1 Background:

Cocaine (benzoylmethylecgonine) is an ester of benzoic acid and ecgonine. Naturally, cocaine is found in the leaves of the coca plant (Erythroxylon coca). The drug can be taken orally, intravenously, or (more commonly) it can be either taken by intranasal insufflation or inhaled as a vapor (“free-basing”)\(^1,5\).

In the body, metabolism to ecgonine methyl ester and ecgonine occurs by the action of serum and liver cholinesterase\(^11\). Benzylecgonine is not produced enzymatically but by simple hydrolysis, both in the body and in aqueous specimens\(^4\). Loss of methyl ester occurs spontaneously, in a process that is dependent on pH and temperature\(^5\). Benzylecgonine may also undergo enzymatic conversion to ecgonine by cholinesterases\(^4\). Other metabolites may occur, including arylhydroxy metabolites\(^14\).

After single intranasal doses of 100 to 150 milligrams of cocaine, plasma concentrations of cocaine reach peaks of 100 to 500 ng/mL after 20 to 60 minutes\(^1,3,19\). Much higher plasma concentrations occur in "street" use, and high brain concentrations of cocaine have been found in post-mortem studies following cocaine overdose\(^8\). The half-life of cocaine in blood is on the order of 30 to 120 minutes; whereas benzoylecgonine has a longer half-life in blood of 7 to 9 hours.

In urine, benzoylecgonine is the major metabolite found\(^7\). Only a few percent of an administered dose of cocaine appears in urine unchanged\(^2\). Benzylecgonine is found in urine soon after cocaine insufflation, and can remain detectable for up to 48 hours\(^2\). When cocaine and alcohol are ingested together, the metabolite cocaethylene is formed\(^10,12\). In acidic urine, the proportion of unchanged cocaine is reported to be increased. Ecgonine methyl ester, norcocaine and arylhydroxy metabolites have also been found in urine\(^7,9,14,17,18,20\).

\(^*\)Taken from DPC Coat-A-Count Cocaine Metabolites in Urine Instruction Manual, with permission.

2 References:


2.22 Instruction Sheet, Cocaine Metabolite Direct RIA Kit, Immunalysis Corporation, June 2001

3 Scope and Applicability:

3.1 This procedure describes the use of the Immunalysis Cocaine Metabolite Direct RIA Kit as a screening test (qualitative determination) for the presence of benzoylecgonine in both ante- and post-mortem urine samples. Benzoylecgonine is the principal urinary metabolite of cocaine.

3.2 If required, blood may be used in place of urine.

3.3 This procedure is intended strictly for In vitro diagnostic use in the context of a program involving an established confirmatory test for cocaine and its metabolites. Gas chromatography/mass spectrometry (GC/MS) is the confirmatory method employed by the SASL.

3.4 Clinical considerations and professional judgement must be applied to any drug of abuse test result, particularly when preliminary positive results are used.

3.5 The detection limit (or "minimum detectable dose") of this procedure is approximately 2 ng/mL

3.6 The threshold for signaling a “Positive” test has been set to 300 ng/mL

4 Summary of the Analytical Method:

Cocaine (benzoylmethylecgonine) can lose its methyl group through hydrolysis, and the benzyl group through the action of pseudocholinesterase. Approximately 70% emerges in the urine over 48 hours, primarily as benzoylecgonine.

The Immunalysis Cocaine Direct RIA Kit procedure is a quantitative radioimmunoassay system, wherein ¹²⁵I-labeled benzoylecgonine competes for a fixed time with the benzoylecgonine present in a sample for antibodies. The sample is mixed in a scintillation vial with a solution containing benzoylecgonine antibody, and radio-labeled benzoylecgonine. Any benzoylecgonine present in the sample competes with the labeled benzoylecgonine for the antibody, decreasing the
amount of labeled benzoylecgonine that subsequently precipitates out. The antibody which has been bound to the benzoylecgonine present in the sample and the added benzoylecgonine is precipitated through the addition of a second antibody-PEG complex. After centrifugation, the supernatant is decanted to terminate the competition and to isolate the antibody-bound fraction of the radio-labeled benzoylecgonine. The presence or absence of benzoylecgonine is determined through the use of a scintillation counter, comparing the number of counts obtained for the sample, to that for a standard with a known methamphetamine concentration.

