



COCAINE / COCAINE METABOLITE DIRECT RIA KIT [¹²⁵I]

concentration in the reaction mixture.

Immunalysis Corporation
Catalog Number 112-0100
Catalog Number 112-0500

100 tubes
500 tubes

INTRODUCTION

A radioimmunoassay (RIA) for benzylecgonine and cocaine in urine is described. A 25 µl aliquot of sample is incubated with a 100 µl dilution of sheep anti-cocaine (serum) antibody (polyclonal) and 200 µl of ¹²⁵I -benzoylecgonine reagent. Separation of the bound ¹²⁵I -benzoylecgonine is by a second antibody complex. The technique is sensitive to 2 ng/ml without dilution or other manipulation. The assay features a 300 ng/ml cut-off.

INTENDED USE

The Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] is intended for detection and semi-quantitation of benzoylecgonine and cocaine at 300 ng/ml or higher.

The Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] 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 (GC-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

Cocaine abuse is widespread and its prevalence may be increasing in all social and age strata (1). The drug is generally inhaled or smoked (1,2). Several methods for measurement of cocaine metabolites in urine exist (3-6). Benzoylecgonine, a major metabolite appears within minutes in urine (3). Since the number and proportion of metabolites vary in subjects, results are expressed in benzoylecgonine equivalents per ml. The Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] is a single incubation assay providing results similar to those obtained by existing methods (4-6). Native (unaltered) cocaine urine concentration is far lower than that of its major metabolite benzoylecgonine. After intra- venous administration of 100mg cocaine urine concentrations ranged from 1.2 - 2.4 ug/ml compared with concentrations ranging from 5 - 55 ug/ml for benzoylecgonine (3). Cocaine was undetectable (at a 50 ng/ml cut-off) 12 hours after administration in comparison with benzoylecgonine which persists hours to days after administration (7). It has been suggested that a benzoylecgonine/cocaine ratio of less than 100 is indicative of use within the past 10 hours (7).

PRINCIPLES OF THE PROCEDURE

The Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] is based upon the competitive binding to antibody of ¹²⁵I radiolabeled antigen and unlabeled antigen, in proportion to their

An unknown specimen is mixed in a test tube with fixed amounts of sheep anti-cocaine antibody (polyclonal) 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 cut-off reference standard (300 ng/ml benzoylecgonine) are indicative of the presence of benzoylecgonine and cocaine in the urine specimen.

The Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] requires only one 60 minute incubation and avoids extraction of urine sample for measurement. It employs a benzoylecgonine 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 Urine Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] are itemized below:

Materials

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

Equipment

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

REAGENTS

Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I]
Contents. Each Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] contains:

Contents	100 tube kit
1 bottle cocaine Antibody	11 ml
1 bottle ¹²⁵ I -Benzoylecgonine	
Reagent	21 ml
1 bottle containing 300 ng/ml BE in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine)	4 ml
1 Bottle Second Antibody-PEG Complex	21 ml

Cocaine Antibody. The antibody solution is anti- cocaine 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-Benzoylecgonine Reagent. The tracer solution contains I-125-benzoylecgonine 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 300 ng of benzoylecgonine dissolved in a synthetic urine matrix.

Normal Control. This bottle contains a drug free synthetic urine

matrix.

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 absorbent 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 radioimmunoassay. Micropipets supplied by "Eppendorf" or "SMI" with disposable tips are excellent when used carefully according to instructions to ensure 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 - 8 °C.

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

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 Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] 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 Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I]. Samples containing radioactive contamination from previous in-vivo diagnostic procedures should be rejected.

Storage and Handling Instructions.

Urine samples should be stored at 2 - 8 °C 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 automated pipettors.

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 µl of Positive Reference Standard and Normal Control to the appropriate tubes.
3. Add 25 µl of each urine specimen to the appropriate tubes.
4. Add 200 µl of I-125 Benzoylcegonine Reagent to each tube.
5. Add 100 µl of blue Anti-Cocaine Serum Reagent to each tube.
6. Add 200 µl 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 up to 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 15 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 300 ng/ml Positive Reference Standard.

