



METHAMPHETAMINE DIRECT RIA KIT [I-125]

Immunalysis Corporation :

Catalog Number 111-0100 100 tubes

Catalog Number 111-0500 500 tubes

INTRODUCTION

A radioimmunoassay (RIA) for d-methamphetamine in urine is described. A 25 ul aliquot of sample is incubated with a 100 ul dilution of rabbit anti-d-methamphetamine antibody and 200 ul of I-125-methamphetamine reagent. Separation of the bound I-125-meth-amphetamine is by a second antibody complex. The technique is sensitive to 10 ng/ml without dilution or other manipulation. The assay features a 1000 ng/ml cut-off.

INTENDED USE

The Immunalysis Methamphetamine Direct RIA Kit [I-125] is intended for detection and semi-quantitation of d-methamphetamine in urine at 1000 ng/ml or higher.

The Immunalysis Methamphetamine Direct RIA Kit [I-125] 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). Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

EXPLANATION OF THE TEST

The Immunalysis Methamphetamine Direct RIA Kit is a specific and sensitive in-vitro test to detect the presence of d-methamphetamine in urine. While the assay will detect amphetamine use, interference by l-methamphetamine and pseudo-ephedrine is virtually nonexistent.

Methamphetamine is a potent central nervous system stimulant(1) with less peripheral actions than amphetamine. 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(2). Alkaline urine reduces the excretion of unchanged methamphetamine to less than 5% of the dose.

The Immunalysis Methamphetamine Direct RIA Kit [I-125] provides a positive reference standard containing 1000ng /ml of d-methamphetamine, a normal reference control, I-125 methamphetamine radioligand, d-methamphetamine antibody and a second antibody complex to precipitate antibody-bound amphetamine.

PRINCIPLES OF THE PROCEDURE

The Immunalysis Methamphetamine Direct RIA Kit [I-125] (for d-methamphetamine measurement) is based upon the competitive

binding to antibody of I-125 radiolabeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. An unknown specimen is mixed in a test tube with fixed amounts of d-methamphetamine antibody and radiolabeled antigen. Antigen present in a patient sample competes with labeled antigen for the limited antibody present. After precipitation of the antigen-antibody complex with a second antibody reagent and centrifugation, the tubes are aspirated or decanted and the pellets containing bound antigen are counted in a gamma scintillation counter. Sample CPM values equal to or less than the CPM value of the reference standard are indicative of the presence of Methamphetamine in the urine specimen. A reference standard containing 1000 ng/ml d-methamphetamine is supplied for use as a cut-off .

The Immunalysis Methamphetamine Direct RIA Kit [I-125] requires only one 60 minute incubation, and avoids extraction of urine sample for measurement. It employs a d-methamphetamine directed antiserum. There is little or no interference with binding proteins(s) or other macromolecules.

Materials and Methods

Materials and equipment required but not supplied with the Immunalysis Methamphetamine Direct RIA Kit [I-125] are itemized below:

Materials

- 12x75 mm Disposable Glass or Plastic Culture Tubes.
- Test Tube Racks.
- Manual micropipets or automated pipetting stations.

Equipment

- Refrigerator (for kit storage).
- Vortex Mixer.
- Interval Timer.
- Centrifuge.
- Gamma Counter calibrated for ¹²⁵I.
- Calculator.

REAGENTS

Immunalysis Methamphetamine Direct RIA Kit I-125 Contents. Each Immunalysis Methamphetamine Direct RIA Kit [I-125] contains:

Contents	100 Test Kit
1 bottle d-methamphetamine Antibody	11 ml
1 bottle I-125-methamphetamine (not more than 10 uCi)	21 ml
1 bottle containing 1000 ng/ml d-methamphetamine in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine)	4 ml
1 bottle Second Antibody-PEG Complex	21 ml

d-Methamphetamine Antibody. The antibody solution is rabbit anti-d-methamphetamine serum diluted in 0.1 M phosphate

buffer, pH 7.0, with BSA 0.1%, sodium chloride 0.9%, and sodium azide 0.1% (colored blue).

I-125-methamphetamine. The tracer solution contains not more than 10 uCi of I-125-methamphetamine in 0.1 M phosphate buffer, pH 7.0, with BSA (0.1%), sodium chloride (0.9%) and sodium azide (0.1%) (colored green).

Positive Reference Standard. This contains 1000 ng /ml of d-methamphetamine dissolved in a synthetic urine.

Normal Control. This bottle contains drug free synthetic urine.

Second Antibody-PEG Complex. The separation reagent contains a second antibody-PEG complex in 0.1 M phosphate buffered saline (colored pink).

Precautions

For In Vitro Diagnostic Use.

Not for Internal or External Use in Humans or Animals.

Radioactive Warning. This radioactive material may be received, acquired, possessed and used by physicians, clinical laboratories and hospitals or forensic and crime laboratories possessing a specific license and only for in-vitro clinical or laboratory tests not involving internal or external administration of the material or the radiation therefrom to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of the State with which the Commission has entered into an agreement for the exercise of regulatory authority.

