AccuSign® MET

One-Step Methamphetamine Test

For *In Vitro* Use Only

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

Catalog No.	DOA-202-35	35 Test Kit
	DOA-202-10	10 Test Kit

Intended Use

The **AccuSign® MET** test is a simple, one-step, immunochromatographic assay for the rapid, qualitative detection of methamphetamine in urine above a cutoff concentration of 1000 ng/mL.

The* AccuSign® MET *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

Methamphetamine is a potent sympathomimetic agent with therapeutic applications. The drug can be taken orally, injected, or inhaled. Acute higher doses lead to enhanced stimulation of the central nervous system and induce euphoria, alertness, reduced appetite, and a sense of increased energy and power.[2] Cardiovascular responses to methamphetamine include increased blood pressure and cardiac arrhythmias. More acute responses include anxiety, paranoia, hallucinations, psychotic behavior, and eventually, depression and exhaustion.

The effects of methamphetamine generally last 2–4 hours, and the drug has a half-life of 9–24 hours in the body. Methamphetamine is excreted in the urine primarily as amphetamine and oxidized and deaminated derivatives.[2] However, 10–20% of methamphetamine is excreted unchanged. Thus, the presence of the parent compound in the urine indicates methamphetamine use. Methamphetamine is generally detectable in the urine for 3–5 days, depending on urine pH level.

Principle

The **AccuSign® MET** test uses solid-phase immunoassay technology for the qualitative detection of methamphetamine in human urine. 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 the drug or its 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 the specific drug position in the Result window, indicating a positive result from positive drug competition. A negative urine sample will generate a line at the specific drug position in the Result window, indicating a negative result from an absence of competition with free drugs.

In addition to the Test line(s) 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.

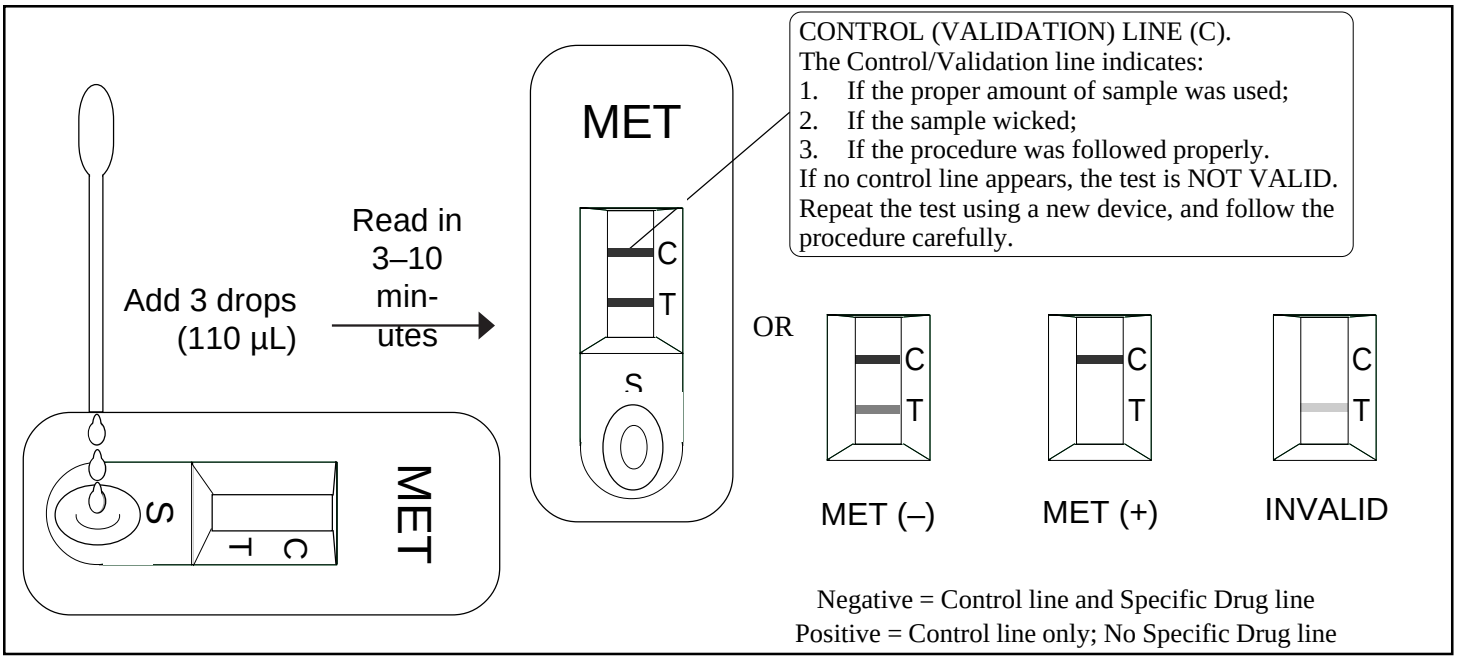
Materials Provided

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

- **AccuSign® MET** device. The test device contains a membrane strip coated with anti-methamphetamine antibody and a dye pad containing colloidal gold coated with drug-protein (from purified bovine protein source) conjugate.
- Disposable specimen dropper.
- 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**® **MET** 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 Test and Control positions.

