T-58012

AccuSign® EDDP

One-Step EDDP Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of EDDP (Methadone Metabolite) in Human Urine

Catalog No.	DOA-213-35	35 Test Kit
	DOA-213-10	10 Test Kit

Intended Use

AccuSign® EDDP is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of EDDP at a cutoff of 100 ng/mL in human urine.

The* AccuSign® EDDP *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

EDDP (2-Ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine) is the primary metabolite of methadone[2]. Methadone is a synthetic analogic drug which possesses many of the pharmacologic properties of morphine. Unlike morphine, however, methadone produces marked sedative effects with repeated administration as a result of drug accumulation[1,2].

Methadone is mainly used in detoxification programs as an oral substitute for heroin or other morphine like drugs to suppress withdrawal symptoms and/or to maintain chronic relapsing heroin addicts. Urinary methadone screening is a well established method for monitoring the compliance of patients undergoing methadone maintenance or detoxification treatment. Using methadone screening as a marker for compliance to methadone administration does however have several shortcomings:

- Positive results are obtained when non-compliant patients spike specimens with methadone to cover up their non-compliance[3,4].
- It may not be able to detect levels in those patients who are maintained on low-dose regimes of around 5 or 10 mg/day, those who are on a reducing dosage protocol, or those who are rapid metabolizers of methadone[5].

The detection of urine EDDP rather than methadone can overcome these limitations[4,6,7], thereby making compliance testing easier particularly in the case of unsupervised sample collection.

