



PCP Direct RIA Kit [¹²⁵I]

Immunalysis Corporation

Catalog Number 108-0100 100 tubes

Catalog Number 108-0500 500 tubes

INTRODUCTION

A radioimmunoassay (RIA) for phencyclidine (PCP) in urine is described. A 50 ul aliquot of sample is incubated with a 100 ul dilution of goat anti-PCP antibody and 200 ul of ¹²⁵I-PCP reagent. Separation of the bound ¹²⁵I-PCP is by a second antibody complex. The technique is sensitive to 1 ng/ml without dilution or other manipulation. The assay features a 25 ng/ml cut-off.

INTENDED USE

The Immunalysis PCP Direct RIA Kit [¹²⁵I] is intended for semi-quantitation of PCP at 25 ng/ml or higher.

The Immunalysis PCP 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

The Immunalysis PCP Direct RIA Kit is a specific and sensitive in-vitro test to detect the presence of phencyclidine and its mono-hydroxylated metabolite, 1-(1-phenylcyclohexyl)-4-hydroxypiperidine, in urine.

PCP may be self administered either by smoking, inhalation, oral ingestion or injection. It is often mixed with other street drugs and used inadvertently. Free PCP and its metabolites are excreted in urine (1,2)

The Immunalysis PCP Direct RIA Kit [¹²⁵I] provides a positive reference standard [25 ng/ml] and a normal control, I-125 PCP reagent, PCP antibody and a second antibody complex to precipitate antibody-bound PCP.

PRINCIPLES OF THE PROCEDURE

The Immunalysis PCP Direct RIA Kit [¹²⁵I] (for phencyclidine 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 (3,4).

An unknown specimen is mixed in a test tube with fixed amounts of goat anti-PCP 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 cut-off reference standard (25 ng/ml PCP) are indicative of the presence of PCP in the urine specimen.

The Immunalysis PCP Direct RIA Kit [I-125] requires only one 60 minute incubation, and avoids extraction of urine sample for measurement. It employs a PCP 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 PCP Direct RIA Kit [¹²⁵I] are itemized below:

Materials

12x75 mm Disposable Glass or Plastic Culture Tubes.

Test Tube Racks.

0.05, 0.2 ml and 0.5 ml micropipets or automated pipetors

Equipment

Refrigerator (for kit storage).

Vortex Mixer.

Interval Timer.

Centrifuge.

Gamma Counter.

Calculator.

REAGENTS

Immunalysis PCP Direct RIA Kit [¹²⁵I] Contents.

Contents	100 Test Kit
1 bottle PCP Antibody	11 ml
1 bottle ¹²⁵ I -PCP Reagent	21ml
1 bottle containing 50 ng/ml PCP in a synthetic urine matrix	5 ml
1 bottle Normal Control in a synthetic urine matrix	5 ml
1 bottle Second Antibody-PEG Complex	21 ml

PCP Antibody. The antibody solution is goat anti-PCP 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-PCP Reagent. The tracer solution contains ¹²⁵I -PCP 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 bottle contains 25 ng/ml of PCP 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 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 - 8°C.

Treatment Required. There is no sample treatment or reagent purification required for the use of the Immunalysis PCP 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 20% indicates deterioration of the tracer.

SPECIMEN COLLECTION

Precautions.

The Immunalysis PCP 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 PCP 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 should be followed in sequence using manual pipettes. Alternatively all the 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 50 ul of Positive Reference Standard and Normal Control to the appropriate tubes.
3. Add 50 ul of each urine specimen to the appropriate tubes.
4. Add 200 ul of ¹²⁵I -PCP to each tube.
5. Add 100 ul of anti-PCP 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 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 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/aspirate 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 25 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 100ul 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 PCP (has a PCP concentration equal to or greater than 25 ng/ml).

If the average sample CPM is greater than the average CPM of the Positive Reference Standard the sample is called NEGATIVE for PCP (less than 25 ng/ml PCP). 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.

PCP ng/ml	Mean CPM
0	128533
12.5	68554
25	54115
50	41563

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 25 ng/ml cut-off to the high point.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Urine samples (42) collected from presumed non-users were tested with the Immunalysis PCP Direct RIA Kit [¹²⁵I]. One hundred percent of these normal urines measured negative at 25 ng/ml. Random RIA positive urine samples (51) were subjected to GC/MS analyses. All samples were confirmed as true positive. At the 25 ng/ml cutoff.