Test Protocol

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

Interpretation of Results

Negative: The appearance of a reddish-purple Control line (**C**) and a line next to Test position (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 a specific drug 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 a reddish-purple Control line and no distinct line next to T indicates the test result is positive for MET (i.e., the specimen contains the drug 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**® **MET** 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 5 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 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® MET** test device has a built-in control. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should appear in the Control 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 is 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® MET** 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 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 practice. For information on how to obtain controls, contact PBM's Technical Services.

Expected Values

AccuSign® MET is a qualitative assay. The amount of methamphetamine and/or their metabolites present in urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain methamphetamine and/or their metabolites above the cutoff concentration.

Performance Characteristics

The **AccuSign® MET** test has been shown to detect an average cutoff of 1000 ng/mL of D-methamphetamine in urine.

The accuracy of **AccuSign® MET** was evaluated in comparison to a commercially available immunoassay, Syva® EMIT® II. A total of 320 samples was tested with both procedures. Overall agreement of 91.3% was observed, as shown below. (Table 1.)

Table 1. Accuracy: Comparison of AccuSign® MET with Syva® EMIT® II

		Syva® EMIT® II (AMP/MET)		
		Positive	Negative	TOTAL
AccuSign®	Positive	108	0	108
MET	Negative	28	184	212
TOTAL		136	184	320
		Relative Sensitivity	Relative Specificity	
Methamphetamine		79.4% (108/136)	> 99.9% (184/184)	

In a separate study, **AccuSign® MET** was evaluated against specimens confirmed as positive by GC/MS. Of 89 samples confirmed as positive, 88 samples were positive when tested with **AccuSign® MET** (98.9% agreement). (Table 2.)

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

		AccuSign®	GC/MS
MET	Positive	88	89
	Negative	1	0

Precision and Accuracy

The precision of **AccuSign® MET** was determined by carrying out the test with serially diluted standard drug solutions. About 98% of the samples containing methamphetamine concentrations 25% over the cutoff level consistently showed positive results.

The study also included over 40 samples containing ± 25% of the cutoff level as a challenge of cutoff precision. These results were found to be consistently in agreement with expected test results.

Distribution of Random Error:

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

Reproducibility

The reproducibility of the test results of **AccuSign® MET** was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 moderately positive samples (containing the drug at a concentration 1.5-2 times the cutoff level), and 5 strongly positive samples (containing the drug at a concentration 4-5 times the cutoff level). The results obtained at these three sites with these controls demonstrated 100% agreement with each other.

Specificity

The following table lists compounds that are detected by the **AccuSign® MET** test. The specificity of the **AccuSign® MET** test was determined by adding the drugs and drug metabolites listed to drug-negative urine specimens and testing with the **AccuSign® MET** 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)
D-Amphetamine	200,000
D,L-Amphetamine	>200,000
(–)Ephedrine	200,000
(±)Ephedrine	200,000
D-Methamphetamine	1,000
p-OH-Methamphetamine	>200,000
Methylenedioxyamphetamine	>200,000
Methylenedioxymethamphetamine	2,000

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

Table 4. Non Cross-Reacting Compounds

Acetaminophen
Acetophenetidin (Phenacetin)
N-Acetylprocainamide
Acetylsalicylate
Aminopyrine
Amitryptyline
Amobarbital
Amoxapine
Amoxicillin
Apomorphine
Aspartame
Atropine
Benzilic acid
Benzoic acid
Benzoylecgonine
Benzphetamine
Butabarbital
Cannabidiol
Chloralhydrate
Chloramphenicol
Chlordiazepoxide
Chlorothiazide
Chlorpromazine
Chlorquine
Cholesterol
Clomipramine
Clonidine
Cocaine hydrochloride
Codeine
Cortisone
(–) Cotinine
Creatinine
Deoxycorticosterone
Dextromethorphan
Diazepam
Diclofenac
Diethylpropion
Diflunisal
Digoxin
Diphenhydramine
Domperidone
Doxylamine
Ecgonine hydrochloride
Ecgonine methylester
Erythromycin
ß-Estradiol
Estrone-3-sulfate
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Glucuronide
Glutethimide
Guaifenesin
Hippuric acid
Hydralazine
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
O-Hydroxyhippuric acid
3-Hydroxytyramine
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Maprotiline
Meperidine
Meprobamate
Methadone
Methaqualone
Methylphenidate
Methyprylon
Morphine-3-ß-D-glucuronide
Nalidixic acid
Nalorphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenelzine
Phenobarbital
Phentermine
Phentoin
L-Phenylephrine
ß-Phenylethylamine
Phenylpropanolamine
Prednisolone
Prednisone
Procaine
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
Quinidine
Quinine
Rantidine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Δ^9-Tetrahydrocannabinol-carboxylic acid
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 for 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.

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 MET	Methamphetamine Test

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