



BUPRENORPHINE (BUP)

Rapid Test (BUP) strip

Introduction

The BUP Rapid Test Dipstick (Urine) is a Rapid chromatographic immunoassay for qualitative detection of Buprenorphine in human urine at a cut-off concentration of 10 ng/mL which is only utilized for in vitro diagnostics (IVD). It merely provides a preliminary result, therefore, an alternative method with more specificity is recommended to be used to confirm the initial results, in which case, the most preferred method is gas chromatography-mass spectrometry (GC-MS). In cases of drug abuse, especially when the test result is positive, clinical and expert observations are required.

Summary

Buprenorphine is a strong painkiller often used to treat addiction. It is sold under brand names Suboxone, Temgesic, Buprenex, and Subutex containing Buprenorphine HCl combined with Naloxone HCl. Therapeutically, Buprenorphine is an alternative treatment for drug addicts. This kind of treatment is a method of medical care used for addicts to drugs such as heroin. In alternative treatment, Buprenorphine is as strong and effective as methadone, but its addiction rate is less than methadone. The concentrations of Buprenorphine and norBuprenorphine in the urine might be less than 10 ng/mL after therapeutic confirmation, but go up to 20 ng/mL for drug abuse. The half-life of Buprenorphine plasma is 2 to 4 hours. While a complete omission of one dose takes around 6 days, the detection source for the main drug in the urine is approximately 3 days. Buprenorphine rapid test is a quick urine screening method which could be performed without laboratory equipment. This test employs monoclonal antibodies for the specific detection of high levels of Buprenorphine in the urine. BUP rapid test result becomes positive when the level of Buprenorphine exceeds 10 ng/mL.

01

Function Method Basis

Rapid Buprenorphine urine test employs an immunochromatographic method based on competitive binding. Substances that may be found in the urine specimen compete with ones metabolized by the liver to bind to antibodies. During the test, the urine specimen flows upward via capillary action. If the amount of Buprenorphine in the urine is below 10 ng/mL, it does not bind to the binding sites of the antibodies coated with gold nanoparticles. Consequently, the antibody conjugated with nanoparticles will be absorbed by the Buprenorphine on the surface of the membrane and will leave a colored line in the test area (T). Should the amount be greater than 10 ng/mL, no colored line will appear in the T area, since all the binding sites of the Buprenorphine antibodies are occupied. So, if the presence of Buprenorphine in the urine specimen is positive, no colored line will be formed in the test area (T) due to drug competition, whereas if the urine specimen contains Buprenorphine amounts lower than the desired level in the test, a colored line will appear in this area. In order to have a procedural control, a colored line always appears in the area of the control line (C), indicating the appropriate volume of the specimen added to the membrane.

Compounds

The test strip contains anti-Buprenorphine monoclonal antibody particles and a combination of conjugated Protein-Buprenorphine. The secondary antibody prepared in goat species is also employed for the control line.

+ **Caution:** for in vitro diagnostic use only.

Do not use after the expiration date. The test must remain in its package until use. All specimens must be considered infectious and all precautions and safety measures for handling and use must be taken.

Storage

The package should be stored at room temperature or in refrigerator (2-30 °C). The test is valid only until the expiration date printed on the package. Keep from freezing.

Specimen Collection and Preparation

+ **Urine collection:** The urine specimen must be collected in a clean and dry container. It could be collected at any time of the day.

02

+ **Specimen storage:** The urine specimen can be stored at 2-8 °C for more than 48 hours, and frozen for long-term storage at -20 °C. Frozen specimens must be thawed and mixed well before testing.

Contents and Required Supplies

Items found in the kit: + Brochure + Test strip

Required items not included in the kit: + Specimen collection container + Timer

User Manual

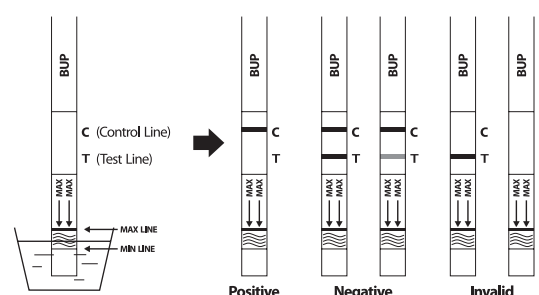
Before testing, allow the test strip and the urine specimen reach room temperature (18-30 °C).

• Bring the pouch to room temperature, before opening it. Remove the test strip from the sealed pouch, and use it as soon as possible.

• Dip the test strip vertically into the urine specimen in the direction indicated by the arrows up to the MAX line, and hold it for at least 10-15 seconds (See illustration).

