T-58011-A

AccuSign® BUP

One-Step Buprenorphine Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Buprenorphine and its Metabolites in Human Urine

Catalog No.	DOA-212-35	35 Test Kit
	DOA-212-10	10 Test Kit

Intended Use

AccuSign® BUP is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of buprenoprhine and its metabolites at a cutoff of 8 ng/mL in human urine.

The* AccuSign® BUP *test provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography, mass spectrometry (GC/MS) is the preferred confirmatory method. Other chemical confirmatory methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.[1]

Summary and Explanation

Buprenorphine is a synthetic derivative of thebaine. Its structure is similar to morphine but has anagonist and agonist properties.[2,3] Buprenorphine has been widely prescribed as a pain-killer since the early 1980s. It has also been used as a substitution treatment for opioid drug dependence as an alternative to methadone. In addition to therapeutic uses, buprenorphine is also abused either sublingually or intravenously.

Buprenorphine is metabolized in the body to norbuprenorphine. Both buprenorphine and norbuprenorphine undergo extensive conjugation to buprenorphine-3-β-D-glucuronide and norbuprenorphine-3-β-D-glucuronide, respectively.[3]These conjugates are subsequently excreted into urine during the course of several days.[3] Concentrations of unconjugated 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.[4] The presence of buprenorphine and its metabolites in urine can be detectable after 1-4 days.[3,5]

Principle

The **AccuSign® BUP** test uses solid-phase chromatographic membrane immunoassay technology for the qualitative detection of buprenorphine and its metabolites. The test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition between the drug conjugates and the drugs which may be present in the urine sample, for binding to antibodies. In the test procedure, a sample of urine is placed in the Sample well of the device and is allowed to migrate upward. If the drug is present in the urine sample, it competes with the drug conjugate bound to the dye, for the limited antibodies immobilized on the membrane. If the level of drug or drug metabolite is above the cutoff level, the drug will saturate the antibodies, thus inhibiting the binding of the dye coated with drug conjugates to the antibodies on the membrane. This prevents the formation of a line on the membrane. Therefore, a drug-positive urine sample will not generate a line at Test (T) position in the Result window, indicating a positive result from positive drug competition. A negative urine sample will generate a line at Test position in the Result window, indicating a negative result from an absence of competition with free drugs. In addition to the Test line that may appear in the Result window, a Control line is present to confirm the viability of the test. This Control line (validation line) should always appear if the test is conducted properly. Polyclonal sheep anti-mouse IgG antibody is immobilized on the control line. The monoclonal antibody-dye conjugates that pass the line will be captured and produce a colored line at the Control position (C). This works as a procedural control, confirming that proper sample volume was used and the reagent system at the Control line and the conjugate-color indicator worked properly. If insufficient sample volume is used, there may not be a Control line, indicating the test is invalid.

Materials Provided

The **AccuSign® BUP** test kit contains all the reagents necessary to perform the tests.

