

# AccuSign® OPI

## One-Step Opiates Test

For *In Vitro* Use Only

### Simple One-Step Immunoassay for the Qualitative Detection of Morphine and Opiates in Human Urine

## **PBM**

Catalog No.	DOA-203-35	35 Test Kit
	DOA-203-10	10 Test Kit

### Intended Use

**AccuSign® OPI** is a simple, one-step immunochromatographic assay intended for use in the qualitative detection of morphine in human urine with a cutoff at 300 ng/mL.

*The AccuSign® OPI 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.<sup>1</sup>*

### Summary and Explanation

Morphine, codeine, and semisynthetic derivatives of morphine belong to the class of drugs called opiates. An opiate exerts its effects on the central nervous system and can produce euphoria, respiratory depression and coma when it is abused. Morphine is the prototype compound of opiates. Morphine is excreted in the urine as unchanged morphine, morphine-3-glucuronide, and other minor metabolites. Heroin is metabolized to morphine and codeine and excreted in the urine with a small amount in unchanged form. Codeine is also excreted as morphine and in the form of conjugates. Although some opiate metabolites appear in the feces, urinary excretion is the primary route of elimination.<sup>1,2,3</sup>

### Principle

The **AccuSign® OPI** test uses solid-phase chromatographic membrane immunoassay technology for the qualitative detection of opiates. 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® OPI** test kit contains all the reagents necessary to perform the tests.

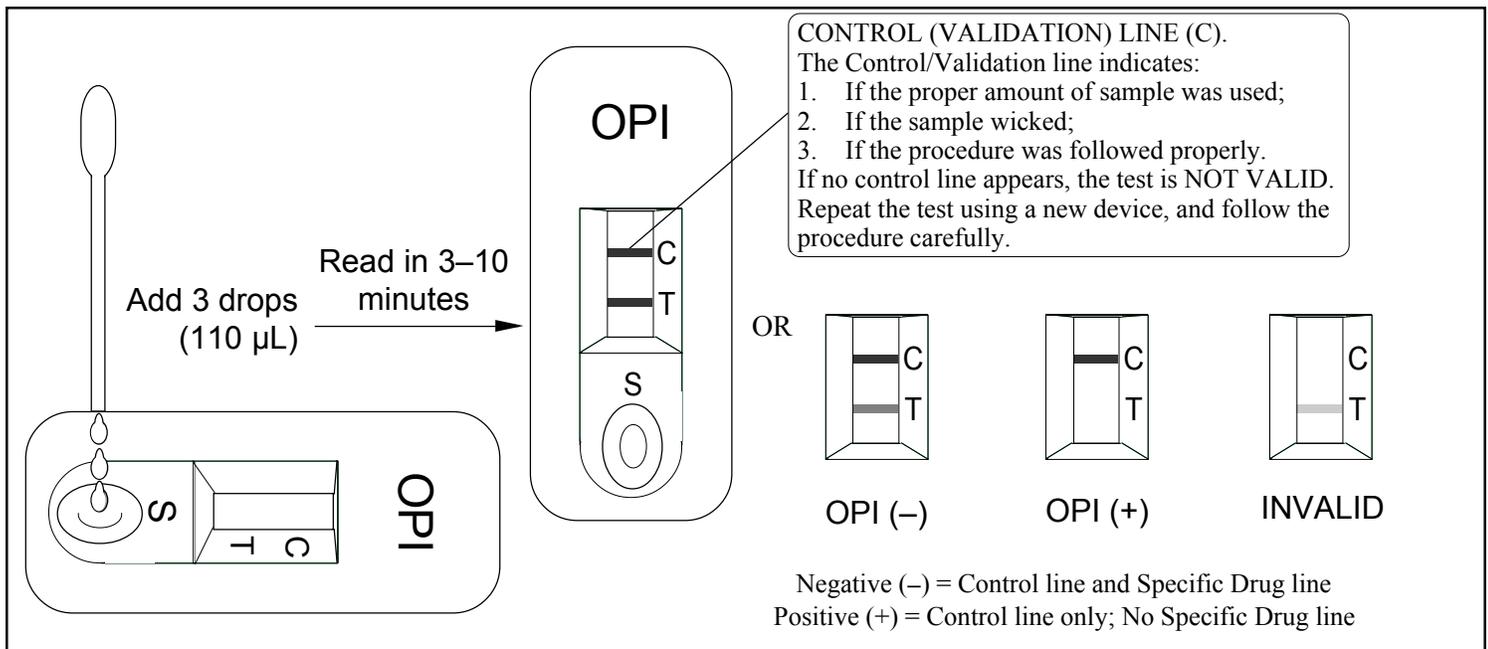
- **AccuSign® OPI** device. The test device contains a membrane strip coated with mouse monoclonal anti-opiate antibody and a pad containing drug-dye conjugate in a protein matrix.
- Disposable specimen dispensers.
- Instructions for use.