• Place the test strip on a flat, non-absorbent surface, set the timer and wait for the colored line to appear. Interpret the results within 5 minutes.

+ Do not interpret the results after 10 minutes.



03

Result Interpretation

Negative: Two lines appear, one line should be in the control area (C) and another colored line should appear in the test area (T). This negative result indicates that the Buprenorphine concentration is below the detectable level of 10 ng/mL.

+ **Attention:** The color shade in the testing area may vary, but it should be considered negative whenever there is even a faint colored line.

Positive: A colored line should appear in the control area (C) and no line should be seen in the test area (T). This positive result shows that the concentration of Buprenorphine is above the detectable level of 10 ng/mL.

Invalid: The control line (C) is not formed. insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem is not solved, stop using the strip and contact the after-sales service immediately.

Quality Control

The quality control of the method is included in the test, and the colored line appearing in the control area (C) is considered as an inner controller which confirms the sufficient volume of the specimen and the correct execution of the test. The test strip does not come with control standards. It is recommended to do the test with positive and negative controls in an accredited laboratory to confirm the method and the correctness of the test.

Limitations

1. Buprenorphine Rapid Test strip provides only preliminary analytical and qualitative results, and therefore, a secondary analytical method must be employed to confirm the results. For the confirmation of the results of this test, gas chromatography-mass spectrometry (GC-MS) is preferred.

2. There is a possibility of error in this technique. As some interfering substances in the urine specimen may lead to wrong results.

3. Adding substances like bleaches or white alum in the urine specimen may cause a wrong result, despite the analytical method used, and should the substances remain, the test should be performed with another urine specimen.

04

4. A positive result indicates the presence of a substance or its metabolites. A negative result, on the other hand, may not necessarily indicate the absence of the substance in the urine. The drug level may be lower than the level detectable by the test.

5. The test is not able to detect the abuse of certain therapeutic cases.

+ **Expected values:** if Buprenorphine concentration is less than 10 ng/mL, the result will be negative and if the concentration is greater than 10 ng/mL, the result will be positive. The cut-off value of the test is 10 ng/mL.

Executive Feature

Accuracy: The results obtained from Hana Gene's BUP Rapid Test strip were compared with those of an available commercial kit. In a study on 94 urine specimens (43 positive and 51 negative), Hana Gene's kit detected 43 of the specimens positive and 51 negative. The results are presented in table below:

Method		other BUP Rapid Test		Total Result
The BUP Rapid Test Dipstick	Results	Positive	Negative	
	Positive	43	0	
	Negative	0	51	
Total Result		43	51	94
% Agreement with this Rapid Test		> 99.9%	> 99.9%	> 99.9%

In order to compare the results of Hana Gene's Buprenorphine Rapid test kit, using GC/MS method, 250 clinical specimens (106 positive; 144 negative) were collected, and Hana Gene's kit detected 105 positive and 144 of the specimens negative. The results are shown in the table below:

Method		GC/MS		Total Result
The BUP Rapid Test Dipstick	Results	Positive	Negative	
	Positive	105	0	
	Negative	1	144	
Total Result		106	144	250
Relative Sensitivity: 99.1%		Relative Specificity: > 99%		
Total Agreement: 99.6%				

05

Analytical Sensitivity:

Some amount of drug-free urine specimens was prepared in the following concentrations of Buprenorphine:

0 ng/mL, 5 ng/mL, 7.5 ng/mL, 10 ng/mL, 12.5 ng/mL, 15 ng/mL and 30 ng/mL. The results show more than 99% accuracy in 50% of specimens greater than the cut-off level, and 50% below cut-off value. This information is summarized in the table below:

BUP Concentration (ng/mL)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
5	-50%	30	30	0
7.5	-25%	30	26	4
10	Cut-off	30	14	16
12.5	+25%	30	3	27
15	+50%	30	0	30
30	3X	30	0	30

Analytical Features:

The following table shows the compounds positively identified by the Buprenorphine test strip in the urine specimen within 5 minutes.

Compound	Concentration (ng/mL)
Buprenorphine	10
Norbuprenorphine	50
Buprenorphine 3-D-Glucuronide	50
3-D-Glucuronide Norbuprenorphine	100

Precision

A study was carried out in three different centers by untrained people making use of three different lots of the product in order to evaluate the intra-rater, inter-rater, and inter-individual reliability.

A panel of coded specimens containing certain Buprenorphine concentrations with GC/MS method was used in each center, at 0 ng/mL, 25% above and below the minimum detection limit, and 50% above and below the minimum detection limit of 10 ng/mL.