5 Sample Handling and Preservation:

5.1 Store the components of the Immunalysis Cocaine Metabolite Direct RIA Kit at 2-8 °C in its original box, in a refrigerator designated for incoming radioactive materials.

5.2 Collect urine without preservative. The specimen can be refrigerated or frozen. If the specimen is cloudy, it should be cleared by filtration or centrifugation before use, and mixed by gentle swirling.

5.3 If adulteration of the specimen is suspected, do not analyze the sample until after speaking with the submitting laboratory.

5.4 The sample must be analyzed within 14 days of receipt.

5.5 Samples containing radioactive contamination from previous in vivo diagnostic procedures are not compatible with this method.

6 Safety:

6.1 The toxicity or carcinogenicity of the reagents and standards used in this method has not been fully established. All of the chemicals should be regarded as a potential health hazard and exposure to these compounds should be avoided.

6.2 The samples, and some of the components of the Immunalysis Cocaine Metabolite Direct RIA Kit contain human source material, or other potentially bio-hazardous materials. All standards and samples must be handled according to the accepted procedures laid out in the SASL document entitled “Blood Borne Pathogen and Bio-Safety”.

6.3 This procedure involves the use of isotopically labeled reagents. These reagents must be used, handled, and disposed of, in accordance with the procedures described in the SASL radiation safety protocol.

6.4 Samples and standards considered to be hazardous must be prepared for disposal by the accepted procedures laid out by SASL protocols.

6.5 Since this procedure has potential exposure to both radioisotopes and bio-hazards, the use of both the Personal Protective Equipment (PPE) and the Engineering Controls (EC) are necessary. Follow the SASL guidelines for the use of the Personal Protective Equipment and Engineering Controls.

6.6 Sodium azide has been added to some of the components of the Immunalysis Cocaine Metabolite Direct RIA Kit, as an antibacterial agent. The concentration of sodium azide is below the regulatory limit of 0.1 g/dL in all lyophilized reagents, when reconstituted with the specified volume or water, as well as in all reagents supplied ready to use. To prevent buildup of explosive metal azides in lead and copper plumbing, the reagents should be discarded down the drain only if diluted and flushed with large volumes of water.

7 Interferences

7.1 The Cocaine Metabolite antiserum is highly specific for cocaine, and its major urinary metabolite benzoylecgonine, with an extremely low cross-reactivity to other compounds that may be present in samples. The following compounds were found to be non-detectable by this procedure at a level of 10,000 ng/mL:
Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic Acid, Atropine, Barbital, Butabarbital, Caffeine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxphene, 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methamphetamine, Methaqualone, Metharbital, Mephenytoin, a-Methyl-a-propylsuccinimide, Mepobarbital, Methyl PEMA, Methsuximide, 4-Methylprimidone, Morphine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Phenobarbital, Phenobarbital, Phenobsuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenoxyzaine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THC-COOH.

7.2 The literature suggests medications containing lidocaine, other local anesthetic agents or structurally related compounds or Phencyclidine might cause a false positive result. The literature also suggest that food or drinks containing coca products might cause a false positive result.

7.3 Other substances and/or factors not listed above, e.g. technical or procedural errors, may interfere with the test and cause false positive results.

8 Equipment and Supplies:

8.1 Immunalysis Cocaine Metabolite Direct RIA Kit. Immunalysis Part Number 106-0100.

8.2 Gamma counter, Logic Systems Model 111 (or equivalent).

8.3 Centrifuge.

8.4 Vortex mixer.

8.5 12x75 mm polypropylene scintillation vials.

8.6 Micro-pipettes: 25 µL, 100 µL, and 200 µL.

8.7 Class A Volumetric Pipettes.

8.8 Class A Volumetric Flasks.

8.9 Foam decanting rack.

9 Reagents:

9.1 Type II Water.

9.2 Stock Benzoylecgonine Solution: The Stock Benzoylecgonine Solution is supplied by the manufacturer in a ready to use liquid form. Store refrigerated. The Stock Benzoylecgonine Solution is stable for 30 days (or until the expiration date marked on the vial) when refrigerated at 2-8 °C.

