AccuSign® MTD

One-Step Methadone Test

For *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Methadone in Urine

Catalog No.	DOA-208-35	35 Test Kit
	DOA-208-10	10 Test Kit

Intended Use

AccuSign® MTD is a simple, one-step immunochromatographic assay for the rapid, qualitative detection of methadone at a cutoff of 300 ng/mL in human urine.

The* AccuSign® MTD *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

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. Overdosage with methadone is characterized by stupor, muscle flaccidity, respiratory depression, cold and clammy skin, pupillary constriction, hypotension, coma and circulatory collapse. Fatalities in adults from methadone overdosage have increased significantly in many urban areas as a result of widespread availability of the drug, both from licit and illicit sources.[1,2]

The major urinary excretion products are methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). In maintenance subjects, 24 hour urinary methadone may account for 5-50% of the dose and EDDP for 3-25%, with large individual variations due to urine pH, urine volume, dose and rate of metabolism. EMDP may account for less than 1% of the dose.[1,2]

Principle

The **AccuSign® MTD** test uses solid-phase chromatographic membrane immunoassay technology for the qualitative detection of methadone. 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.

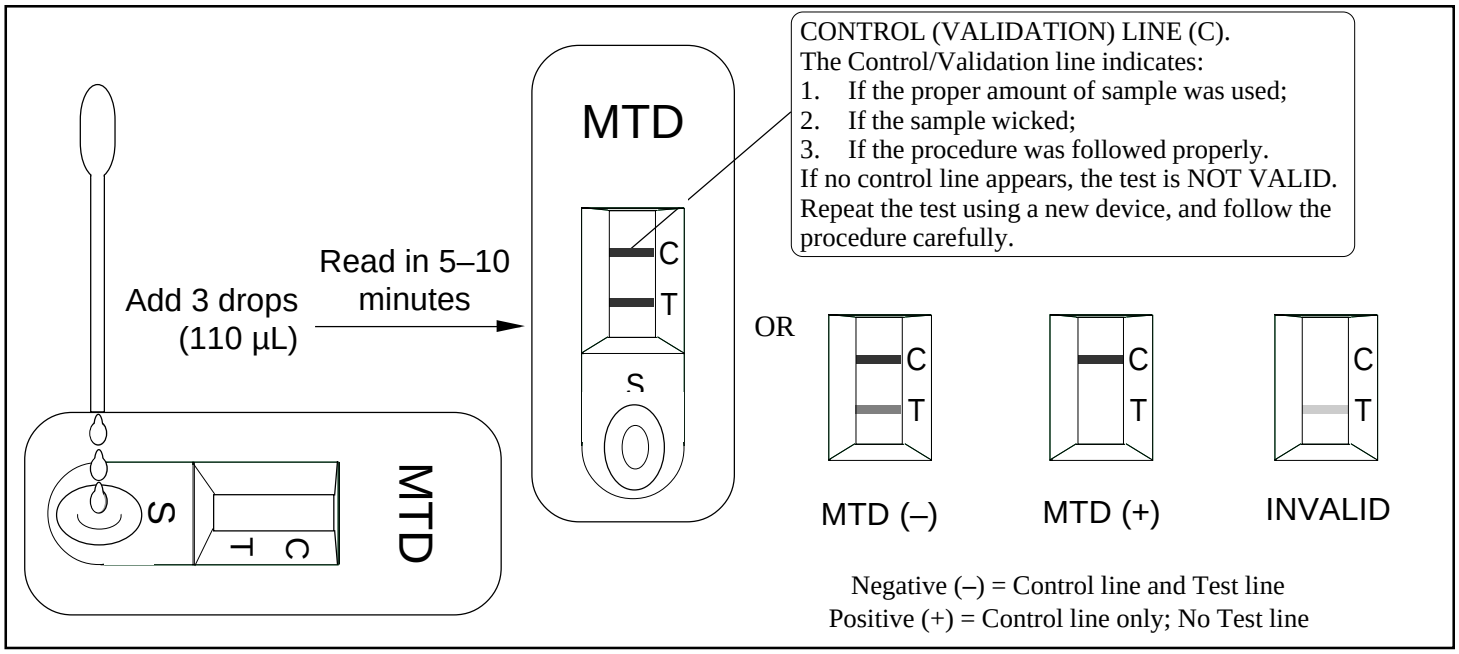
Materials Provided

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

- **AccuSign® MTD** device. The test device contains a membrane strip coated with monoclonal anti-methadone 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® MTD** 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® MTD** 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® MTD** pouch and label the **AccuSign® MTD** device with the patient ID.
2. Holding the dropper vertically, dispense 3 drops (110 µL) of the urine sample into the Sample well (**S**).
3. Read the result after 5 minutes, but within10 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 MTD (i.e., the specimen contains MTD 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® MTD** 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 which are not listed in Table 3 below, 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 conju

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

Performance Characteristics

The **AccuSign® MTD** test has been shown to detect methadone at an average cutoff of 300 ng/mL in urine.

