Marijuana Detection Times: Does the Methodology Validate the Conclusions?

To the Editor:

A recent article (1) reported an evaluation of a number of commercially available immunoassays and their ability to detect marijuana metabolites after a single, controlled dose of tetrahydrocannabinol (THC). The authors reported two major conclusions: 1. Significant differences exist between commercially available immunoassays and 2. Even the best performing immunoassay only provided a one- to two-day window of detection when using a 50-ng/mL cut-off.

The authors were very careful to control the clinical aspects of the study, to the point of sequestering the volunteers on the clinical ward for the duration of the study. However, it appears that less care was given to an equally important aspect of the study—the analytical variables.

In the article, the authors described the analytical protocol as follows: “Individual urine specimens (N = 957) were collected throughout the three week study. Each specimen was analyzed under blind conditions by immunoassay according to the manufacturer's instructions. All urine specimens were also analyzed for THCCOOH by GC–MS with a 15-ng/mL cut-off according to a previously published procedure (25).”

From this description and from the absence of any notation to the contrary, the reader would assume that all the assays were performed at the same time using fresh specimens. However, based upon discussions with the authors, it is apparent that this was not the case and that the study protocol failed to control three important methodological variables that would certainly contribute to the discrepancies observed among the immunoassay results.

The methodological variables in the study which contribute to the inconsistencies reported by the authors include: 1. Immunoassays were performed in at least three different laboratories. The radioimmunoassay (RIA) and gas chromatographic–mass spectrometric (GC–MS) testing were performed at the Navy Drug Screening Laboratory at Norfolk, VA; the OnLine immunoassays were performed at Roche Diagnostic Systems’ facilities in Nutley, NJ; and the other immunoassays were conducted at other sites. 2. Immunoassays were performed at different times, which ranged from a few months to a number of years after specimen collection. Based upon information from the authors, all the samples were collected between March and October 1990, and the RIA and GC–MS assays were performed between March 1991 and March 1992. The OnLine assays were run over a three day period in November 1991. 3. Although all aliquots were frozen (−30°C), different volumes were taken for the various assays. For example, a 2.5-mL aliquot was taken for the OnLine assays, but a 30-mL aliquot was taken for the RIA and GC–MS assays.

The instability of marijuana metabolites in urine is well known, although not well documented. Cody reported this effect at the 1988 TIAFT meeting (2). Several variables contribute to the degradation of marijuana metabolites. Among the most important are the number of freeze/thaw cycles of the sample; the type of storage container used for the urine sample; the ratio of the sample volume to the container size; and the time between sample collection, storage, and testing.

To publish acceptable data on a comparison of cannabinoid immunoassays, it is very important that all these factors be controlled. Retaining all aliquots under identical conditions and performing all the comparison assays at the same time would be the optimal experimental protocol to accomplish this. These safeguards were not followed with the aliquots collected during this study. Therefore, the scientific validity of the data is open to question, and the results are not representative of the true performance of the various immunoassays evaluated.

The second conclusion of the authors, that there is a one- to two-day window for the detection of marijuana in urine after a single, controlled dose of THC, must also be questioned as the analytical data was not collected under optimal conditions. Analyzing frozen, 2.5-mL aliquots many months after sample collection is not representative of current routine testing practices in workplace or other drug testing programs.

The premise of this study was one of value, and the authors successfully controlled one of the most difficult aspects—the sample collection protocol. Unfortunately, they failed to satisfactorily control an equally important aspect of the study—the analytical variables. As such, the conclusions reached in this study are of questionable value.

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References


The authors reply:

We would like to respond to the Letter to the Editor from S.J. Salamone concerning our recent article (1). Dr. Salamone was concerned that we did not perform all ten assays at the same time on fresh specimens. As a result, Dr. Salamone questioned the analytical aspects of the analysis of cannabinoid metabolites by the nine different commercially available immunoassays and the analysis of 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THCCOOH) by gas chromatography–mass spectrometry (GC–MS).

Dr. Salamone, who is responsible for the development and production of the Roche OnLine™ immunoassay product line, contacted us after publication of the article, and we supplied him with details of the collection, storage, and analysis of the urine specimens.

Six subjects were confined to the closed research ward of the Addiction Research Center, Baltimore, MD, for four to six weeks during the study. Each individual urine void was collected from the six research subjects over a period of four weeks (n = 957) and refrigerated immediately. Each void was measured, poured into the appropriate storage container, and frozen at −30°C on the day of collection. Three milliliters of urine was pipetted into each of four 4-mL polypropylene cryotubes; 15 mL was poured into a 30-mL polypropylene screw cap bottle; and 30 mL was poured into a 60-mL polypropylene screw cap bottle. After the seven month protocol was complete, the frozen specimens were assembled, coded, and randomized. This procedure ensured that all specimens were randomized for analysis within the large batch to eliminate any potential based on subject, dose, time of collection, or individual calibration. We consider it an important aspect of our validity studies that all assays are performed under blind conditions. Codes were not broken until the completion of the assay. Specimens were shipped frozen on dry ice to the analytical laboratory when the work was not performed in-house.

The assay comparison study was performed over a period of approximately one year after the completion of the clinical study. GC–MS analyses were performed by the Navy Drug Screening Laboratory, Jacksonville, FL, between March 1991 and March 1992. The Roche Diagnostic Systems OnLine assays (Somerville, NJ) were performed at the Roche Diagnostic Systems Laboratory in Nutley, NJ by Roche laboratory personnel over three days in November 1991. Oversight of the analytical process was provided by Dr. Huestis. The analysis of specimens by the Diagnostic Products Milenia™ immunoassay occurred at the Ochsner Foundation Hospital in Jefferson, LA in late 1991 and early 1992. The EMIT® d.a.u.™ 100, 50, 20 and EMIT II 100 and 50 from Syva Co. (San Jose, CA); the Diagnostic Reagents DRI (Mountain View, CA); and Abbott Diagnostics ADx® (Abbott Park, IL) assays were performed in the analytical laboratories of the Addiction Research Center, Baltimore, MD (in-house), in 1991 and early 1992. One of the reasons for the extended analytical time period, in addition to the large number of specimens tested, was the introduction of new commercial cannabinoid assays, including the EMIT II, DRI, and OnLine products. Specimens were thawed just prior to analysis for all of the assays.

Dr. Salamone indicated in his letter that the use of different laboratories could contribute to noted inconsistencies between assays. Each laboratory routinely performed the type of immunoassay analysis that was tested, and in the case of the Roche OnLine assay, the tests were performed by Roche Diagnostics research personnel. Personnel were unaware of the identity of the specimens; therefore, no manipulation of results could have occurred. The analyses of the DRI and Milenia assays were among the last assays to be performed, and the Milenia cannabinoid assay produced the most sensitive and most specific immunoassay test results in comparison with the GC–MS “gold standard” data. These data were not published because the Milenia product line was withdrawn by Diagnostic Products prior to publication of the article.

The stability of cannabinoids in urine over time is an important issue. Dr. Salamone refers to the work of Cody (2) that was presented at the 25th International Meeting of TIAFT in Groningen, The Netherlands. The author discusses the stability of cannabinoid metabolites in spiked quality control samples and in human urine specimens found to be positive for cannabinoid metabolites. Urine quality control pools were found to be stable for a period of at least six months. More variability was noted in the routine human urine specimens, despite identical storage conditions. Some routine specimens retained essentially the same amount of THCCOOH over an extended period of time; however, many