Cocaine/Benzoylecgonine ELISA

INTENDED USE

The Diagnostic Automation Inc. (DAI) Cocaine Metabolite ELISA Kit is a specific and sensitive in-vitro test to detect the presence of Benzoylecgonine (BE) in forensic samples such as whole blood, serum, plasma and urine.

SUMMARY AND EXPLANATION

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 Metabolite 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 DAI Cocaine Metabolite ELISA Kit 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).

TEST PRINCIPLE

The DAI Cocaine Metabolite ELISA Kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. A 10 µl aliquot of a diluted unknown specimen is incubated with a 100 µl dilution of enzyme (Horseradish peroxidase) labeled Benzoylecgonine derivative in micro-plate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample. The technique is sensitive to 1 ng/ml.

MATERIALS AND COMPONENTS

Materials provided with the test kits
1. Microwells coated plate 12x8x1
2. BE-Conjugate 12 ml
3. Immunalysis Positive Reference Standard 2 ml
4. Negative Standard 1 ml
5. TMB Substrate 12 ml
6. Stop Reagent 11 ml

Materials required but not provided:
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

PRECAUTION

1. Potential biohazardous materials:
   - The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
   - This test kit is designed for research use only.
   - Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
   - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
   - It is recommended that serum samples be run in duplicate.
   - Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. The DAI Benzoylecgonine ELISA Kit is to be used with human forensic samples, such as whole blood, oral fluids, serum, urine and plasma. Has not tested all possible applications of this assay.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2 - 4 °C until use. Samples should be well mixed before assay.
4. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with Blue Ice or equivalent.
ASSAY PROCEDURE

All reagents must be brought to room temperature (18-26°C) before use. The procedure as described below may be followed in sequence using manual pipettes. Alternatively all reagents may be added using an automated pipette.

1. Dilute specimens, to the necessary range with Phosphate Buffered Saline pH 7.0. (Urine samples 1:10 for a benzoylecgonine cutoff level of 300 ng/ml.) The dilution factor can be adjusted based on the laboratory’s cutoff.
2. Add 10 µl of appropriately diluted calibrators and standards to each well in duplicate.
3. Add 10 µl of the diluted specimens in duplicate (recommended) to each well.
4. Add 100 µl of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
5. Incubate for 30 minutes at room temperature, preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash well 6 times with 350 µl distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amount of hemoglobin (some postmortem samples) use 10M Phosphate buffered saline pH 7.0-7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.
7. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate, does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well.
8. Add 100 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
9. Incubate for 30 minutes at room temperature, preferably in the dark.
10. Add 100 µl of Stop Solution to each well, to change the blue color to yellow.
11. Measure the absorbance at a dual wavelength of 450 nm and 650 nm. Wells should be read within 1 hours of yellow color development.
12. Wells with blank values or no optical density above the background level (1.0 absorbance) should be run with every plate.

The following data represent a typical dose/response curve.

<table>
<thead>
<tr>
<th>Benzoylecgonine (ng/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>0.52</td>
</tr>
<tr>
<td>25</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
<td>0.27</td>
</tr>
</tbody>
</table>

The dose/response curve shown above should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or a cutoff calibrator should be run with every plate.

PERFORMANCE CHARACTERISTICS

1. Accuracy

60 whole blood samples and 35 urine samples collected from presumed non-users were tested in the DAI Cocaine Metabolite ELISA Kit. All the samples were found to be positive i.e. above the cut-off of 50 ng/ml.

2. Precision

The precision of the DAI Cocaine Metabolite ELISA Kit has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.

3. Intra-assay Precision

Intra-assay precision was determined with reference controls. A 0, 10, 25, and 50 ng/ml standard was assayed five times in the same assay. The results are tabulated as follows:

<table>
<thead>
<tr>
<th>Benzoylecgonine (ng/ml)</th>
<th>Mean Abs</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.377</td>
<td>0.115</td>
<td>4.84</td>
</tr>
<tr>
<td>10</td>
<td>1.009</td>
<td>0.078</td>
<td>7.73</td>
</tr>
<tr>
<td>25</td>
<td>0.695</td>
<td>0.045</td>
<td>6.47</td>
</tr>
<tr>
<td>50</td>
<td>0.518</td>
<td>0.049</td>
<td>9.51</td>
</tr>
</tbody>
</table>

4. Sensitivity

Assay sensitivity based on the minimum Benzoylecgonine concentration required to produce a four standard deviation from assay A0 is 1 ng/ml.

5. Specificity

The specificity of the DAI Cocaine Metabolite ELISA was determined by generating inhibition curves for each of the compounds listed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. ng/ml equivalent to 50ng</th>
<th>Cross-reactivities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoylecgonine</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Cocaine</td>
<td>750</td>
<td>66</td>
</tr>
<tr>
<td>Cofaetylene</td>
<td>&gt;5000</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Norcocaine</td>
<td>&gt;5000</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Egcgonine</td>
<td>&gt;5000</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Egcgonine Methyl</td>
<td>&gt;10000</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Ester</td>
<td>&gt;10000</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

6. Cross-Reactivity with Unrelated Drugs

Aliquots of a human urine matrix were spiked with the following compounds at a concentration of 2,000 ng/ml. None of these compounds gave values in the assay that were equal to or greater than the assay sensitivity level (1 ng/ml).

Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminoprine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbitul, Butabarbital, Caffeine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxphene, 5,5'-Diphenylhydantoin, 10-11- Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Etohitoin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytino, a-Methyl-a-Propylsuccinimide, Mephobarbital, Methyl PEMA, Methoximide, 4-Methylprimidone, Morphine, Meperidine, Nicinamide, Norethindrone, N-Normethoximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenypropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THC-COOH.
STORAGE

1. Store the kit at 2-8 °C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

REFERENCES


DISCLAIMER

This ELISA kit has not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of the buyer to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

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