This kit contains radioactive material which should be handled according to the following guidelines:

- (1) All radioactive materials should be stored and used in specially designated areas.
- (2) No pipetting should be done by mouth.
- (3) There should be no smoking or eating within the work area.
- (4) Hands should be washed after using radioactive materials.
- (5) Work should be carried out on a surface covered with absorbant materials.
- (6) Any spills of radioactive material should be cleaned immediately and all contaminated materials disposed of as radioactive waste. Contaminated surfaces should be washed with a detergent.

Sodium Azide Hazard. Sodium azide can violently react with the copper, lead, brass or solder in plumbing systems. Since sodium azide has been added in a concentration of 0.1% to the buffer, standards and antibody, these reagents should be disposed into the drain system together with copious amounts of water. Copper-free and lead-free plumbing is recommended.

General. Precise pipetting is the essence of successful radio immunoassay. Micropipets supplied by "Eppendorf" or "SMI" with disposable tips are excellent when used carefully according to instructions to insure the necessary accuracy. New automatic dispensers improve reliable delivery.

Storage. The expiration date of the kit is stated on the label. The kit can be expected to perform satisfactorily until the

expiration date if stored in the refrigerator at 2 - 4 degrees centigrade.

Treatment Required. There is no sample treatment or reagent purification required for the use of the Immunalysis Methamphetamine Direct RIA Kit [I-125].

Indications of Deterioration. A drop of greater than 25% in the zero-dose (maximum bound %) for a constant incubation time indicates deterioration of the antibody or tracer. A significant shift of the standard curve to the right would result from deterioration of the standards. Non-specific binding above 15% indicates deterioration of the tracer.

SPECIMEN COLLECTION

Precautions.

The Immunalysis Methamphetamine Direct RIA Kit [I-125] is to be used with human urine samples.

Additives.

Urine specimens to which sodium fluoride has been added or preservatives necessary to prevent bacterial growth do not affect the assay.

Interfering Substances.

There are no commonly abused drugs which alter the values obtained with the Immunalysis Methamphetamine Direct RIA Kit [I-125]. Samples containing radioactive contamination from previous in vivo diagnostic procedures should be rejected.

Storage and Handling Instructions.

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. Urine samples should be shipped refrigerated with Blue Ice or equivalent.

DETAILS OF THE PROCEDURE.

All reagents must be brought to room temperature before use.

The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added simultaneously using an automated pipettor. After simultaneous addition of all reagents proceed directly to step 7 below.

1. Set up and label as many duplicate tubes as are required for the Positive Reference Standards, the Normal Control and the urine specimens to be assayed.
2. Add 25 ul of Positive Reference Standard and Normal Control to the appropriate tubes.
3. Add 25 ul of each urine specimen to the appropriate tubes.
4. Add 200 ul of I-125 methamphetamine to each tube.
5. Add 100 ul of blue Anti-d-methamphetamine Serum Reagent to each tube; mix well on a vortex-type mixer.
6. Add 200 ul of Second Antibody Reagent (**shake well before use**) to each tube.
7. Gently vortex mix all tubes and incubate for 60 minutes or any interval upto 3 hours at room temperature (25°C). Standards, samples and controls must be incubated together for the same time period. The assay rack may be covered with parafilm.
8. Centrifuge the tubes for 10 minutes, at approximately 1200-

2500 x g in a swinging bucket rotor, or at least 3500-4000g in a fixed angle head rotor. Centrifugation time may be extended, if necessary, to optimize formation of suitable pellets.

9. Decant supernatant, drain (optional) and blot each tube.
10. Count each tube in a gamma scintillation counter to obtain counts per minute (CPM).
11. Compare average counts per minute obtained from each unknown specimen with the average CPM obtained from the 1000 ng/ml Positive Reference Standard.

Should you desire to determine NSB, use 100ul of Normal control in place of antibody. It is recommended that at least one in-house positive quality control sample be included with every assay run.

Sodium Periodate (50 ul) should be added to all tubes if interference from ephedrine or pseudoephedrine is suspected.

RESULTS

If the average sample CPM is equal to or less than the average CPM of the Positive Reference Standard the sample is **POSITIVE** for methamphetamine (has a methamphetamine concentration equal to or greater than 1000 ng/ml). If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called **NEGATIVE** for methamphetamine (less than 1000 ng/ml Methamphetamine).

Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding CPM (ordinate). Values for unknown samples are obtained by interpolation from the curve.

THE FOLLOWING DATA REPRESENT A TYPICAL DOSE/RESPONSE CURVE. NOTE THAT THE ENTIRE CLINICAL RANGE IS PERFECTLY LINEAR WITHOUT MANIPULATION.

d-Methamphetamine ng/ml	Mean CPM
0	137243
250	90530
500	67190
1000	43576

The dose/response curve shown above should not be used in assay calculations. One generated at time of assay is done easily and is suitable for calculation of drug concentration in sample. The dose/response plot is sharp and linear from the low point through the 500 ng/ml cut-off.

