False positivity for marijuana in immunoassay analysis due to Efavirenz use.
A case report

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Abstract: Cross-reaction is an important problem with immunoassays that may lead to interferences and potentially yield wrong results. This case study describes a false positive tetrahydrocannabinol (THC) screening with CEDIA immunoassay reagents after the use of efavirenz for anti-retroviral therapy. A 31-year-old HIV (+) probationer transsexual was admitted to Ege University Institute of Drug Abuse, Toxicology and Pharmaceutical Sciences Toxicology Laboratory due to marijuana abuse. He had been using 600 mg/day Efavirenz to overcome his disease. As the routine application in the laboratory, the urine specimens of the probationer were first screened for drugs of abuse using CEDIA reagents. The analysis results were all found to be positive. Subsequently, the urine specimens were also analyzed for their THC content with another urine screening immunoassay kit using DRI (Diagnostic Reagent Inc.) reagents. The results were found to be negative. In order to ensure a correct result, the confirmation of the analysis was performed with the gas chromatography-mass spectrometer for the urine samples and the results were found to be below the detection limit (LOD <1.97 ng/mL). Additionally a hair sample belonging to probationer was obtained, analyzed with a fully validated method and the result was also found to be negative. Therefore, it was concluded that the result obtained with CEDIA reagents was false positive for THC which is, and was caused by Efavirenz use by the probationer. The results demonstrated that there is a strong need for the careful interpretation of the analysis results by an experienced forensic toxicologist to decrease the chances of obtaining wrong results.

Key Words: cross-reaction, Efavirenz, immunoassay, urine, hair.
Therapy with 600 mg/day Efavirenz and Tenofovir (due to marijuana abuse) undergoing anti-retroviral therapy was 31-year-old transsexual, HIV (+) probationer. Given the widespread inclusion of EFV in first-line antiretroviral therapy, the use of THC assays that are subject to EFV interference for probation system is problematic.

Different types of enzymes are used in CEDIA (Cloned Enzyme Donor Immunoassay) and DRI (Diagnostic Reagents, Inc) methods. These screening tests work at different wavelengths and have different specificities [6]. The CEDIA Multi-Level THC assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system, based on the bacterial enzyme-galactosidase that has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme which, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically [7]. The DRI assay uses specific antibodies, and is based on the competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme and free drug from urine sample for a fixed amount of specific antibody binding sites. In the absence of free drug in the urine the specific antibody binds the enzyme-labeled causing a decrease in enzyme activity [8]. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm. In a previous study [4], it was reported that the cross reactions with THC immunoassays was a result of the interaction of EFV metabolite with the antibody complexes used in the assays. Due to these cross-reactions encountered with immunoassay methods [9], it is very important to use different improved kit systems to reduce the misinterpretation of a patient’s results.

**CASE REPORT**

A 31-year-old transsexual, HIV (+) probationer (due to marijuana abuse) undergoing anti-retroviral therapy with 600 mg/day Efavirenz and Tenofovir (United States Department of Health and Human Services guidelines currently recommends the use of Efavirenz in combination with tenofovir/emtricitabine) to overcome his disease [10]. According to Turkish Penalty Code, individuals who have received probation undergo a 6-week treatment program and are subjected to a urine drug screening test and a clinical interview [11]. During the program, the urine specimens of the probationers are analyzed for three times. The urine sample of our HIV (+) probationer was taken under the chain of custody at Institute of Drug Addiction, Toxicology and Pharmaceutical Sciences in Ege University with 15 day intervals. The results of the study can be seen in Table 1. The creatinine concentration and pH of the urine samples were measured to assess possible dilution and to check sample integrity. All values were within the acceptable limits. In addition, no oxidizing agent (glutaraldehyde, nitrite, etc.) was found in the samples.

According to the routine methodology in the laboratory, the urine specimens of the probationer were first screened for drugs of abuse using CEDIA reagents on MGC240 analyser. The analysis results were all found to be positive for THC-COOH (cut-off level: 50 ng/mL). Since it is known that several urine screening immunoassays yields false positive results due to EFV, the results of the analysis were suspicious to the laboratory staff. Subsequently, the urine specimens (all three) were also analyzed for their THC content with another urine screening immunoassay kit using DRI (Diagnostic Reagent Inc.) reagents. The results were found to be below the cut-off value (50ng/mL). At this point, the confirmation of the analysis results has become mandatory with the gas chromatography-mass spectrometer (GC-MS) available at the laboratory. To analyze the urine specimen, the sample is first hydrolyzed and then prepared for analysis using a solid phase extraction (SPE) procedure. After sample derivatization with PFPA/PFPOH, the sample is analyzed. All of the GC-MS results were found to be below the detection limit (LOD <1.97 ng/mL). In order to ensure a correct result, a hair sample belonging to probationer was obtained with voluntary consent form at the third week of the probation programme. The hair sample was analyzed with a fully validated method. It was washed and cut into small pieces and spiked with deuterated internal standard. The sample is then hydrolyzed at 90 °C in 1 M NaOH for 15 minutes. THC-COOH was isolated by a liquid-liquid extraction (LLE) with n-hexane:ethyl acetate (9:1). Aqueous solution was acidified and dried extracts were

<table>
<thead>
<tr>
<th>Urine Sample</th>
<th>CEDIA (cut-off:50ng/mL)</th>
<th>DRI (cut-off: 50ng/mL)</th>
<th>GC-MS (LOQ:1.97ng/mL)</th>
<th>pH (4-9)</th>
<th>Creatinine (&gt;25mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62 ng/mL</td>
<td>&lt; 50</td>
<td>&lt; LOQ</td>
<td>6.0</td>
<td>74.4</td>
</tr>
<tr>
<td>2</td>
<td>77 ng/mL</td>
<td>&lt; 50</td>
<td>&lt; LOQ</td>
<td>6.0</td>
<td>112.0</td>
</tr>
<tr>
<td>3</td>
<td>67 ng/mL</td>
<td>&lt; 50</td>
<td>&lt; LOQ</td>
<td>6.0</td>
<td>133.7</td>
</tr>
</tbody>
</table>
derivatized. After derivatization procedure, aliquot was reconstituted with ethyl acetate, analyzed by GC-MS and result was found to be below the detection limit. As a result, it was concluded that the result obtained with CEDIA reagents is false positive which is caused by Efavirenz use of the probationer.

**DISCUSSION**

Interference in immunoassay is one of the important factors that may result in uncertainty for the forensic analysis. Our findings demonstrate that CEDIA immunoassay reagents used for the detection of THC-COOH are susceptible to cross-reaction errors resulting from the presence of Efavirenz in human urine. Moreover, when another urine screening immunoassays method (DRI reagents) is used, no false-positive results were observed. Therefore, when using urine screening methods with immunoassays, the laboratories should be aware of the potential for interference. The immunoassays techniques only provide useful preliminary clinical information, but the results should be viewed as 'presumptive positive' until confirmed by an independent chemical technique like chromatography.

Given the increasing incidence of HIV infection among substance users and the increasing use of complex combination antiretroviral regimens, the risk of adverse drug interactions with possibly fatal consequences cannot be overlooked or ignored. Physicians should be able to determine whether the positive test results could be related to proper use of a prescription drug or from abuse. Moreover, this work has shown that the more methods become sophisticated and technically advanced, the greater the need for a close contact between the clinician and laboratory to reduce false results in the analysis. In addition, the results demonstrated that there is a strong need for the careful interpretation of the analysis results by an experienced forensic toxicologist in the laboratories to reduce wrong results.

**Conflict of interest.** The authors declare that they have no conflict of interest concerning this article.

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**References**