not reuse
	Caution, consult accompanying documents		CE Mark

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 San Diego, CA 92121
 tel 858-481-5031
 toll free 888-882-7739
 www.cliawaived.com