06

BUP Concentration (ng/mL)	n per site	LOT A		LOT B		LOT C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
5	10	10	0	10	0	10	0
7.5	10	9	1	9	1	8	2
12.5	10	1	9	1	9	1	9
15	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Buprenorphine with concentrations of 5 ng/mL and 15 ng/mL was added to 15 urine specimens with low, normal, and high specific gravity. Then pure specimens and specimens with certain Buprenorphine concentrations were checked by the test strip. The results show that the specific weight and gravity do not affect the test results.

Effect of Urinary pH

The pH of the negative diluted urine specimen was changed from 5 to 9, and the specimens were given Buprenorphine concentrations of 5 ng/mL and 15 ng/mL. The adjusted pH of the urine was done in a duplicate rapid Buprenorphine test whose results show that the pH range does not interfere with the test.

Cross Reactivity

In order to assess the interferences with positive and negative Buprenorphine test results, a study was carried out. The following compounds did not show any interference in the test results with 100 µg/ml Buprenorphine concentration:

Acetamidophenol 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 EMDP D-Methamphetamine Prednisolone Amobarbital Ephedrine L-Methamphetamine Prednisone Amoxapine (1r,2s)-(-)Ephedrine Methaqualone 5 beta- Amoxicillin (-)-ψ-Ephedrine Methoxyphenamine pregnane3α17α- L-Amphetamine (±)-Epinephrine (-) 3,4-Methylenedioxy- 21triol 21 Ampicillin Erythromycin amphetamine (MDA) Procaine Apomorphine β-Estradiol (+) 3,4 Methylenedioxy- Promazine Aspartame Estrone-3-sulfate methamphetamine Promethazine Atropine Ethanol (Ethyl alcohol) Methylphenidate D,L-Propanolol Benzilic acid Ethyl-p-aminobenzoate Methpyrrolon D-Propoxyphene Benzoic acid Etodolac Methaqualone D-Pseudoephedrine Benzoylcgonine Famprofazone Metoprolol

07

Quinacrine Benzphetamine Fenfluramine Morphine sulfate Quinidine Bilirubin Fenoprofen Morphine- Quinine (±)-Brompheniramine Fentanyl 3-β-D-glucuronide Ranitidine Buspirone Fluoxetine Nalidixic acid Riboflavin Caffeine Furosemide Nalorphine Salicylic acid Cannabidiol Gentisic acid Naloxone Secobarbital Cannabinol D (+) Glucose Naltrexone Serotonin Chloralhydrate Guaiacol Glyceryl Ether Methylpyrrolon (5-hydroxytyramine) Chloramphenicol Guaiacol Glyceryl Ether Metoprolol Sodium chloride Chlordinapoxide carbamate Nimesulide Sulfamethazine Chloroquine Hemoglobin Norcodeine Sulindac Chlorothiazide Hydralazine Morphine sulfate Temazepam (+)-Chlorpheniramine Hydrochlorothiazide Alpha- Tetracycline (±)-Chlorpheniramine Hydrocodone Naphthaleneacetic Acid Tetrahydrocortisone, Chlorpromazine Hydrocortisone Norethindrone 3-acetate Chlorprothixene Hydromorphone Normorphine Tetrahydrozoline Cholesterol p-Hydroxyamphetamine D-Norpropoxyphene Thebaine Cimetidine O-Hydroxyhippuric acid Noscapine Theophylline Clomipramine p-Hydroxymethamphetamine D,L-Octopamine Thiamine Clonidine p-Hydroxynorephedrine Orphenadrine Thioridazine Cocaine HCl Hydroxyzine Oxalic acid (chlorpromazine) Codeine 3-Hydroxytyramine Oxazepam L-Thyroxine Cortisone Ibuprofen Oxolinic acid Tolbutamide (-) Cytidine Imipramine Oxycodone Cis-Tramadol Creatinine Iproniazid Oxymetazoline Trazodone Cyclobarbitol (-)-Isoproterenol Oxymorphone Triamterene Cyclobenzaprine Isoxsuprine Papaverine Trifluoperazine Deoxycorticosterone Kanamycin Pemoline Trimethobenzamide (-) Deoxyephedrine Ketamine Penicillin-G Trimethoprim R (-) Deprenyl HCl Ketoprofen Pentazocine Trimipramine Dextromethorphan Labetalol Pentobarbital Phencyclidine Diazepam L-Ascorbic acid Perphenazine D, L-Tryptophan Diclofenac L-Ephedrine Triethylamine Tyramine Dicyclomine L-Epinephrine Phenelzine D, L-Tyrosine Diflunisal Levorphanol Pheniramine Uric acid Digoxin Lidocaine Phenobarbital Verapamil 4-Dimethylaminopyridine Lindane Phenothiazine Zomepirac Diphenhydramine (hexachlorocyclohexane) Phentermine

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