The accuracy of **AccuSign® MTD** was evaluated in comparison to a commercially available immunoassay (Syva® EMIT® II) at a cutoff of 300 ng/mL. A total of 89 samples was tested by both procedures. Complete agreement was observed in 98% of the samples as shown below (Table 1).

Table 1. Accuracy: Comparison of AccuSign® MTD with Syva® EMIT® d.a.u.

		Syva® EMIT® d.a.u. (MTD)		
		Positive	Negative	TOTAL
AccuSign® MTD	Positive	23	0	23
	Negative	1	65	66
TOTAL		24	65	89
		Relative Sensitivity	Relative Specificity	
		95.8% (23/24)	> 99% (65/65)	

The false-negative sample was analyzed by GC/MS and contained the drug at 302 ng/mL. In a separate study, **AccuSign® MTD** was evaluated against 17 specimens confirmed as positive by GC/MS. The range of drug values were 302 to 947 ng/mL for MTD. The results demonstrate the excellent correlation of **AccuSign® MTD** with GC/MS.

Precision and Accuracy

The precision of the **AccuSign® MTD** assay was determined by carrying out the test with serially diluted standard drug solutions using 3 lots of product on 3 different dates. Results for all samples containing methadone concentrations below the cutoff level were negative. About 98% of the spiked samples containing methadone concentrations 25% above the cutoff level (375 ng/mL) showed positive results. All samples containing methadone concentrations 50% above the cutoff level showed positive results.

Distribution of Random Error:

Twenty (20) blind samples prepared by spiking various concentrations of methadone 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® MTD** assay was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (600 ng/mL methadone), and 5 strongly positive samples (1,200 ng/mL methadone). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

Compounds that are detected by the **AccuSign® MTD** test are listed below. Drugs and metabolites were spiked to drug negative specimens and tested by **AccuSign® MTD** 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)**
Diphenhydramine	100,000
Doxylamine	>100,000
EDDP	>100,000
EMDP	>100,000
Imipramine	>100,000
LAAM	2,000
Meperidine	>100,000
Methadone	300
Nor-LAAM	10,000

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

Table 3. Non Cross-Reacting Compounds

Acetaldehyde	Aspartame	Cholesterol
4-Acetamidophenol	Atropine	Clomipramine
Acetone	Benzilic acid	Clonidine
Acetophenetidin (Phenacetin)	Benzocaine	Cocaine
	Benzoic acid	Codeine
N-Acetylprocainamide	Benzoylecgonine	Cortisone
	Benzphetamine	(–) Cotinine
Acetylsalicylic acid	Bilirubin	Creatinine
Albumin	Butabarbital	Deoxycorticosterone
Aminopyrine	Caffeine	
Amitryptyline	Cannabidiol	Dextromethorphan
Amobarbital	Cannabinol	Dextropropoxyphene
Amoxapine	Chloralhydrate	
Amoxicillin	Chloramphenicol	Diazepam
D,L-Amphetamine	Chlordiazepoxide	Diclofenac
L-Amphetamine	Chlorothiazide	Diethylpropion
Ampicillin	Chlorpheniramine	Diflunisal
Apomorphine	Chlorpromazine	Digoxin
Ascorbic acid	Chloroquine	Domperidone

Ecgonine
Ecgonine methylester
(+) Ephedrine
(±) Ephedrine
(–) Ephedrine
(–) Ψ Ephedrine
Epinephrine
Erythromycin
ß-Estradiol
Estriol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Guaiacol glycerol ether
Glucose
Glucuronide
Glutethimide
Guaifenesin
Hemoglobin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketones
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Lysergic acid diethylamide
Maprotiline
Melanin
Meprobamate
Methadone
D-Methamphetamine
p-Hydroxymethamphetamine
Methaqualone
Methoxyphenamine
(±) 3,4-Methylenedioxyamphetamine
(±) 3,4-Methylenedioxymethamphetamine
Methylphenidate
Methyprylon
Morphine
Morphine-3-ß-D-glucuronide
Nalidixic acid
Nalorphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
(–) Norpseudoephedrine
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
ß-Phenethylamine
Phenobarbital
Phenothiazine
Phentermine
Phentoin
L-Phenylephrine
D,L-Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
D-Pseudoephedrine
L-Pseudoephedrine
Quinidine
Quinine
Rantidine
Salicylic acid
Secobarbital
Serotonin
Sodium chloride
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Δ^8-THC
Δ^9-THC
11-nor-Δ^9-THC-9-COOH
Tetrahydrocortisone
Tetrahydrozoline
Thebaine
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.* 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.

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 MTD	Methadone Test

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