It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve can be established by preparing dilutions of the positive reference control with the negative control. Should you desire to determine NSB, use 100µl of Normal control in place of antibody.

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 benzoylecgonine (has a benzoylecgonine concentration equal to or greater than 300 ng/ml).

If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for benzoylecgonine (less than 300 ng/ml benzoylecgonine). 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.

Benzoylecgonine ng/ml	Mean CPM
0	125813
75	89527
150	70543
300	55187

The dose/response curve shown above should not be used in assay calculations. A dose/response curve generated at time of assay is suitable for calculation of drug concentration in sample. The dose/response plot is sharp and linear from the low point through the 300 ng/ml cut-off to the high point.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Urine samples (47) collected from presumed non-users were tested in the Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I]. One hundred percent of these normal urine samples measured negative with a 300 ng/ml cutoff.

Random RIA positive urine samples (55) were subjected to GC-MS analyses. All samples were confirmed as true positive relative to a 300 ng/ml cutoff.

Precision

The precision of the Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I] 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 75, 150, and 300 ng/ml standard was assayed five times in the same assay. The results are tabulated in Table 1.

Table 1

Intra-assay Precision (Reference Controls)

Benzoylecgonine (ng/ml)	Mean CPM	S.D.	C.V.%
75	88952	707	0.8
150	73470	899	1.2
300	55556	897	1.6

Intra-assay CVs for Reference Controls ranged from 0.8% to 1.6%

Inter-assay Precision

Inter-assay precision was performed on reference controls. A 75, 150, and 300 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)

BE (ng/ml)	No of deter-minations	Mean CPM	S.D.	C.V.%
75	10	89367	1138	1.3
150	10	71754	1352	1.9
300	10	54701	1230	2.3

Reference Control inter assay CVs ranged from 1.3 to 2.3%. The average CV was 1.83%.

Sensitivity

Assay sensitivity based on the minimum benzoylecgonine concentration required to produce a four standard deviation from assay Bo is 2 ng/ml. A conservative 300 ng/ml cut off is recommended.

Specificity

The specificity of the Immunalysis Radioimmunoassay for Cocaine Metabolite [¹²⁵I] for various metabolites was determined by generating inhibition curves for each of the compounds 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.

Table 3
Cross-reactivities related drugs

Compound	Approx. ng/ml equivalent to 300ng cocaine	Cross-reactivities at 50% Inhibition
Benzoyllecgonine	300	100
Cocaine	273	109
Ecgonine	16666	1.8
Ecgonine Methyl Ester	300000	<0.1

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 (<5 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbitol, Butabarbital, Caffeine, 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, Methamphetamine, Metharbital, Mephentoin, α -Methyl- α -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

Recovery

Aliquots of a human urine matrix were spiked with benzoyllecgonine to give a final theoretical concentration of 75, 150 and 300 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.

TABLE 4
Recovery

Spiked urine concentration	X concentration (ng/ml)	% Recovery
75	74.6	99
150	149.13	99
300	294.0	98

Expected Values

The urine level of benzoyllecgonine in humans who have not used cocaine within the last two weeks is usually negative, i.e. below 300 ng/ml by the Immunalysis Cocaine / Cocaine Metabolite Direct RIA Kit [¹²⁵I]. User levels range from 150 to 2000+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine metabolite level depends on the individual metabolism, the amount and purity of the cocaine used and the time elapsed since last use.

Interpretation

Benzoyllecgonine appears in the urine within minutes after drug use and may persist for 4-7 days. This Immunalysis highly sensitive, single step, single incubation assay, characterized by a sharp linear plot through the 300 ng/ml cut-off from the low point throughout the high concentration points, reliably documents cocaine use.

Limitations of the Procedure

Samples containing radioactive contamination from previous in-vivo diagnostic procedures should be rejected. Clinical consideration and professional judgement should be applied to any drug abuse test, particularly when preliminary positive results are used. There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results e.g. technical or procedural errors.

REFERENCES

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This package conforms to the conditions and limitations specified in 49 CFR 173.421 for radioactive material, excepted package - limited quantity of material, UN2910.

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June 2001