

mation of the measurement's performance (8). These 20 results are, however, insufficient for reliable quality-control planning. Further updating of the initial estimation as new results are obtained is obligatory.

Through the calculation of CIs for critical errors with the above formulas, it is possible to quantify the reliability of quality judgments. This analysis highlights the significant variability that may exist in such judgments because of the random nature and limited quantity of quality control data. Caution should be exercised when interpreting such data and making decisions on an appropriate quality-control strategy.

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**Wojciech Gernand**

*Department of  
Laboratory Diagnostics  
Medical University of Lublin  
ul. Chodzki 1  
20-093 Lublin, Poland  
E-mail gernand@wp.pl*

#### Characterization of Interference with 6 Commercial $\Delta^9$ -Tetrahydrocannabinol Immunoassays by Efavirenz (Glucuronide) in Urine

To the Editor:

We observed that antiretroviral therapy with efavirenz (EFV) produces urine samples that screen positive for  $\Delta^9$ -tetrahydrocannabinol (THC) exposure, despite the absence of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid (THC metabolite). These observations were made when we were using immunoassay-based reagents to screen for drugs of abuse in patients enrolled in a study on the effects of inhaled marijuana on HIV-related neuropathy. Extensive anecdotal literature exists regarding the interference of EFV with antibody-based assays for THC metabolites in urine. In product literature, the manufacturer of EFV (Sustiva<sup>®</sup>; BMS Virology) states that EFV may interfere with THC metabolite immunoassays (1). An earlier letter to *Clinical Chemistry* discusses the interference of EFV with an ELISA for estradiol (2). We therefore characterized the occurrence of EFV cross-reactivity with several commercial reagents used to screen for THC exposure.

We hypothesized that cross-reactivity with THC immunoassays was the result of the interaction of EFV metabolite (and not a parent drug) with the antibody complexes used in the assays. The majority of EFV in urine exists as an 8-position hydroxylated metabolite [8-hydroxy-efavirenz (EFV-8-OH)] and/or its 8-ether glucuronide (EFV-8-G) (3). Findings with 2 different THC immunoassays [Instant-View MultiDrug Screen Urine Test (Alfa Scientific Designs, Inc.) and Cannabinoids (THCA/CTHC) Direct ELISA Kit (Immunoanalysis Corporation)] revealed that cross-reactivity was attributable to EFV-8-G, and not EFV-8-OH or a parent drug. Initial studies were performed after isolation of the parent drug and metabolites from EFV tablets by organic solvent extraction and from EFV-positive urine by HPLC fraction collection, respectively. Naive urine was then

supplemented with EFV-8-OH, EFV, or E-8-G. Urine containing EFV-8-OH or EFV alone exhibited negligible and no cross-reactivity, respectively, whereas naive urine supplemented with EFV-8-G exhibited a threshold-bound, concentration-independent cross-reactivity similar to that observed in urine from patients undergoing EFV therapy. Furthermore, hydrolysis of EFV-positive urine by either enzymatic (glucuronidase) or acidic thermal treatment abolished the interference. These initial observations support the hypothesis that EFV interference with THC immunoassays is mediated through cross-reactivity involving EFV-8-G, supporting the need to characterize the EFV-related interference with THC immunoassays.

Urines from 8 individuals undergoing antiretroviral therapy with 600 mg EFV/day were randomized for analysis by 6 different instrument-based THC immunoassays. Patients providing urine for these studies gave informed consent before analyses, and the studies were performed in accordance with guidelines established by the University of California, San Diego, Institutional Review Board and Human Subjects Committee. Hydrolyzed duplicates of each sample were also prepared by the addition of 50  $\mu$ L of concentrated HCl/mL of urine, followed by heating to 80 °C in a laboratory microwave oven (20 s at a relative power setting of high). Concentrations of EFV, EFV-8-OH, and EFV-8-G were determined by HPLC with ultraviolet detection. The purity and identity of each HPLC peak were confirmed by nanospray tandem mass spectrometry (MS/MS) (3). 11-Nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid was quantified by isotope-dilution gas chromatography-mass spectrometry (GC-MS) (4). Table 1 presents data for the above concentrations and the responses of 6 commercial THC immunoassays to urine from patients undergoing EFV therapy. These data show that immunoassays performed with reagents from Microgenics Corporation (Cedia<sup>®</sup> Dau MultiLevel THC), BioSite Incorporated (Triage<sup>®</sup> TOX Drug Screen), and Im-

**Table 1. Concentrations of THC metabolite and EFV metabolites in patient urines tested for THC metabolites by immunoassay reagents from multiple vendors.**