### 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®** 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® OPI** 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 windows T and C.

### Test Protocol

1. For each test, open one **AccuSign® OPI** pouch.
2. Holding the dropper vertically, dispense 3 full drops (110 µL) of the urine sample into the Sample well (**S**) and label the **AccuSign®** device with the patient ID.
3. Read the result in 3–10 minutes.

## 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 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 OPI (i.e., the specimen contains OPI 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® OPI** test device.

## Limitations

- The test is designed for use with human urine only.
- There is a possibility that factors such as technical or procedural errors, as well as other substances in the urine sample which are not listed in Table 4 below, may interfere with the test and cause erroneous results.
- Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the method of analysis. If adulteration is suspected, the test should be repeated with a new sample.
- The test must be read within 10 minutes of sample application. The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period.
- Urine samples obtained after the consumption of food or tea derived from or containing poppy seed may produce a positive result.

## User Quality Control

**Internal Control:** Each **AccuSign®** test device has a built-in control. The Control line is an internal positive process control. A distinct reddish-purple Control line should always appear in the 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 working. 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 process control.

The positive and negative process controls contained in each AccuSign<sup>®</sup> 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 in the Control window, 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 and assay procedure are performing properly. 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<sup>®</sup> OPI** is a qualitative assay. The amount of opiates present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain morphine or opiates above the cutoff concentration.

## Performance Characteristics

The SAMHSA has suggested that the screening cutoff for positive samples be 300 ng/mL for morphine. The **AccuSign<sup>®</sup> OPI** test has been shown to detect morphine in urine at an average cutoff of 300 ng/mL.

The accuracy of **AccuSign<sup>®</sup> OPI** was evaluated in comparison to a commercially available immunoassay (Syva<sup>®</sup> EMIT<sup>®</sup> II) at a cutoff of 300 ng/mL. A total of 966 samples was tested by both procedures. Complete agreement was observed in 99% of the samples as shown below. (Table 1.)

**Table 1. Accuracy: Comparison of AccuSign<sup>®</sup> OPI with Syva<sup>®</sup> EMIT<sup>®</sup> II**

		Syva <sup>®</sup> EMIT <sup>®</sup> II (OPI)		
		Positive	Negative	TOTAL
<b>AccuSign<sup>®</sup> OPI</b>	Positive	249	0	249
	Negative	1	716	717
<b>TOTAL</b>		250	716	966
		Relative Sensitivity	Relative Specificity	
<b>Opiates</b>		99.6% (249/250)	> 99% (716/716)	

In a separate study, **AccuSign<sup>®</sup> OPI** was evaluated against specimens confirmed as positive by GC/MS. The results below demonstrate the excellent correlation of **AccuSign<sup>®</sup> OPI** with GC/MS (99% agreement). (Table 2.)

**Table 2. Accuracy: Comparison of AccuSign<sup>®</sup> OPI with GC/MS Assay**

		AccuSign <sup>®</sup>	GC/MS
<b>OPI</b>	Positive	73	74
	Negative	1	0

## Precision and Accuracy

The precision of the **AccuSign<sup>®</sup> OPI** assay was determined by carrying out the test with serially diluted standard drug solutions. Ninety-five percent (95%) of the samples containing drug concentrations 25% over the cutoff level consistently showed positive results.

The study also included over 40 samples  $\pm$  25% cutoff level. These results were found to be consistently in agreement with predicate test results.

### Distribution of Random Error:

Twenty (20) blind samples prepared by spiking various concentrations of morphine were separately tested by two operators. The test results from the two operators showed complete agreement.

## Reproducibility

The reproducibility of the test results of the **AccuSign<sup>®</sup> OPI** assay was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (a concentration 1.5 times the cutoff level), and 5 strongly positive samples (i.e., a concentration 3 times the cutoff level). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

## Specificity

The **AccuSign<sup>®</sup> OPI** test detects morphine and other opiates in urine.

The following table lists compounds that are detected by the **AccuSign<sup>®</sup> OPI** test. The specificity of the **AccuSign<sup>®</sup> OPI** test was determined by adding the drugs and drug metabolites listed to drug-negative urine specimens and testing with the **AccuSign<sup>®</sup> OPI** test kit. The results are expressed in terms of the concentration required to produce a positive result. (Table 3.)

**Table 3. Specificity**

Compound	Concentration (ng/mL)
Codeine	300
Dihydrocodeine	300
Hydrocodone	500
Hydromorphone	500
Lavofloxacin	100,000
Levophanol	5000
Meperidine	>100,000
Morphine	300
Morphine-3- $\beta$ -D-glucuronide	300
Nalorphine	15,000
Naloxone	>100,000
Norcodeine	>100,000
Oxycodone	5,000
Oxymorphone	20,000
Thebaine	10,000
Tramadol	>100,000

The following compounds show no cross-reactivity when tested with **AccuSign<sup>®</sup> OPI** at a concentration of 100  $\mu$ g/mL. (Table 4.)