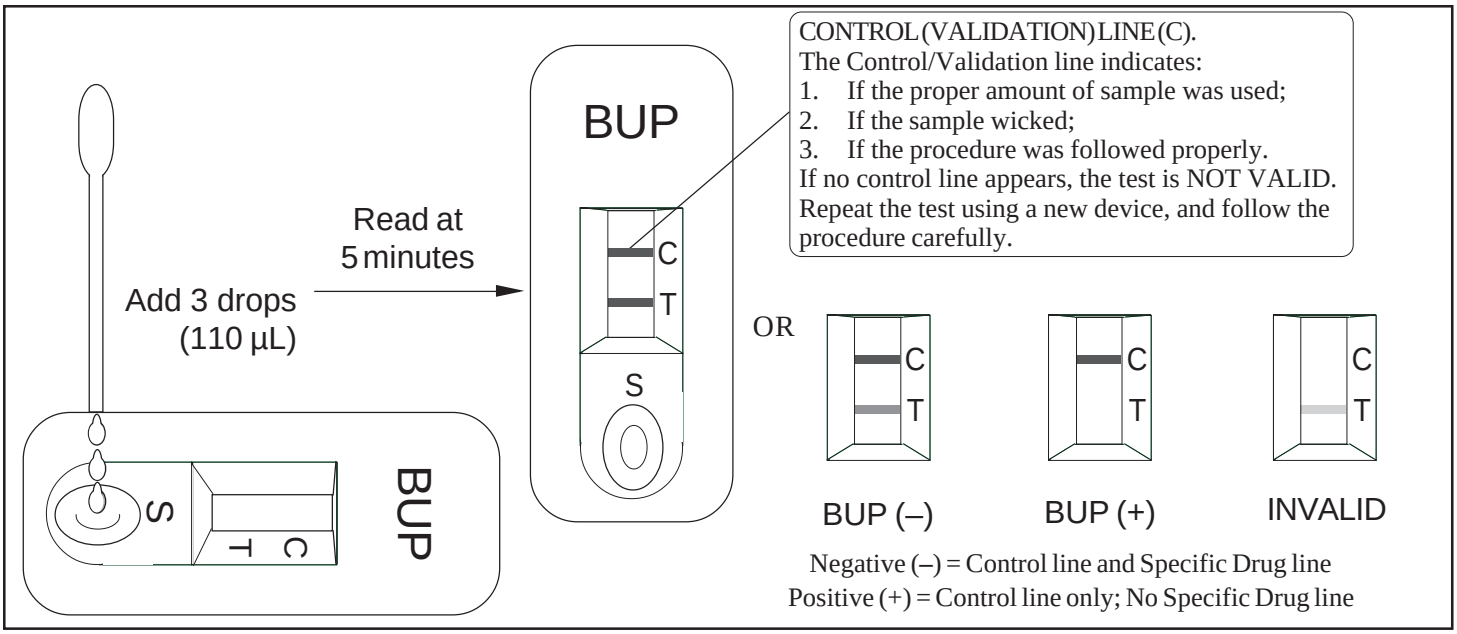
- **AccuSign® BUP** device. The test device contains a membrane strip coated with monoclonal anti-buprenorphine antibody and a pad containing dye coated with drug conjugates.
- Disposable specimen dispensers.
- Instructions for use.

Precautions

- For *in vitro* diagnostic use only.
- Avoid cross contamination of urine samples by using a new urine specimen container and dropper for each urine sample.
- The test kit does not contain any HIV or hepatitis infective components.
- Urine specimens are potentially infectious. Proper handling and disposal methods should be established according to good laboratory practices.
- The **AccuSign® BUP** device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.
- Do not use the test kit after the expiration date.

Storage and Stability

The **AccuSign® BUP** test kit should be stored at 2–30°C (35–86°F) in the original sealed pouch. The expiration dating was established under these storage conditions.



Specimen Collection and Preparation

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed immediately, specimens should be refrigerated (2–8°C) or frozen. Specimens should be brought to room temperature before testing. Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing to settle before testing.

Test Procedure

The test procedure consists of adding the urine sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. For each test, open one **AccuSign® BUP** pouch and label the **AccuSign® BUP** device with the patient ID.
2. Holding the dropper vertically, dispense 3 drops (110µL) of the urine sample into the Sample well (S).
3. Read the result at 5 minutes of sample addition.

Interpretation of Results

Negative: The appearance of a reddish-purple Control line (**C**) and a line next to T indicates a negative test result; i.e., no drug above the cutoff level has been detected. The color intensities of the Control line and the Test line may not be equal. *Any faint line at the T position in the Result window, visible in 10 minutes, should be interpreted as negative. A negative test result does not indicate the absence of drug in the sample; it only indicates the sample does not contain drug above the cutoff level in qualitative terms.*

Positive: The appearance of only a reddish-purple Control line and no distinct line next to T indicates the test result is positive for BUP (i.e., the specimen contains BUP at a concentration above the cutoff level). *A positive test result does not provide any indication of the level of intoxication or urinary concentration of the drug in the sample; it only indicates the sample contains drug above the cutoff level in qualitative terms.*

Invalid: A distinct Control line (**C**) should always appear. The test is invalid if no Control line forms at the **C** position. Such tests should be repeated with a new **AccuSign® BUP** test device.

Limitations

- The test is designed for use with unadulterated urine only.
- There is a possibility that factors such as technical or procedural errors, as well as other substances in the urine sample, may interfere with the test and cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results. If adulteration is suspected, the test should be repeated with a new sample.
- The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.
- Urine samples obtained from donors consuming high doses of codeine based medications may produce a positive result.