Patient	nor-THCOOH, <sup>a</sup> μg/L	EFV, <sup>b</sup> mg/L	EFV-8-OH, <sup>b</sup> mg/L	EFT-8-G, <sup>b</sup> mg/L	Immunoassay result <sup>c</sup>					
					BioSite	Dade-Behring	OraSure	Immunalysis	Abbott	Cedia-Dau
1	1.4	<0.1	3.8	39.6	THC+	Neg	Neg	THC+	Neg	THC+
2	<0.1	0.1	23.7	11.2	THC+	Neg	Neg	THC+	Neg	THC+
3	<0.1	<0.1	94.8	0.9	Neg	Neg	Neg	THC+	Neg	Neg
4	0.7	<0.1	4.4	3.6	Neg	Neg	Neg	THC+	Neg	THC+
5	3.4	0.1	27.0	1.8	Neg	Neg	Neg	THC+	Neg	THC+
6	0.4	<0.1	20.4	2.6	Neg	Neg	Neg	THC+	Neg	THC+
7	<0.1	<0.1	65.5	14.0	THC+	Neg	Neg	THC+	Neg	THC+
8	<0.1	<0.1	13.0	16.6	THC+	Neg	Neg	THC+	Neg	THC+

<sup>a</sup> nor-THCOOH, 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid, as measured by GC-MS.

<sup>b</sup> Concentrations of EFV, EFV-8-OH, and EFT-8-G were determined by HPLC with ultraviolet detection, and peak purity and identity were confirmed by nanospray MS/MS.

<sup>c</sup> THC+, positive test result indicating THC metabolite concentration greater than the stated cutoff value (50 μg/L); Neg, negative test result indicating THC metabolite concentration below the stated cutoff value.

munalysis Corporation [Cannabinoids (THCA/CTHC) Direct ELISA Kit] were subject to interference attributable to urinary EFV-8-G. Reagents from these 3 vendors produced test results indicating the presence of THC metabolite above the immunoassay cutoff value of 50 μg/L, although GC-MS analysis gave a measured THC metabolite concentration <5 μg/L in all samples. False-positive findings from the above immunoassays were reversed by acid hydrolysis of urine before re-testing, with a single exception (patient 1). Nanospray MS/MS of acid-treated urine verified the conversion of EFV-8-G into EFV-8-OH in all cross-reacting samples except for the single sample that remained cross-reactive; this sample exhibited incomplete hydrolysis (20% hydrolysis). There were no occurrences of false-positive findings in immunoassays performed with reagents from Dade-Behring Incorporated (Syva<sup>®</sup> DAT), OraSure Technologies (Cannabinoids Intercept MicroPlate EIA), and Abbott Laboratories (AxSYM Cannabinoids Reagents).

Despite the existence of extensive anecdotal literature concerning interference by EFV in urinary THC immunoassays, these data are the first characterization of the nature and extent of this interference with commonly used "Drugs of Abuse" screening reagents. These findings demonstrate that some, but not all, immunoassay reagents used for the

detection of THC metabolite are susceptible to cross-reaction errors resulting from the presence of EFV (metabolite) in human urine.

We sincerely appreciate the clinical support provided by the University of California, San Diego, HIV Neurobehavioral Research Center. Microgenics Corporation (Fremont, CA), BioSite Incorporated (San Diego, CA), Immunalysis Corporation (Pomona, CA), and OraSure Technologies (Bethlehem, PA) graciously provided complimentary assays or reagents. These studies were funded in part by the Center for Medicinal Cannabis Research, University of California, San Diego.

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Steven Rossi<sup>1\*</sup>  
Tony Yaksh<sup>1</sup>  
Heather Bentley<sup>2</sup>  
Geoffrey van den Brande<sup>2</sup>  
Igor Grant<sup>3</sup>  
Ronald Ellis<sup>4</sup>

<sup>1</sup> Departments of Anesthesiology  
<sup>3</sup> Psychiatry, and <sup>4</sup> Neurosciences  
University of California, San Diego  
La Jolla, CA

<sup>2</sup> HIV Neurobehavioral  
Research Center  
Center for Medicinal  
Cannabis Research  
San Diego, CA

\* Address correspondence to this author at: Department of Anesthesiology, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0818. Fax 619-543-6070; e-mail srossi@ucsd.edu.

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**Metabolic Syndrome: Older than Usually Assumed, But Still Too Young to Die**

To the Editor:

In a recent issue of the Journal, Dr. Gerald Reaven informed us of the death of the metabolic syndrome (1).