



Amphetamine ELISA Kit

Catalog Number KA0926

96 assays

Version: 02

Intended for research use only

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Table of Contents

Introduction	3
Intended Use	3
Background	3
Principle of the Assay	3
General Information	4
Materials Supplied	4
Storage Instruction	4
Materials Required but Not Supplied	4
Precautions for Use	4
Assay Protocol	6
Sample Preparation	6
Assay Procedure	6
Data Analysis	7
Calculation of Results	7
Performance Characteristics	7
Resources	10
Reference	10
Plate Layout	11

Introduction

Intended Use

The Amphetamine 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 (1). Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Background

The Amphetamine ELISA Kit is a specific and sensitive in-vitro test to detect the presence of d-amphetamine in samples such as whole blood, oral fluids, serum, plasma and urine. While the assay will detect amphetamine use, interference by l-amphetamine and pseudo-ephedrine is virtually nonexistent. Amphetamine is a potent central nervous system stimulant. The (+)-isomer also referred to as d-amphetamine is three to four times more potent than the (-)-isomer, l-amphetamine (2). Amphetamine may be metabolized and excreted as the p-hydroxy isomer. Amphetamines act by inducing euphoria, irritability, anxiety and paranoia. Urinary excretion rates are influenced by the urinary pH with acidic urine favoring the excretion of unchanged drug (2). Up to 80% of a given dose may be excreted unchanged, especially in acid urine. Alkaline urine reduces the excretion of unchanged amphetamine to less than 5% of the dose.

Principle of the Assay

The Amphetamine Direct ELISA Kit (for d-amphetamine 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-amphetamine derivative in micro-plate 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 Amphetamine ELISA Kit avoids extraction of urine sample for measurement. It employs a d-amphetamine 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-amphetamine	96 (8x12) wells
d-Amph-Conjugate	12 ml
Positive Reference Standard	2 ml
Negative Standard	1 ml
TMB Substrate	12 ml
Stop Reagent	11 ml

Storage Instruction

- ✓ The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactory until the expiration date is stored in the refrigerator at 2-4 °C.
- ✓ Store the kit at 2-8 °C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose test reagents to heat, sun, or strong light.

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

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

- ✓ This test kit is designed for Research Use Only.
- ✓ Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- ✓ The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- ✓ It is recommended that serum samples be run in duplicate.
- ✓ 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. Any deviation from this may yield invalid data.

Assay Protocol

Sample Preparation

1. The Amphetamine Direct 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.
3. Urine samples should be stored at 2-4 °C until use. Samples should be well mixed before assay.
4. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

Assay Procedure

All reagents must be brought to room temperature (18-26 °C) 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 Buffered Saline pH 7.0. (Urine samples 1:20 for a cutoff level of 500 ng/ml.) The dilution factor can be adjusted based on the laboratories 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 preferably in the dark (18-26 °C), after addition of enzyme conjugate to the last well.
6. Wash well 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 amount 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 hours of yellow color development.

Data Analysis

Calculation of Results

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 amphetamine. If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called NEGATIVE for amphetamine.

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.

✓ Example of a standard curve

The following data represent a typical dose/response curve.

d-amphetamine (ng/ml)	Absorbance
0	2.459
10	0.891
25	0.431
50	0.255

The dose/response curve shown above 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.

Performance Characteristics

✓ Accuracy

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

✓ Precision

The precision of the Amphetamine ELISA Kit has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting 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. The results are tabulated in Table 1.

Amphetamine (ng/ml)	Mean Abs.	S.D.	C.V.%
0	2.399	0.115	4.8
10	0.897	0.095	10.6
25	0.458	0.061	13.32
50	0.271	0.022	8.12

✓ Sensitivity

Assay sensitivity based on the minimum amphetamine concentration required to produce a four standard deviation from assay A₀ is 1 ng/ml.

✓ Specificity

The specificity of the ELISA for Amphetamine was determined by generating inhibition curves for each of the compounds listed below the antisera cross-reactivities are listed in Table 2.

Compound	Approx. ng/ml equivalent to 25 ng amphetamine	Cross-reactivities percentage
L- Amphetamine	855	2.9
Hydroxyamphetamine HCl	57	44
L-Methamphetamine HCl	1250	2
dl-MDA	10	250
(methylenedioxyamphetamine) d-Methamphetamine HCl	417	6.5
dl-HMA	100	25
(hydroxyl methoxyamphetamine) Phenteramine	28	89
Fenfluramine	>2500	<1
d-Ephedrine	>2500	<1
L-Ephedrine	>2500	<1
d-Phenylpropanolamine	>2500	<1
L-Phenylpropanolamine	>2500	<1
dl-MDMA	>2500	<1
(methylene dioxymethamphetamine) dl-MDEA	>2500	<1
(methylene dioxyethylamphetamine) d-pseudoephedrine	>2500	<1
L-Pseudoephedrine	>2500	<1
dl-MBDB	>2500	<1
(N-methyl-3,4 methylene dioxyphenyl-2-butanamine) Tyramine	>2500	<1
Methylphenidate	>2500	<1

✓ Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 5,000 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, Barbitol, Benzoyllecgonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Metharbital, Mephénytoin, “-Methyl-“-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH.

Resources

Reference

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3. J. Caldwell and P.S. Sever. The Biochemical Pharmacology of Abused Drugs. Clinical Pharmacology and Therapeutics. 16: 625- 638 (1974).
4. R. C. Baselt. In: Advances in Analytical Toxicology, Vol.1. p.87 - 93. Ed. R. C. Baselt, Biomedical Publications, Foster City, CA (1984).

Plate Layout

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