User Quality Control

Internal Control: Each **AccuSign®** test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should always appear at the C position, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test has been performed correctly and the device is working properly, the background in the result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

The positive and negative procedural controls contained in each **AccuSign®** test device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear at the Control position, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing process. For information on how to obtain controls, contact PBM's Technical Services.

Expected Values

AccuSign® BUP is a qualitative assay. The amount of buprenorphine and its metabolites present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain burprenorphine and its metabolites above the cutoff concentration.

Performance Characteristics

The **AccuSign® BUP** test has been shown to detect buprenorphine and its metabolite buprenorphine-3-β-D-glucuronide at an average cutoff of 8 ng/mL in urine.

The accuracy of **AccuSign® BUP** was evaluated in comparison to a commercially available semi-quantitative immunoassay (CEDIA® Buprenorphine Assay from Microgenics Corporation). A total of 86 samples was tested by both procedures. Complete agreement was observed in 97% of the samples as shown below (Table 1). Values of less 8 ng/mL with the CEDIA® Buprenorphine Assay were reported as negative, whilst values of 8ng/mL and above were reported as positive.

Table 1. Accuracy: Comparison of AccuSign® BUP with CEDIA® Buprenorphine Assay

		CEDIA® Buprenorphine Assay		
		Positive	Negative	Total
AccuSign® BUP	Positive	34	1	35
	Negative	2	49	51
TOTAL		36	50	86
Relative Sensitivity			94.4% (34/36)	
Relative Specificity			98.0% (49/50)	

The false negative sample was analyzed by GC/MS and contained the drug at 9.6 ng/mL. The 2 false positive samples contained zero and 0.9 ng/mL by GC/MS.

Specificity

The specificity of **AccuSign® BUP** test was determined by adding the compounds structurally related to buprenorphine to negative urine specimens and testing with the **AccuSign® BUP** test kit. The results are expressed in terms of the concentration required to produce a positive result (Table 2).

Table 2. Specificity

Compound	Concentration(ng/ml)
Buprenorphine	8
Buprenorphine-3-β-D-glucuronide	10
Codeine	70,000
Dextromethorphan	>200,000
Dihydrocodeine	200,000
EDDP	>200,000
EMDP	>200,000
Heroin	>50,000
Hydrocodone	>200,000
Hydromorphone	>200,000
Imipramine	>200,000
LAAM	>200,000
Meperidine	>200,000
Methadone	>200,000
Morphine	>200,000
Morphine-3-D-glucuronide	>200,000
Morphine-6-D-glucuronide	>50,000
Nalorphine	5,000
Naloxone	>200,000
Naltrexone	>200,000
Norbuprenorphine	>50,000
Norbuprenorphine-3-β-D-glucuronide	>50,000
Noroxycodone	>200,000
Noroxymorphine	>50,000
(+)Norpropoxyphone	>200,000
Oxycodone	>200,000
Oxymorphone	>200,000

References

1. Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse*. Rockville, MD: National Institute on Drug Abuse (NIDA), Research Monograph 73;1986.
2. Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man.* 2nd Ed., Davis, CA: Biomedical Publ.;1982.
3. Cone EJ, Gorodetzky W, Yousefnejad D, Buckwald WF, and ohnson RE. The Metabolism and Excretion of Buprenorphine in Humans. *Drug Metab. Dispos.* 1984;12:577-581.
4. Debrabandere MVB, and Daenens P. High-Performance Liquid Chromatography with Electrochemical Detection of Buprenorphine and its Metabolite in Urine. *J. Chromatogr.* 1991;564:557-566.
5. Blom Y, Bondesson U, and Anggard E. Analysis of Buprenorphine and its N-dealkylated Metabolite in Plasma and Urine by Selected-ion Monitoring. *J. Chromatogr.* 1985;338:89-98.

Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
	"Use By" date in year-month-day format
	Temperature Limitation
	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
PIP	Transfer Pipette
IFU	Instructions for Use
TEST DRUG	One-step immunochromatographic Assay for the Detection of Drugs of Abuse in Urine
TEST BUP	Buprenorphine Test

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