Note: The Stock Benzoylecgonine Solution is the radioactive component of the procedure. The Solution, along with anything contacted by the solution, must be handled properly, and must be disposed of as a radioactive waste.

9.3 Benzoylecgonine Antibody: The Benzoylecgonine Antibody is a sheep anti-benzoylecgonine serum, supplied by the manufacturer in a ready to use liquid form. The Benzoylecgonine Antibody is stable for 30 days when refrigerated at 2-8 °C.

9.4 Second Antibody: The Second Antibody is complexed with PEG, and is supplied by the manufacturer in a ready to use liquid form. The Second Antibody is stable for 30 days when refrigerated at 2-8 °C.
9.3 Benzoylecgonine-Free Urine:

10 Standards:

10.1 Primary Benzoylecgonine Standard, 1,000 µg/mL: Cerilliant Catalog Number S-XXX, or equivalent.

10.2 Benzoylecgonine Calibration Standards, 0, 100, 250, 500, and 1,000 ng/mL benzoylecgonine (as the free base): The Calibration Standards are supplied by the manufacturer in a ready to use liquid form. The Calibration Standards are supplied as a set of 6 vials. The vials are labeled “A” through “F”, with “A” being the 0 ng/mL and “F” being the 5,400 ng/mL Standard. The calibration standards are stable for 30 days (or until the expiration date marked on the vial) after opening, when refrigerated at 2-8 °C. If necessary, the Calibration Standards may be stored for up to 6 months by freezing -20 °C.

10.3 Benzoylecgonine Control, 1,000 ng/mL secobarbital: Add 900 µL of the Barbiturate-Free Urine (Section 9.3) to a clean screw-top sample vial. Add 100 µL of the 10,000 ng/mL Barbiturate Calibration Standard (Section 10.1) to the vial. Mix the contents by inverting the vial 15 times. The Control is stable for 30 days (or until the expiration date marked on the vial) after opening, when refrigerated at 2-8 °C. If necessary, the Control may be stored for up to 6 months by freezing -20 °C.

10.4 Benzoylecgonine Positive Standard, 300 ng/mL: The Benzoylecgonine Positive Standard is supplied by the manufacturer in a ready to use liquid form. The Positive Standard is stable for 30 days (or until the expiration date marked on the vial) after opening, when refrigerated at 2-8 °C.

10.5 Benzoylecgonine Negative Standard, 0 ng/mL secobarbital: The Benzoylecgonine Negative Standard is supplied by the manufacturer in a ready to use liquid form. The Negative Standard is stable for 30 days (or until the expiration date marked on the vial) after opening, when refrigerated at 2-8 °C.

11 Procedure

Note: All components must be at room temperature (15-28 °C) before performing the procedure.

Note: During the following additions, it is important to pipette directly to the bottom of the tubes.

11.1 On Receipt of the Immunalysis Kit:

11.1.1 Record the date of receipt of the kit on a new log sheet.

11.1.2 Label 6 new polypropylene scintillation vials with the concentrations of the standards.

11.1.3 Pipette 25 µL of each of the Benzoylecgonine Calibration Standards (Section 10.2) into separate vials.

11.1.4 Add 200 µL of the Stock Benzoylecgonine Solution (Section 9.2) to each of the vials.

Note: The pipette tip must be discarded of in the container designated for solid radioactive wastes.

11.1.5 Add 100 µL of the Benzoylecgonine Antibody to each of the vials.

11.1.6 Add 200 µL of the Second Antibody to each of the vials.

11.1.7 Vortex each of the vials.

11.1.8 Incubate the vials for one hour at room temperature (15-28 °C).

11.1.9 Centrifuge the vials for 20 minutes.

11.1.10 Thoroughly decant the liquid from all of the vials. Remove all of the visible moisture. Allow the tubes
to drain for 2 or 3 minutes. Strike the vials sharply on absorbent paper to dislodge the last of the residual moisture.

**Note:** All of the supernatant liquid must be disposed of in the shielded bottle designated for the disposal of liquid radioactive wastes. The absorbent paper must also be disposed of in the shielded box designated for the disposal of solid radioactive wastes.

11.1.11 Using the gamma counter, count the standards for 1.5 minutes. Record the count for each of the vials.

**Note:** Once counted, the liquid free tubes must be disposed of in the shielded box designated for the disposal of solid radioactive wastes.

