



BARBITURATES DIRECT RIA KIT [I-125]

Immunoanalysis Corporation:

Catalog Number 110-0100 100 tubes

Catalog Number 110-0500 500 tubes

INTRODUCTION

A radioimmunoassay (RIA) for Barbiturate in urine is described. A 25 ul aliquot of sample is incubated with a 100 ul dilution of sheep anti-Secobarbital antibody and 200 ul of I-125-barbiturate reagent. Separation of the bound I-125-barbiturate is by a second antibody complex. The technique is sensitive to 5 ng/ml without dilution or other manipulation. The assay features a 200 ng/ml cut-off.

INTENDED USE

The Immunoanalysis Barbiturate Direct RIA Kit [I-125] is intended for detection and semi-quantitation of Barbiturate in urine at 200ng/ml or higher.

The Immunoanalysis Barbiturate 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 Immunoanalysis Barbiturate Direct RIA Kit is a specific and sensitive in-vitro test to detect the presence of Barbiturates in urine. Barbiturates - derivatives of Barbituric acid - are sedative drugs which at low doses induces relaxation and at high doses induce coma and even death(2). Barbiturates are usually administered orally but may also be taken intravenously or intramuscularly and are absorbed rapidly. The metabolism of Barbiturates is mainly in the liver, a number of metabolic pathways have been described which include oxidation, desulfuration and ring cleavage. Because the number and the proportion of the various Barbiturate metabolites varies with each individual the results are expressed in terms of the equivalents of the standard, Secobarbital/ml.

The Immunoanalysis Barbiturate Direct RIA Kit [I-125] provides a positive reference standard of 200 ng/ml of secobarbital and a normal reference control, I-125 barbiturate radioligand, Secobarbital antibody and a second antibody complex to precipitate antibody-bound barbiturate.

PRINCIPLES OF THE PROCEDURE

The Immunoanalysis Barbiturate Direct RIA Kit [I-125] is based upon the competitive binding to antibody of I-125 radio-labeled 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 Secobarbital 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 barbiturate in the urine specimen. A reference standard containing 200 ng/ml Secobarbital is supplied for use as a cut-off

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

MATERIALS AND METHODS

Materials and equipment required but not supplied with the Immunoanalysis Barbiturate 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

Immunoanalysis Barbiturate Direct RIA Kit I-125 Contents.

Each Immunoanalysis Barbiturate Direct RIA Kit [I-125] contains:

Contents	100 Test Kit
1 bottle Secobarbital Antibody	11 ml
1 bottle I-125-barbiturate (not more than 10 uCi)	21 ml
1 bottle containing 200 ng/ml Secobarbital in synthetic urine	4 ml
1 bottle Normal Control (synthetic urine)	4 ml
1 Bottle Second Antibody-PEG Complex	22 ml

Secobarbital Antibody. The antibody solution is sheep anti-Secobarbital 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-barbiturate. The tracer solution contains not more than 10 uCi of I-125-barbiturate 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 Standards. This contains 200 ng of

Secobarbital dissolved in a synthetic urine with 0.1% sodium azide.

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 hospital 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 Barbiturate 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 Barbiturate 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 Barbiturate 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 the green I-125 barbiturate to each tube.
5. Add 100 ul of blue Anti-Secobarbital Serum Reagent to each tube.
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 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

200 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 each assay run.

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 Barbiturate (has a Barbiturate concentration equal to or greater than 200 ng/ml). If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for Barbiturate (less than 200 ng/ml Barbiturate). 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.

Secobarbital ng/ml	Mean CPM
0	150,748
50	104,291
100	77,916
200	59,008

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 200 ng/ml cut-off.

Interpretation.

Barbiturates and their metabolites appear in urine within hours after drug use and may persist for days. Thus a positive result documents Barbiturate use. GC/MS is recommended for confirmation.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Fifty urine samples collected from presumed non-users were tested in the Immunalysis Urine Barbiturate RIA Kit [I-125]. One hundred percent of these normal urines measured negative at 200 ng/ml. Fifty samples which were previously confirmed positive for Barbiturate by GC-MS employing a cut-off of 200 ng/ml, were tested using the Immunalysis Barbiturate Direct RIA Kit [I-125]. All of the samples were found to be positive i.e. above the cut-off of 200 ng/ml. 100 % of GC/MS confirmed positive samples were positive by RIA at a 200 ng/ml cut-off.

Precision

The precision of the Immunalysis Barbiturate 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 50, 100, and 200 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)

Secobarbital (ng/ml)	Mean CPM	S.D.	C.V.%
50	104291	1576.13	1.51
100	77916	1544.05	1.98
200	59008	1827.48	3.10

Intra-assay CVs for Reference Controls ranged from 1.51 to 3.10%

Inter-assay Precision

Inter-assay precision was performed on reference controls. A 50, 100 and 200 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)

Secobarbital (ng/ml)	No of determinations	Mean CPM	S.D.	C.V.%
50	10	104490	1247.70	1.19
100	10	79003	1464.75	1.85
200	10	58383	1938.57	3.32

Reference Control interassay CVs ranged from 1.19 to 3.32 %.

Sensitivity

Assay sensitivity based on the minimum Barbiturate concentration required to produce a four standard deviation from assay Bo is 5 ng/ml. A conservative 200 ng/ml cut off is recommended.

Specificity

The specificity of the Immunalysis Radioimmunoassay for Barbiturates [I-125] for various barbiturates 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 with Related Drugs

Compound	Approx. ng/ml equivalent to 200 ng secobarbital	Cross-reactivities at 50% Inhibition
Aprobarbital	357	56
Butabarbital	455	44
Barbital	1050	19
Amobarbital	445	45
Butalbital	541	37
p-Hydroxyphen-barbital	556	36
Pentobarbital	370	54
Diallylbarbituric acid	498	40
Phenobarbital	295	68
Barbituric Acid	>10000	<1
Hexobarbital	>10000	<1
Mephobarbital	>10000	<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, Ascorbic acid, Atropine, Benzoylcegonine, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Mephenytoin, "-Methyl"-propylsuccinimide, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phensuximide PEMA, Primidone, Phencyclidine, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, THC-COOH

Recovery

Normal urines were spiked with Secobarbital to give a final theoretical concentration of 100, 200 and 400 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 Secobarbital	Observed Secobarbital concentration (ng/ml)	% Recovery
50	47.2	94.4
100	107.5	107.5
200	192.1	96.1

Interfering Substances

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

Expected Values

The urine level of Barbiturate in humans who have not used Barbiturates within the last two weeks is usually negative, i.e. below 200 ng/ml by the Immunalysis Barbiturate Direct RIA Kit [I-125]. User levels range from 200 to 400+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine Barbiturate level depends on the individual metabolism, the amount and purity of the Barbiturates used and the time elapsed since last use.

Interpretation

Barbiturates appear in the urine within hours after drug use and may persist for days(3). Thus this Immunalysis highly sensitive, single step, single incubation assay, characterized by a sharp linear plot through the 200 ng/ml cut-off from the low point throughout the high concentration points, reliably documents recent Barbiturate 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. S.C. Harvey, In : The Pharmacological Basis of Therapeutics, 5th Ed., L.S. Goodman and A. Gilman edd. (New York, Macmillan, 1975) (pp102-23)
3. R.C. Baselt. In : Advances in Analytical Technology, Vol.1. Randall C. Baselt ed. (Biomedical Publications, Foster City, CA. 93-97).

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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