INTENDED USE

The Diagnostic Automation Inc. (DAI) Opiates ELISA 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 judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

SUMMARY AND EXPLANATION

The DAI Opiates ELISA is a specific and sensitive in-vitro test to detect the presence of Opiates in samples such as whole blood, oral fluids, serum, plasma and urine.

Heroin/morphine abuse is a major problem in society (2). In the body, both heroin (diacetyl morphine) and morphine are largely converted to morphine-3-glucuronide (MG)(3). The DAI Opiates ELISA measures heroin, morphine, codeine, hydrocodone and their metabolites.

TEST PRINCIPLE

The DAI Opiates ELISA (for morphine equivalents measurement) 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 morphine 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 0.25 ng/mL. The DAI Opiates ELISA avoids extraction of urine sample for measurement. It employs an Opiates directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

MATERIALS AND COMPONENTS

Materials provided with the test kits
1. Microwells with polyclonal anti-morphine 12 x 8 x 1
2. Morphine-Conjugate 12 ml
3. Immunalysis Positive Reference Standard 2 ml
4. Negative Standard 1 ml
5. TMB Substrate 12 ml
6. Stop Solution 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 450nm.
5. Absorbance paper or paper towel
6. Graph paper.

PRECAUTION

1. Not for internal or external use in humans or animals. There should be no eating or drinking within work area. Always wear gloves and a protective lab coat.
2. This test kit is designed for research use only.
3. Do not pipette by mouth. Handle all specimens and reagents as potentially infectious and biohazardous. Do not add sodium azide to samples as preservative. Do not use external controls containing sodium azide. Bring all reagents to room temperature.
4. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue. Do not pour chromogenic substrate back into container after use.
5. Do not freeze reagent. Do not mix reagents from different lot numbers.
6. Keep reagents out of direct sunlight. Handle stop solution with care, as it is corrosive.
7. Viscous samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting. Ensure the bag containing the micro-plate strips and dessicant is well sealed if only a few strips are used.

SPECIMEN COLLECTION & HANDLING

1. The DAI Opiates ELISA kit is to be used with human samples, such as whole blood, oral fluids, serum, plasma and urine. Not all possible applications of this assay have been tested. Cutoff criteria is important in deciding the sample dilution.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2 - 40 °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.
4. The expiration date of the kit is stated on the label.
5. The kit can be expected to perform satisfactorily until the expiration date if stored in the refrigerator at 2 – 40 °C.
ASSAY PROCEDURE

1. 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 pipettor.

2. Dilute specimens, to the necessary range with Phosphate Buffer Saline pH 7.0. (urine samples are normally diluted 1:20 for a cutoff level of 300 ng/ml of morphine.) The dilution factor can be adjusted based on the laboratory’s cutoff.

3. Add 10 µl of appropriately diluted calibrators and standards to each well in duplicate.

4. Add 10 µl of the diluted specimens in duplicate (recommended) to each well.

5. Add 100 µl of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.

6. Incubate for 60 minutes at room temperature (18-26 °C) preferably in the dark, after addition of enzyme conjugate to the last well.

7. Wash the wells 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 amounts of hemoglobin (some Postmortem samples), use 10 mM Phosphate buffered saline pH 7.0-7.4. This will lower potential nonspecific binding of hemoglobin to the well, thus lowering background color.

8. 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.

9. Add 100 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.

10. Incubate for 30 minutes at room temperature, preferably in the dark.

11. Add 100 µl of Stop Solution to each well, to change the blue color to yellow.

12. Measure the absorbance at a dual wavelength of 450 nm and 650 nm.

13. Wells should be read within 1 hour of yellow color development.

The following data represent a typical dose/response curve.

<table>
<thead>
<tr>
<th>Morphine (ng/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.669</td>
</tr>
<tr>
<td>5</td>
<td>1.238</td>
</tr>
<tr>
<td>10</td>
<td>0.794</td>
</tr>
<tr>
<td>25</td>
<td>0.133</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.

RESULTS

If the average sample absorbance is equal to or less than the average absorbance of the laboratory morphine positive reference standard the sample is POSITIVE for Opiates. If the average sample absorbance is greater than the average absorbance of the laboratory morphine positive reference standard the sample is called NEGATIVE for Opiates.

Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve.

PERFORMANCE CHARACTERISTICS

1. Accuracy

40 whole blood samples and 40 urine samples collected from presumed non-users were tested in the DAI Opiates ELISA Kit. One hundred percent of these normal samples measured negative at 25 ng/mL for whole blood and 300 ng/mL for urine. Fifty whole blood samples which were previously confirmed positive for Opiates by GC-MS employing a cut-off of 25 ng/mL, were tested in the DAI Opiates ELISA Kit. All of the samples were found to be positive i.e. above the cut-off of 25 ng/mL.

2. Precision

The precision of the DAI Morphine 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, 5, 10 and 25 ng/ml standard was assayed five times in the same assay. The results are tabulated in the following table.

<table>
<thead>
<tr>
<th>Morphine ng/ml</th>
<th>Mean Abs</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.654</td>
<td>0.21</td>
<td>7.9</td>
</tr>
<tr>
<td>5</td>
<td>1.198</td>
<td>0.145</td>
<td>12.1</td>
</tr>
<tr>
<td>10</td>
<td>0.806</td>
<td>0.093</td>
<td>11.54</td>
</tr>
<tr>
<td>25</td>
<td>0.099</td>
<td>0.011</td>
<td>11.1</td>
</tr>
</tbody>
</table>

4. Sensitivity

Assay sensitivity based on the minimum morphine concentration required to produce a four standard deviation from assay Ao is 0.25 ng/mL.

5. Specificity

The specificity of the ELISA for Opiates was determined by generating inhibition curves for each of the compounds listed below The antisera cross-reactivities are listed in Table.
6. 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 (0.25 ng/mL). Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbital, Benzylecgonine, Butobarbital, Caffeine, Cocaine, Carbamazepine, Chloroquine, Chloropromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, Diphenylhydantoin, 10,11-Dihydrocarbamazepine, Diazepam, Doxepin, EMED, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoxin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, LAAM, Lidocaine, LSD, MDA, MDMA, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytoin, a-Methyl-a-propylsuccinimide, Meprobamate, Methyl PEMA, Methoximide, 4-Methylpramidine, Meperidine, Niacinamide, Norethindrone, N-Normethsuximide, Nor LAAM, Nortriptiline, PCP, Phenobarbital, Phenoximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenypropanolamine, Procaine, Propanoxyphe, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline, THCCOOH, Tramadol, O-desmethyl Tramadol, N-Desmethyl Tramadol

REFERENCES

2. Drugs on the Job. Time Magazine, March 17, 1986