Precision

The precision of the Immunalysis PCP 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 12.5, 25, and 50 ng/ml standard was assayed five times in the same assay. The results are tabulated in Table 1.

Table 1

PCP (ng/ml)	counts 0.1 min	S.D.	C.V.%
12.5	65090	1296	1.99
25	52615	1463	2.78
50	40175	842	2.09

Intra-assay CVs for Reference Controls ranged from 1.99 to 2.78%.

Inter-assay Precision

Inter-assay precision was performed on reference controls. A 12.5, 25 and 50 ng/ml reference control was assayed in ten separate runs and a SD and CV determined. Results are tabulated in Table 2

Table 2
Inter-assay Precision (Reference controls)

PCP (ng/ml)	No of determinations	Mean (ng/ml)	Mean counts	S.D.	C.V.%
12.5	10	12.7	65234	1043	1.59
25	10	26.1	53127	1115	2.10
50	10	51.3	41013	763	1.85

Reference Control interassay CVs ranged from 1.59 to 2.10 %. The average CV was 1.85%.

Sensitivity

Assay sensitivity based on the minimum PCP concentration required to produce a four standard deviation from assay Bo is 1 ng/ml. A conservative 25 ng/ml cut off is recommended.

Specificity

The following compounds were tested for cross-reactivity in the Immunalysis Radioimmunoassay for PCP. The compounds tested were prepared in a human urine matrix. The results are expressed as that amount of the compound capable of giving a result equivalent to 25 ng/ml of PCP.

Table 3

Crossreactivity

Compound	Approx ng/ml equivalent to 25 ng PCP/ml	% Cross reactivity
Phencyclidine (PCP)	25	100
1-[1-(2-thienyl)cyclohexyl]-piperidine	50	50
1-[1-(2-thienyl)cyclohexyl]-morpholine	125	20
1-(1-phenylcyclohexyl)pyrrolidine	250	10
1-(1-phenylcyclohexyl)morpholine	312.5	8

Cross-Reactivity with Unrelated Drugs

A human urine matrix was spiked with the following compounds at a concentration of 10,000 ng/ml. None of these compounds gave values in the assay that are equal to or greater than the assay sensitivity level (2.5 ng/ml).

Amphetamine, Amobarbital, Barbitol, Butabarbital, Caffeine, Cocaine, delta-9-THC, 9-carboxy-THC, Benzoylcegonine, Carbamazepine, Codeine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Ethosuximide, Ethotoin, Glutethimide, Hexobarbita, Lidocaine, Methadone-Primary, Methaqualone, Methadone Metabolite, Metharbital, Mephentoin, Methyl-x-propylsuccinimide, Mephobarbita, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Methamphetamine, N-Normethsuximide, Phenobarbita, Phensuximide, PEMA, Primidone

Recovery

Aliquots of a human urine matrix were spiked with PCP to give a final theoretical concentration of 12.5, 25 and 50 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

Spiked urine concentration	X Concentration PCP ng/ml	% Recovery
12.5	12.38	99.04%
25	25.9	103.6%
50	50.9	101.8%

Interfering Substances

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

Expected Values

The urine level of PCP in humans who have not used PCP within the last two weeks is usually negative, i.e. below 25 ng/ml by the Immunalysis PCP Direct RIA Kit (I-125). User levels range from 25 to 50+ ng/ml. It is difficult to document that chronic, moderate or even occasional users have been restricted to a single exposure. The urine PCP level depends on the individual metabolism, the amount and purity of the PCP used and the time elapsed since last use.

Interpretation

PCP appears in the urine within minutes after drug use and may persist for days. This Immunalysis highly sensitive, single step, single incubation assay, characterised by a sharp linear plot through the 25 ng/ml cut-off from the low point throughout the high concentration points, reliably documents recent or distant PCP use.

Limitations of the Procedure

Samples containing radioactive contamination from previous in-vivo diagnostic procedures should be rejected.

The Immunalysis PCP Direct RIA 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 (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgement should be applied to any drug abuse test, particularly when preliminary positive results are used.

There is the 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.

4. Yalow, R.S., Circ. Res.,(Suppl). 116-128 (1973).

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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February 2003**

REFERENCES

1. Urine Testing for Drugs of Abuse, National Institute on Drug Abuse Research Monograph 73, 1986.
2. Budd, R.D. and Leung, W.J., Clin. Toxicol. 18: 85-90 (1981).
3. Yalow, R.S. and Berson, S.A., Nature, 184: 1648-1649 (1959).