**Table 4. Non Cross-Reacting Compounds**

4-Acetamidophenol	(-) Ephedrine	Noroxymorphone
Acetophenetidin (Phenacetin)	(-) Ψ Ephedrine	D-Norprop- oxyphene
N-Acetylprocain- amide	Erythromycin	(-) Norpseudo- ephedrine
Acetylsalicylic acid	β-Estradiol	Noscapine
Aminopyrine	Estrone-3-sulfate	Nylidrin
Amitriptyline	Ethyl-p-amino- benzoate	D,L-Octopamine
Amobarbital	Fenoprofen	Oxalic acid
Amoxapine	Furoximide	Oxazepam
Amoxicillin	Gentisic acid	Oxolinic acid
D,L-Amphetamine	Glutethimide	Oxymetazoline
L-Amphetamine	Guaifenesin	Papaverine
Apomorphine	Hippuric acid	Penicillin-G
Aspartame	Hydralazine	Pentazocaine
Atropine	Hydrochlorothiazide	Pentobarbital
Benzilic acid	Hydrocortisone	Perphenazine
Benzoic acid	O-Hydroxyhippuric acid	Phencyclidine
Benzoylcegonine	3-Hydroxytyramine	Phendimetrazine
Benzphetamine	Ibuprofen	Phenelzine
Butabarbital	Imipramine	Phenobarbital
Cannabidiol	Imipramine	Phentermine
Cannabinol	Iproniazid	Phentoin
Chloralhydrate	(-) Isoproterenol	L-Phenylephrine
Chloramphenicol	Isoxsuprine	β-Phenylethyl- amine
Chlordiazepoxide	Ketamine	Phenylpropanol- amine
Chlorothiazide	Ketoprofen	Prednisolone
Chlorpromazine	Labetalol	Prednisone
Chlorquine	Lidocaine	Promazine
Cholesterol	Loperamide	Promethazine
Clomipramine	Loxapine succinate	D,L-Propranolol
Clonidine	Maprotiline	Propiomazine
Cocaine	Meprobamate	D-Propoxyphene
Cortisone	Methadone	D-Pseudo- ephedrine
(-) Cotinine	p-Hydroxymetham- phetamine	Quinidine
Creatinine	Methaqualone	Quinine
Deoxycorticosterone	Methoxyphenamine	Rantidine
Dextromethorphan	(±) 3,4-Methylene- dioxy- amphetamine	Salicylic acid
Diazepam	(±) 3,4-Methylene- dioxymeth- amphetamine	Secobarbital
Diclofenac	Methylphenidate	Serotonin
Diethylpropion	Methyprylon	Sulfamethazine
Diflunisal	Nalidixic acid	Sulindac
Digoxin	Naltrexone	Temazepam
Diphenhydramine	Naproxen	Tetracycline
Domperidone	Niacinamide	Δ <sup>8</sup> -THC
Doxylamine	Nifedipine	Δ <sup>9</sup> -THC
Ecgonine	Norethindrone	11-nor-Δ <sup>9</sup> -THC-9- COOH
Ecgonine methyl- ester		
(+) Ephedrine		
(±) Ephedrine		

Tetrahydro- cortisone	Tolbutamide	D,L-Tryptophan
Tetrahydrozoline	Triamterene	Tyramine
Thiamine	Trifluoperazine	D,L-Tyrosine
Thioridazine	Trimethoprim	Uric acid
D,L-Thyroxine	Trimipramine	Verapamil
	Tryptamine	Zomepirac

## References

- Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse*. National Institute on Drug Abuse (NIDA), Research Monograph 73; 1986.
- Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man*. 2nd Ed., Davis, CA: Biomedical Publ.; 1982; p.488.
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## Symbols Key

	Manufactured by
	CE Mark
	Authorized Representative
	In Vitro Diagnostic Medical Device
	Catalog Number
	Consult Instructions for Use
	Batch Code
	"Use By" date in year-month-day format
	Temperature Limitation
	Contains sufficient for <n> tests
	Do not reuse
	Contents
	Test Device
	Transfer Pipette
	Instructions for Use
	One-step immunochromatographic Assay for the Detection of Drugs of Abuse in Urine
	Opiates Test

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MTPromedi Consulting GmbH  
Altenhofstrasse 80  
66386 St. Ingbert  
Germany  
+49-68 94-58 10 20



Princeton BioMeditech Corporation  
4242 US Hwy 1  
Monmouth Jct., NJ 08852, U.S.A.  
1-732-274-1000 www.pbmc.com