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

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.[1]

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.[1,2,3]

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.

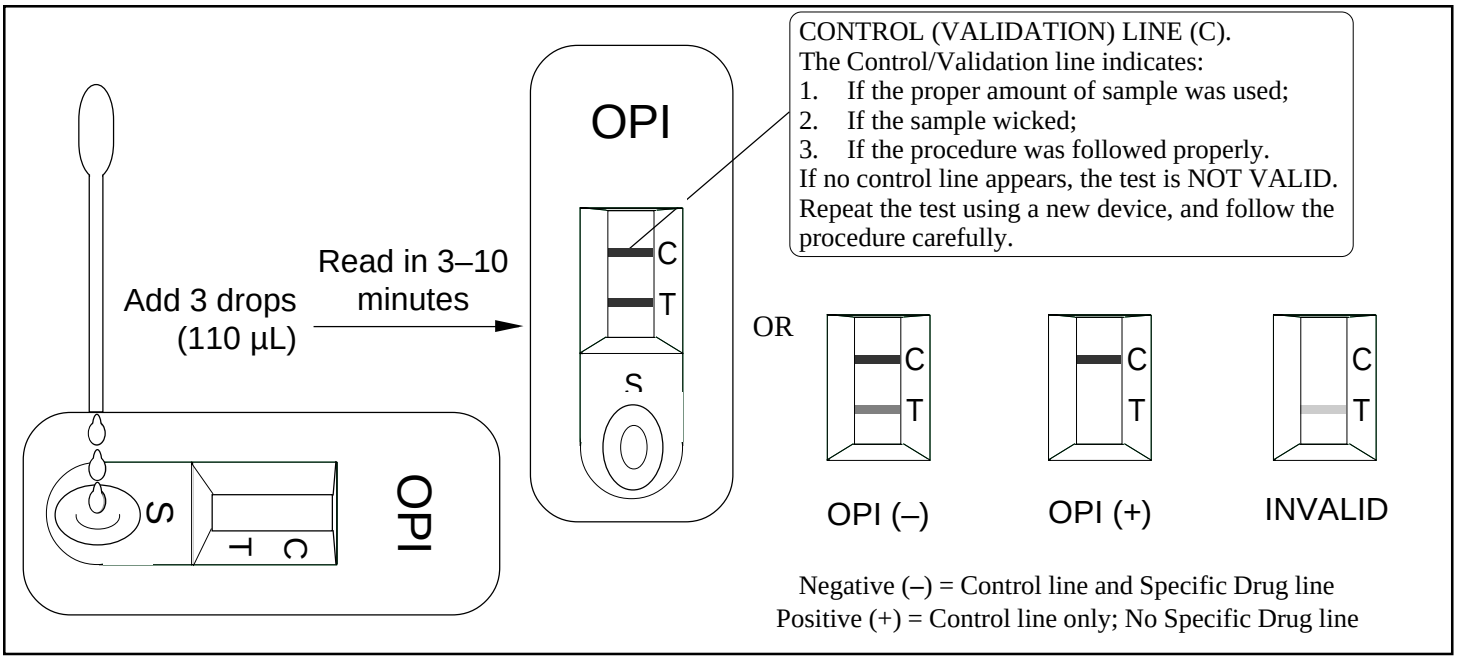
- **AccuSign® OPI** device. The test device contains a membrane strip coated with mouse monoclonal anti-opiate antibody and a pad containing drug-dye conjugate in a protein matrix.
- Disposable specimen dispensers.
- Instructions for use.

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® 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® 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® OPI** test has been shown to detect morphine in urine at an average cutoff of 300 ng/mL.

The accuracy of **AccuSign® OPI** was evaluated in comparison to a commercially available immunoassay (Syva® EMIT® 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® OPI with Syva® EMIT® II

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

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

Table 2. Accuracy: Comparison of AccuSign® OPI with GC/MS Assay

		AccuSign®	GC/MS
OPI	Positive	73	74
	Negative	1	0

Precision and Accuracy

The precision of the **AccuSign® 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 ± 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® 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® OPI** test detects morphine and other opiates in urine.

The following table lists compounds that are detected by the **AccuSign® OPI** test. The specificity of the **AccuSign® OPI** test was determined by adding the drugs and drug metabolites listed to drug-negative urine specimens and testing with the **AccuSign® 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)	% Cross-reactivity
Codeine	300	100
Morphine-6-ß-D-glucuronide	300	100
Hydrocodone	500	60
Hydromorphone	600	50
Levophanol	5,000	6
Meperidine	80,000	0.4
Morphine	300	100
Morphine-3-ß-D-glucuronide	500	60
Nalorphine	1,000	30
Naloxane	100,000	0.3
Norcodeine	60,000	0.5
Oxycodone	20,000	1.5
Oxymorphone	60,000	0.5
Procaine HCl	100,000	0.3
Thebaine	5,000	6

The following compounds show no cross-reactivity when tested with **AccuSign® OPI** at a concentration of 100 µg/mL. (Table 4.)

Table 4. Non Cross-Reacting Compounds

4-Acetamidophenol
Acetophenetidin (Phenacetin)
N-Acetylprocainamide
Acetylsalicylic acid
Aminopyrine
Amitryptyline
Amobarbital
Amoxapine
Amoxicillin
D,L-Amphetamine
L-Amphetamine
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzoylecgonine
Benzphetamine
Butabarbital
Cannabidiol
Cannabinol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Cocaine
Cortisone
(–) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diethylpropion
Diflunisal
Digoxin
Diphenhydramine
Domperidone
Doxylamine
Ecgonine
Ecgonine methylester
(+) Ephedrine
(±) Ephedrine
(–) Ephedrine
(–) Ψ Ephedrine
Erythromycin
ß-Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Glutethimide
Guaifenesin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocortisone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Lidocaine
Loperamide
Loxapine succinate
Maprotiline
Meprobamate
Methadone
p-Hydroxymethamphetamine
Methaqualone
Methoxyphenamine
(±) 3,4-Methylenedioxyamphetamine
(±) 3,4-Methylenedioxymethamphetamine
Methylphenidate
Methyprylon
Nalidixic acid
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norethindrone
Noroxymorphone
D-Norpropoxyphene
(–) Norpseudoephedrine
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxymetazoline
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
Phenobarbital
Phentermine
Phentoin
L-Phenylephrine
ß-Phenylethylamine
Phenylpropanolamine
Prednisolone
Prednisone
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
D-Pseudoephedrine
Quinidine
Quinine
Rantidine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Δ^8-THC
Δ^9-THC
11-nor-Δ^9-THC-9-COOH
Tetrahydrocortisone
Tetrahydrozoline
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Trimipramine
Tryptamine
D,L-Tryptophan
Tyramine
D,L-Tyrosine
Uric acid
Verapamil
Zomepirac

References

1. Hawks RL, Chiang CN, eds. *Urine Testing for Drugs of Abuse.* 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; p.488.
3. Tietz, Norbert W. *Textbook of Clinical Chemistry.* W.B. Saunders Company. 1986, p. 1735.

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
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	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 OPI	Opiates Test

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CE

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