



HEROIN / MORPHINE DIRECT RIA KIT [¹²⁵I]

(Opiates)

Immunalysis Corporation

Catalog Number 107-0100 100 tubes

Catalog Number 107-0500 500 tubes

INTRODUCTION

A radioimmunoassay (RIA) for heroin/morphine in urine is described. A 25 µl aliquot of sample is incubated with a 100 µl dilution of rabbit anti-morphine (serum) antibody and 200 µl of ¹²⁵I-morphine reagent. Separation of the bound ¹²⁵I-morphine is by a second antibody-Polyethylene Glycol 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 Heroin/Morphine Direct RIA Kit [¹²⁵I] is intended for detection and semi-quantitation of opiates. For Forensic use only.

The Immunalysis Heroin / Morphine 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 (GS-MS) is the preferred confirmatory method (1). Professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLES OF THE PROCEDURE

The Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I] (for heroin, morphine, codeine and MG measurement) is based upon the competitive binding to antibody of ¹²⁵I 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 morphine 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 morphine in the urine specimen. A reference standard containing 300 ng/ml morphine is supplied for use as a cut-off.

The Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I] requires only one 60 minute incubation and avoids extraction of urine sample for measurement. It employs a morphine 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 Heroin/Morphine Direct RIA Kit [¹²⁵I] 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 Urine Heroin/Morphine Direct RIA Kit I-125

Contents.

Each Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I] contains:

Contents	100 Test Kit
1 bottle rabbit anti morphine Antibody	11 ml
1 bottle [¹²⁵ I -morphine]	21 ml
1 bottle containing 300ng/ml morphine in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine)	4 ml
1 Bottle Second Antibody-PEG Complex	21 ml

Morphine Antibody. The antibody solution is rabbit anti-morphine 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-Morphine. The tracer solution of [¹²⁵I -morphine 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/ml of morphine dissolved in a synthetic urine.

Normal Control. This bottle contains drug free synthetic urine equivalent to normal human urine.

Second Antibody-PEG Complex. The separation reagent contains a goat anti rabbit 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 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 -8⁰ C.

Treatment Required. There is no sample treatment or reagent purification required for the use of the Immunalysis Heroin/Morphine 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 Heroin/Morphine 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 Heroin/Morphine 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 ⁰ 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 an automated pipettor

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 ¹²⁵I- Morphine Reagent to each tube.
5. Add 100 µl of blue Anti-Morphine Serum Reagent to each tube; mix well on a vortex-type mixer.
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 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 300 ng/ml Positive Reference Standard.

It is recommended that at least one in house 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 100 µl 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 morphine. If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for morphine. 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.

Morphine ng/ml	Mean CPM
0	70815
75	30546
150	17069
300	12622

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 to the high point and through the 300 ng/ml cut-off.

Interpretation.

Heroin/Morphine and their metabolites appear in urine within an hour after drug use and may persist for a week or more. Thus a positive result reliably documents heroin/morphine use. GC/MS is recommended for confirmation.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

One hundred samples collected from presumed non-users were tested in the Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I]. One hundred percent of these normal urines measured negative at 300 ng/ml. Fifty samples found to be positive for morphine using the Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I] at a 300 ng/ml cut-off were analysed by GC/MS. All of the samples were confirmed positive.

Precision

The precision of the Immunalysis Heroin/Morphine 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)

Morphine (ng/ml)	Mean CPM	S.D.	C.V.%
75	29318	788	2.68
150	15971	361	2.27
300	11777	462	3.92

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)

Morphine (ng/ml)	No of determinations	Mean CPM	S.D.	C.V.%
75	10	30455	905	2.97
150	10	16079	622	3.86
300	10	11515	401	3.48

Sensitivity

Assay sensitivity based on the minimum morphine concentration required to produce a four standard deviation from assay Bo is 2 ng/ml.

Specificity

The specificity of the Immunalysis Radioimmunoassay for Morphine (I-125) 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. The antisera cross-reactivities are listed in Table 3.

TABLE 3

Cross Reactivities with Related Drugs

Compound	Approx. ng/ml equivalent to 300 ng/ml morphine	Cross-reactivities
Morphine	300	100
Codeine	222	135
Morphine 3-glucuronide	967	31
Morphine 6-glucuronide	1504	20
Ethyl morphine HCl	652	46
Hydrocodone	1071	28
Dihydromorphine	1667	18
6-acetyl-morphine	909	33
Thebaine	6000	5
Meperidine HCl	30000	1
Hydromorphone HCl	1578	16
Oxycodone HCl	2500	12

Cross-Reactivities 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 (2 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbitol, Benzoylcegonine, Butabarbital, Caffeine, Cocaine, Carbamazepine, Chloroquine, Chloropromazine, Carbolmal, 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, Mephentanyl,

a-Methyl-a-propylsuccinimide, Mephobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH

Recovery

Normal urines were spiked with morphine 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	Recovered-morphine concentration (ng/ml)	%
75	78.4	104.5
150	143.8	95.9
300	314.2	104.7

Expected Values

The urine level of morphine in humans who have not used morphine within the last two weeks is usually negative, i.e. below 300 ng/ml by the Immunalysis Heroin/Morphine Direct RIA Kit [¹²⁵I]. User levels range from 150 to 300+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine level depends on the individual metabolism, the amount and purity of the heroin/morphine used and the time elapsed since last use(4).

Interpretation

Morphine appears in the urine within minutes after drug use and may persist upto a week or more. Thus this Immunalysis highly sensitive, single step, single incubation assay, characterised by a sharp linear plot through the 300 ng/ml cut-off from the low point throughout the high concentration points, allows detection of early or late heroin/morphine 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.
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4. R.C. Baselt. In : Advances in Analytical Technology, Vol.1. Randall C. Baselt edd. (Biomedical Publications, Foster City, CA. 112- 116).

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

IMMUNALYSIS CORPORATION
Pomona, CA 91767
(909) 394 2203
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