Interpretation.

d-Methamphetamine and its metabolites appear in urine within hours after drug use and may persist for days. Thus a positive result documents methamphetamine use. GC/MS is recommended for confirmation.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Fifty urine samples collected from presumed non-users were

tested in the Immunalysis Methamphetamine Direct RIA Kit [I-125]. One hundred percent of these normal urines measured negative at 500 ng/ml. Fifty-two samples which were previously confirmed positive for amphetamine by GC-MS employing a cut-off of 1000 ng/ml, were tested in the Immunalysis Methamphetamine Direct RIA Kit [I-125]. All of the samples were found to be positive i.e. above the cut-off of 1000 ng/ml.

Precision

The precision of the Immunalysis Methamphetamine Direct RIA Kit [I-125] 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 250, 500 and 1000 ng/ml standard was assayed five times in the same assay. The results are tabulated in Table 1.

Table 1

Methamphetamine (ng/ml)	Mean CPM	S.D.	C.V.%
250	90530	2201	2.43
500	67190	2512	3.74
1000	43576	2048	4.70

Intra-assay CVs for Reference Controls ranged from 2.43 to 4.70%

Inter-assay Precision

Inter-assay precision was performed on reference controls. A 250, 500 and 1000 ng/ml reference control was assayed in ten separate runs over a 24 hour period and a SD and CV determined. Results are tabulated in Table 2.

Table 2

Inter-assay Precision (Reference controls)

ethamphetamine (ng/ml)	No of determinations	Mean CPM	S.D.	C.V.%
250	10	91976	1853	2.02
500	10	67212	1760	2.62
1000	10	42143	1941	4.61

Reference Control interassay CVs ranged from 2.02 to 4.61%. The average CV was 3.08%.

Sensitivity

Assay sensitivity based on the minimum methamphetamine concentration required to produce a four standard deviation from assay Bo is 10 ng/ml. A conservative 1000 ng/ml cut off is recommended.

Specificity

The specificity of the Immunalysis Radioimmunoassay for Methamphetamine was determined by generating inhibition curves

for each of the compounds listed below and then determining by extrapolation the percentage cross-reactivity at assay cut-off (approximately 50 percent B/Bo). The antisera cross-reactivities are listed in Table 3.

Methamphetamine Antiserum	Cross-reactivities
Compound reactivities	Approx. ng/ml equivalent to 1000 ng/ml d-methamphetamine
d,l-methamphetamine	1333
MDMA	657
d-amphetamine	>100000
l-amphetamine	>100000
p-hydroxyamphetamine	>100000
MDA	>100000
d-ephedrine	>100000
l-ephedrine	>100000
d,l-ephedrine	>100000
beta - phenethylamine	>100000
phentermine	>100000
d-phenylpropanolamine	>100000
d,l-phenylpropolamine	>100000
d-pseudoephedrine	>100000
l-pseudoephedrine	>100000
tyramine	>100000

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 (25 ng/ml).

Acetaminophen, Acetylsalicylic acid, Aminopyrine, Ampicillin Amobarbital, Ascorbic acid, Atropine, Barbitol, Benzoylcgonine, 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, Mephentoin, "-Methyl"-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH.

Recovery

Normal urines were spiked with d-methamphetamine to give a final theoretical concentration of 250, 500 and 1000 ng/ml. Each of these controls were assayed in replicates of 10 within a test run, and the subsequent experimental concentration and recovery calculated. The results are tabulated in Table 4.

Spiked urine concentration -methamphetamine	Observed d-methamphetamine concentration (ng/ml)	% Recovery
250	256.1	102.4
500	488.6	97.7
1000	1012.1	101.2

Interfering Substances

There are no known interfering substances which alter the values obtained with the Immunalysis Methamphetamine Direct RIA Kit (I-125).

Expected Values

The urine level of methamphetamine in humans who have not used methamphetamine within the last two weeks is usually negative, i.e. below 1000 ng/ml by the Immunalysis Methamphetamine Direct RIA Kit [I-125]. User levels range from 500 to 1000+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine methamphetamine level depends on the individual metabolism, the amount and purity of the methamphetamine used and the time elapsed since last use(3).

Interpretation

Methamphetamine appears in the urine within hours after drug use and may persist for days. Thus this Immunalysis highly sensitive, single step, single incubation assay, characterized by a sharp linear plot through the 1000 ng/ml cut-off from the low point throughout the high concentration points, reliably documents recent or distant Methamphetamine use.

Limitations of the Procedure

Samples containing radioactive contamination from previous in-vivo diagnostic procedures should be rejected. It is possible that other substances and/or factors may interfere with the test and cause false results e.g. technical or procedural errors.

Clinical consideration and professional judgement should be applied to any drug abuse test, particularly when preliminary positive results are used.

REFERENCES

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.

This package conforms to the conditions and limitations specified

in 49CFR 173.421 for radioactive material , excepted package-limited quantity of material UN 2910.

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