Cannabinoids (THCA/CTHC) ELISA

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**
1. Microwells coated with polyclonal anti-carboxy THC 12x8x1
2. THC-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

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

**SUMMARY AND EXPLANATION**
The DAI Cannabinoids ELISA Kit is a specific and sensitive in-vitro test to detect the presence of cannabinoids in samples such as whole blood, serum, plasma and urine. Δ9-THC (a member of the cannabinoid family) is the primary psychoactive ingredient of marijuana (1). Cannabinoid metabolites appear in urine two to four hours after a marijuana smoke and may persist for days (up to thirty) (1-3). Thus a urine assay reasonably serves to detect cannabis use even though a considerable period may have elapsed since smoking or ingestion of marijuana.

**TEST PRINCIPLE**
The DAI Cannabinoids 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 carboxy-THC (THCA) 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. The DAI THC ELISA Kit avoids extraction of urine or blood sample for measurement. It employs a polyclonal high affinity, purified carboxy-THC antibody. Due to the proprietary method of orienting the antibody on the polystyrene micro-plate much higher sensitivity is achieved compared to passive adsorption. This results in extremely small sample size reducing matrix effects and interference with binding proteins(s) or other macromolecules.

**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
2. This test kit is designed for research use only.
3. Do not add sodium azide to samples as preservative. Do not use external controls that contain sodium azide.
4. Viscous samples should always be diluted in phosphate-buffered saline or distilled water prior to pipetting. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that serum samples be run in duplicate.
7. 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 Cannabinoids ELISA Kit is to be used with human samples, such as whole blood, oral fluids, serum, urine and plasma. Has not tested all possible applications of this assay. The cutoff criteria are important in deciding the sample dilution. It is recommended to dilute most blood samples either 1:5 or 1:10 depending on the cutoff used by the laboratory.
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, and should be gently mixed.
1. Dilute specimens, to the necessary range with Phosphate Buffered Saline pH 7.0. (Urine samples 1:10 for a THCA cutoff level of 50 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 60 minutes at room temperature (18-26°C) preferably in the dark, after addition of enzyme conjugate to the last well.
6. Wash well 6 times with 350 µl of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
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.
12. Wells should be read within 1 hour of yellow color development.

The following data represent a typical dose/response curve.

<table>
<thead>
<tr>
<th>CTHC (ng/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.985</td>
</tr>
<tr>
<td>2</td>
<td>1.413</td>
</tr>
<tr>
<td>5</td>
<td>0.955</td>
</tr>
<tr>
<td>10</td>
<td>0.751</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 THCA/CTHC positive reference standard the sample is POSITIVE for cannabinoids. If the average sample absorbance is greater than the average absorbance of the laboratory THCA/CTHC positive reference standard the sample is called NEGATIVE for cannabinoids.

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

35 whole blood samples and 60 urine samples collected from presumed non-users were tested in the DAI Cannabinoids ELISA Kit. One hundred percent of these normal samples measured negative at 20 ng/ml of THCA equivalents for whole blood and 50 ng/ml of THCA equivalents for urine. Forty whole blood samples which were previously confirmed positive for cannabinoids by GC-MS employing a cut-off of 10 ng/ml THCA were tested in the DAI Cannabinoids ELISA Kit. All the samples were found to be positive i.e. above the cut-off of 20 ng/ml.

2. Precision

The precision of the DAI Cannabinoids 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, 2, 5, and 10 ng/ml standard was assayed eight times in the same assay. The results are tabulated as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Abs</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>THCA</td>
<td>1.905</td>
<td>0.139</td>
<td>7.3</td>
</tr>
<tr>
<td>Δ9-THC</td>
<td>1.114</td>
<td>0.103</td>
<td>9.4</td>
</tr>
<tr>
<td>Δ8-THC</td>
<td>0.752</td>
<td>0.066</td>
<td>8.8</td>
</tr>
<tr>
<td>Δ11-THC</td>
<td>0.549</td>
<td>0.042</td>
<td>7.7</td>
</tr>
</tbody>
</table>

4. Sensitivity

Assay sensitivity based on the minimum THCA concentration required to produce a four standard deviation from assay zero dose response (A0) is 1 ng/ml.

5. Specificity

The specificity of the DAI Cannabinoids 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 10 ng/ml THCA</th>
<th>Cross-reactivities</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-nor-9-carboxy-Δ8-THC</td>
<td>11</td>
<td>110</td>
</tr>
<tr>
<td>Δ9-THC</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>Δ8-THC</td>
<td>22</td>
<td>45</td>
</tr>
<tr>
<td>11-hydroxy Δ9-THC</td>
<td>&gt; 1000</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>8,11-Dihydroxy Δ9-THC</td>
<td>&gt; 1000</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Cannabinol</td>
<td>&gt; 1000</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Cannabidiol</td>
<td>&gt; 1000</td>
<td>&lt; 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.
Acetaminophen, Acetylsalicylic acid, Amphetamine, Aminopyrine, Ampicillin, Amobarbital, Ascorbic acid, Atropine, Barbital, Butabarbital, Caffeine, Cocaine, Carbamazepine, Codeine, Chloroquine, Chlorpromazine, Carbromal, Desipramine, Dextromethorphan, Dextropropoxyphene, 5,5-Diphenylhydantoin, 10-11-Dihydrocarbamazepine, Diazepam, Ethosuximide, Estriol, Estrone, Estradiol, Ethotoxin, Glutethimide, Hexobarbital, Ibuprofen, Imipramine, Lidocaine, LSD, Methadone, Methadone-primary metabolite, Methaqualone, Methamphetamine, Metharbital, Mephenytoin, Methyl propylsuccinimide, Methyl PEMA, Methsuximide, 4-Methylprimidon, Morphine, Meperidine, Niacinamide, Norethindrone, Noradrenaline, N-Normethsuximide, Phenobarbital, Phensuximide, PEMA, Primidone, Phencyclidine, Pentobarbital, Phenothiazine, Phenylpropanolamine, Procaine, Quinine, Secobarbital, Tetracycline, Tetrahydrozoline.

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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