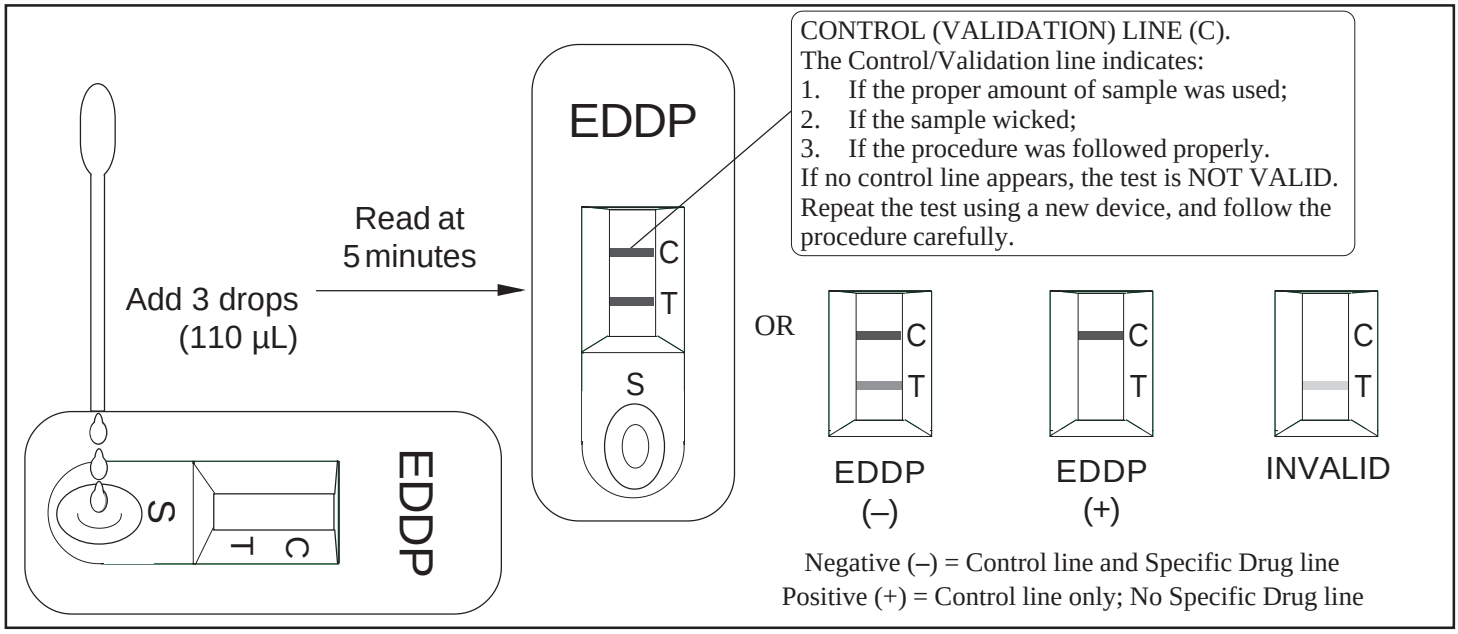
Principle

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

- **AccuSign® EDDP** device. The test device contains a membrane strip coated with monoclonal anti-EDDP antibody and a pad containing dye coated with drug-protein 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® EDDP** 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® EDDP** 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 allowed 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® EDDP** pouch and label the **AccuSign® EDDP** 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 EDDP (i.e., the specimen contains EDDP 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® EDDP** 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.

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 your vendor or 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 your vendor or PBM.

Expected Values

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

Performance Characteristics

The **AccuSign® EDDP** test has been shown to detect EDDP at an average cutoff of 100 ng/mL in urine.

The accuracy of **AccuSign® EDDP** was evaluated in comparison to a commercially available semi-quantitative immunoassay (CEDIA® Methadone Metabolite (EDDP) Assay from Microgenics Corporation). A total of 247 samples were tested by both procedures. Complete agreement was observed in 94.3% of the samples as shown below (Table 1). Values of less than 100 ng/mL with the CEDIA® Methadone Metabolite (EDDP) Assay were reported as negative, whilst values of 100 ng/mL and above were reported as positive.

Table 1. Accuracy: Comparison of AccuSign® EDDP with CEDIA® Methadone Metabolite (EDDP) Assay

		CEDIA® Methadone Metabolite (EDDP) Assay		
		Positive	Negative	TOTAL
AccuSign® EDDP	Positive	104	9	113
	Negative	5	129	134
	TOTAL	109	138	247

Relative Sensitivity	Relative Specificity
95.4% (104/109)	93.5% (129/138)

Specificity

Compounds that are detected by the **AccuSign® EDDP** test are listed below. Drugs and metabolites were spiked to drug negative specimens and tested by **AccuSign® EDDP** for specificity. The results are expressed in terms of the concentration required to produce a positive result (Table 2).

Table 2. Specificity

Compound	Concentration (ng/mL)
EDDP	100
EMDP	>200,000
Methadone	>200,000
alpha-levo-Acetylmethadol	>200,000
alpha-levo-Noracetylmethadol	>200,000

The following compounds show no cross-reactivity when tested with **AccuSign® EDDP** at a concentration of 100 µg/mL (Table 3).

Table 3. Non Cross-Reacting Unrelated Compounds

Acetaminophen	Guaiacol glycerol ether
Acetylsalicylate	Hydrochlorothiazide
Aminopyrine	Imipramine
Amitriptyline	Lidocaine
Amobarbital	Methadone
Amoxapine	p-OH Methamphetamine
Ampicillin	Methaqualone
Apomorphine	Methylenedioxymetham
Ascorbic acid	phetamine
Atropine	Methyprylon
Benzocaine	Naproxen
Butabarbital	Norethindrone
Chlordiazepoxide	Penicillin
Chlorpheniramine	Pentobarbital
Chlorpromazine	Phencyclidine
Chloroquine	Phenolbutazone
Dextropropoxyphene	Phenylpropanolamine
Diazepam	Prednisone
Diphenylhydantoin	Secobarbital
Epinephrine	Tetracycline
Erythromycin	Tetrahydrozoline
Estriol	Trifluoperazine
Gentisic acid	Tryptamine
Glutethimide	Zomepirac

References

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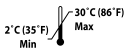
6. George S, Parmar S, Meadway C and Braithwaite RA. Application and Validation of a Urinary Methadone Metabolite (EDDP) Immunoassay to monitor Methadone Compliance. *Ann Clin Biochem.* 2000;37:350-354.
7. Preston KL, Epstein DH, Davoudzadeh D and Huestis MA. Methadone and Metabolite Urine Concentrations in Patients Maintained on Methadone. *J Anal Toxicol.* 2003;27:332-341.

Symbols Key

	Manufactured by
	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 EDDP	EDDP Test

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EC REP

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