11.1.12 Plot and perform a linear regression of the resulting data.

11.1.13 Attach the results of the regresional analysis to the log sheet, and file the log sheet in the binder for RIA logs.

### 11.2 Analytical Procedure:

11.2.1 Label three new polypropylene scintillation vials as “Negative”, “Positive”, and “Control”. Label a vial for each of the samples.

11.2.2 Record the Lot Number of the kit, and the date the kit was put into service.

11.2.3 Pipette 25 µL of the Benzoylcegonine Negative Standard (Section 10.5) into the vial marked “Negative”.

11.2.4 Pipette 25 µL of the Benzoylcegonine Positive Standard (Section 10.4) into the vial labeled “Positive”.

11.2.5 Pipette 25 µL of the Benzoylcegonine Control (Section 10.3) into the vial labeled “Control”.

11.2.6 Pipette 25 µL of the urine sample into the “Sample” vials.

11.2.7 Add 200 µL of the Stock Benzoylcegonine Solution (Section 9.2) to each of the vials.

**Note:** The pipette tip must be discarded of in the container designated for solid radioactive wastes.

11.2.8 Add 100 µL of the Benzoylcegonine Antibody (Section 9.3) to each of the vials.

11.2.9 Add 200 µL of the Second Antibody (Section 9.4) to each of the vials.

11.2.10 Vortex each of the vials.

11.2.11 Incubate the vials for one hour at room temperature (15-28 °C).

11.2.12 Centrifuge the vials for 20 minutes.

11.2.13 Thoroughly decant the liquid from all of the tubes. Remove all of the visible moisture. Allow the tubes to drain for 2 or 3 minutes. Strike the tubes sharply on absorbent paper to shake off the last of the residual moisture.

**Note:** All of the supernatant liquid must be disposed of in the shielded bottle designated for the disposal of liquid radioactive wastes. The absorbent paper must be disposed of in the shielded box designated for the disposal of solid radioactive wastes.

11.2.14 Using the gamma counter, count the standards and the samples for 1.5 minutes. Record the count for each of the tubes.
12 Quality Control:

12.1 The regresional analysis for the calibration curve created in Section 11.1 (On Receipt of the Immunalysis Kit) should exhibit a correlation coefficient of 0.99 or greater. If the fit is less than 0.99, the calibration curve should be re-run.

12.2 Samples should be divided into batches of not more than 10 samples. A Negative, Positive, and Control should be run with each batch of samples.

12.3 The counts obtained for the Control should be compared to the calibration curve created in Section 11.1. If the concentration calculated for the control is more than 10% from the expected value, the batch of samples should be re-run.

12.4 The air displacement pipettes must be calibrated and maintained according to the SASL procedure entitled “Air Displacement Pipettes”.

12.5 Do not use the standards or reagents beyond their expiration dates.

Note: All of the parts must be disposed of in the shielded bottle designated for the disposal of liquid radioactive wastes. The absorbent paper must be disposed of in the shielded box designated for the disposal of solid radioactive wastes.

12.6 The procedure calls for centrifuging at 3000×g for 15 minutes. Lower accelerations are satisfactory only if the centrifugation time is increased appropriately, for example, centrifuging for 30 minutes at 1500×g. A high-speed, refrigerated centrifuge is desirable but not essential. Use the equation in Section 13.2 to calculate the acceleration of the centrifuge.

13 Calculations:

13.1 Compare the counts for the “Sample” to those for the “Positive”.

13.1.1 If the counts for the “Sample” are greater than the counts for the “Positive”, the result is negative (i.e. within the precision of the procedure, the sample contains less than 300 ng/mL benzoylecgonine).

13.1.2 If the counts for the “Sample” are lower than the counts for the “Positive”, the result is positive, and the sample contains more than 300 ng/mL benzoylecgonine, within the precision of the procedure.

13.2 Relating the acceleration to the speed of a centrifuge:

\[ F = 28.38 \times \left( \frac{S}{1000} \right)^2 \times r \]

where:
\[ F = \text{Force (g)} \]
\[ S = \text{Speed (rpm)} \]
\[ r = \text{Radius of the rotor (inches)} \]
## Authorizing Signatures:

Author: Jeffrey A. Boon

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