Methamphetamine ELISA Kit

Catalog Number KA0934

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

The Methamphetamine ELISA Kit provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GS-MS) is the preferred confirmatory method. Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Background

The Methamphetamine ELISA Kit is a specific and sensitive in-vitro test to detect the presence of d-methamphetamine in samples such as whole blood, oral fluids, serum, plasma and urine. The assay will detect amphetamine use, interference by l-methamphetamine and pseudo-ephedrine is virtually nonexistent. Methamphetamine is a potent central nervous system stimulant with less peripheral actions than mphetamine. The (+)-isomer also referred to as d-methamphetamine is ten times more potent than the (-)-isomer, l-methamphetamine. Amphetamines act by inducing euphoria, irritability, anxiety and paranoia Methamphetamine is metabolized to its active metabolite amphetamine (via N-demethylation) and is further metabolized by hydroxylation and deamination of amphetamine. Urinary excretion rates are influenced by the urinary pH with acidic urine favoring the excretion of unchanged drug. Alkaline urine reduces the excretion of unchanged methamphetamine to less than 5% of the dose.

Principle of the Assay

The Methamphetamine ELISA Kit (for d-methamphetamine measurement) is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. A 10 µl aliquot of a diluted unknown specimen is incubated with a 100 µl dilution of enzyme (Horseradish peroxidase) labeled d-methamphetamine derivative in microplate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 1 ng/ml. The Methamphetamine ELISA Kit avoids extraction of urine sample for measurement. It employs a d-methamphetamine directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size, reducing matrix effects and interference with binding proteins(s) or other macromolecules.

General Information

Materials Supplied

List of component

Component	Amount
Microwells coated with polyclonal anti-d-methamphetamine	12x8x1
d-Meth-Conjugate	12 ml
Immunalysis Positive Ref. Std	2 ml
Neg Std	1 ml
TMB Substrate	12 ml
Stop Solution	11 ml

Storage Instruction

- ✓ Urine samples should be stored at 2-4 degrees centigrade until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided.
- ✓ The expiration date of the kit stated on the label.
- ✓ The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2-4℃.

Materials Required but Not Supplied

- ✓ 12x75 mm Disposable Glass or Plastic Culture Tubes to pre-dillute (if required).
- ✓ Manual or electronic micropipettes (single channel or multi channel) or automated pipetting stations.
- ✓ Refrigerator (for kit storage).
- ✓ Interval Timer.
- ✓ Wash bottle or Plate Washer.
- ✓ Micro-plate reader capable of reading at 450 nm and 650nm.

Precautions for Use

- ✓ Precautions

1. Potential biohazardous materials:

   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in

Microbiological and Biomedical Laboratories." 1984.

2. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Control sera and sample diluents contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Assay Protocol

Sample Preparation

1. The Methamphetamine ELISA Kit is to be used with human samples, such as whole blood, oral fluids, serum, urine and plasma. Has not tested all possible applications of this assay. Cutoff criteria are important in deciding the sample dilution.
2. Specimens to which sodium azide has been added affect the assay.

Assay Procedure

All reagents must be room temperature (18-26℃) before use.
The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipettor.

1. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (Urine samples are normally diluted 1:20 for a methamphetamine cutoff of 500 ng/ml.) The dilution factor and volume added can be adjusted based on the laboratory's cutoff.
2. Add 10 µl of appropriately diluted calibrators and standards to each well in duplicate.
3. Add 10 µl of the diluted specimens in duplicate (recommended) to each well.
4. Add 100 µl of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
5. Incubate for 60 minutes at room temperature (18-26℃) preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash the wells 6 times with 350 µl distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amounts of hemoglobin (some Postmortem samples), use 10 mM Phosphate buffered saline pH 7.0-7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.
7. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate, does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.
8. Add 100 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
9. Incubate for 30 minutes at room temperature, preferably in the dark.
10. Add 100 µl of Stop Solution to each well, to change the blue color to yellow.
11. Measure the absorbance at a dual wavelength of 450 nm and 650 nm.
12. Wells should be read within 1 hour of yellow color development.
13. The following data represent a typical dose/response curve.

Data Analysis

Calculation of Results

The dose/response curve shown below should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or a cutoff calibrator should be run with every plate.

The following data represent a typical dose/response curve.

d-methamphetamine (ng/ml)	Absorbance
0	1.519
10	0.649
25	0.471
50	0.359

If the average sample absorbance is equal to or less than the average absorbance of the laboratory positive reference standard the sample is POSITIVE for methamphetamine. If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called NEGATIVE for methamphetamine.
Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve.

Performance Characteristics

✓ Accuracy

Sixty whole blood samples and 40 urine samples collected from presumed non-users were tested in the Methamphetamine ELISA Kit. One hundred percent of these normal samples measured negative at 50 ng/ml for whole blood and 500 ng/ml for urine. Fifty five whole blood samples which were previously confirmed positive for methamphetamine by GC-MS employing a cut-off of 50 ng/ml, were tested in the ethamphetamine ELISA kit. All of the samples were found to be positive i.e. above the cut-off 50 ng/ml.

✓ Precision

The precision of the Methamphetamine ELISA Kit has been verified by assessment of mean, standard deviation (SD) and coefficients of variation (CV) in data resuling from repetitive assays.

✓ Intra-assay Precision

Intra-assay precision was determined with reference controls.
A 0, 10, 25, and 50 ng/ml standard was assayed five times in the same assay.

Methamphetamine (ng/ml)	Mean Abs	S.D.	C.V.%
0	1.652	0.089	5.4
10	0.697	0.055	7.9
25	0.504	0.0614	12.2
50	0.366	0.0391	10.7

✓ Sensitivity

Assay sensitivity based on the minimum methamphetamine concentration required to produce a four standard deviation from assay Ao is 1 ng/ml.

✓ Specificty

The specificity of the ELISA Methamphetamine was determined by generating inhibition curves for each of the compounds listed below The antisera cross-reactivities are listed in Table.

Compound	Approx. ng/ equivalent to 50ng/ml d-methamphetamine	Cross-reactivities
d,l-methamphetamine	77	65
l-methamphetamine	625	8
d, l-MDMA	37	135
d-amphetamine	2500	2
l-amphetamine	1500	3.4
p-hydroxyamphetamine	>5000	<1.0
d, l-MDA	>5000	<1.0
d, l-MDEA	500	10
d, l-MBDB	1000	5
d, l-HMA	>5000	<1.0
fenfluramine	1000	5
d-ephedrine	4300	1.2
l- ephedrine	>5000	<1.0
d, l- ephedrine	>5000	<1.0
beta-phenethylamine	>5000	<1.0
phentermine	>5000	<1.0
d-phenylpropanolamine	>5000	<1.0
d-phenylpropolamine	>5000	<1.0
d-pseudoephedrine	>5000	<1.0

✓ Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 10,000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (1 ng/ml). Acetaminophen, Acetylsalicylic acid, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid Atropine, Barbital, Benzoylecgonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chloropromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydro carbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Metharbital, Mephenytion, “-Methyl-“-propylsuccinimide, Mephobarbital, Methal PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOH.

Resource

Reference

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph, 73, 1986.
2. R.C. Baselt. In: Advances in Analytical Technology, Vol.1. Randall C. Baselt edd. (Biomedical Publications, Foster City, CA. 87-93).
3. Driscoll, R.C., Barr, F.S., Gragg, B.J. and G.W. Moore. Determination of Therapeutic Blood Levels of Methamphetamine by GC. J.Pharm. Sci 60:1492.1971

Plate Layout

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