TECHNICAL NOTE

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Comparison of Abbott Fluorescence Polarization Immunoassay (FPIA) and Roche Radioimmunoassay for the Analyses of Cannabinoids in Urine Specimens

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ABSTRACT: Abbott fluorescence polarization immunoassay (FPIA) and Roche Abuscreen radioimmunoassay (RIA) were compared qualitatively with 142 urine specimens containing 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid. Similar qualitative results were obtained in 132 specimens. When discrepent results were observed, all negative results were within 20% of the 100 ng/mL cut-off. We concluded that FPIA and RIA give comparable results to each other.

KEYWORDS: toxicology, immunoassay, marijuana, urine, cannabinoids

Screening for marijuana use is a major component of drug abuse testing in civilian and military laboratories. The major urinary metabolite of marijuana, 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (carboxy THC) is readily detectable by commercially available immunoassay systems, such as enzyme multiplied immunoassay technique (EMIT®), radioimmunoassay (RIA), and fluorescence polarization immunoassay (FPIA). For many years, the military drug testing laboratories have used Roche Abuscreen RIA as an initial screening test for cannabinoid use. The administrative cut-off established by the military was 100 ng/mL; all specimens which screened by RIA greater than 100 ng/mL were defined as positive and sent for confirmation by gas chromatography/mass spectrometry (GC/MS).

In 1990, the Department of Defense considered a change in its screening methodology

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in all of its drug testing laboratories from Roche Abuscreen RIA to Abbott HTDx FPIA. The following report is a comparison of these two immunoassays at the 100-ng/mL cut-off with GC/MS.

Experimental Procedure

Materials

Urine specimens were obtained from the Forensic Toxicology Drug Testing Laboratory, Fort Meade, Maryland. HTDx FPIA reagents were obtained from Abbott Diagnostics, Inc. Abuscreen RIA for cannabinoids was acquired from Roche Diagnostic Systems. Carboxy THC and d_3-carboxy THC, each 0.1 mg/mL were obtained from Research Triangle Institute. All acids, bases, and salts were Baker reagent grade and all solvents were Fisher pesticide grade. Tetramethylammonium hydroxide (TMAH) and iodomethane were purchased from Eastman Kodak Company.

Methods

FPIA—The Abbott TDx was operated in accordance with the operator's manual. A six-point calibration curve was generated using the calibrators provided by Abbott. Any urine specimen producing a result greater than the highest calibrator was manually diluted with drug free urine and reanalyzed. Quality control specimens were run with each carousel.

RIA—The analysis was performed as specified in the package insert. The pellets were counted on an ICN 4/200 Plus gamma counter. A negative calibrator and a 100-ng/mL positive calibrator were run in duplicate at the beginning of the batch. Negative and positive quality control specimens were run every 15 samples throughout the batch.

GC/MS Analysis—Carboxy THC was quantitated on a Hewlett Packard 5890 gas chromatograph equipped with a 5970 mass selective detector. A DB-5 (15-m by 0.2-mm inside diameter [ID], 0.25-μm film thickness) column provided chromatographic separation. Helium was the carrier gas flowing at 1 mL/min. The injector temperature was 270°C, the transfer line was 280°C, and the oven temperature was 255°C (isothermal). The mass spectrometer was operated in the selected ion monitoring mode with six ions being monitored: 313, 357, and 372 for carboxy THC and 316, 360, and 375 for d_3-carboxy THC. The ratio used for quantitation was 357/360. A blank, four standards, and a quality control were run with each batch.

To 3-mL blank, standard, control, or unknown specimen were added 0.3-mL 10*N* potassium hydroxide and incubated at 50 to 60°C for 15 min. After neutralization and acidification with phosphate buffer and concentrated hydrochloric acid, the specimens were extracted with hexane:ethyl acetate (7:1). The organic layers were separated and evaporated to dryness. The residues were dissolved in 0.1-mL 5% TMAH in dimethylsulfoxide and 0.01-mL iodomethane. After 5 min, 0.2 mL of 0.1*N* hydrochloric acid (HCl) and 2-mL iso-octane were added. After mixing and centrifuging, the iso-octane layer was concentrated to about 0.025 mL and chromatographed.

Results and Discussion

A total of 142 specimens were analyzed by FPIA and RIA using the 100-ng/mL cut-off. The samples represented a range of concentrations above and below this administrative screening cut-off. Drug-free samples were not investigated since previous studies showed that each of the immunoassay methods in this comparative study were able to

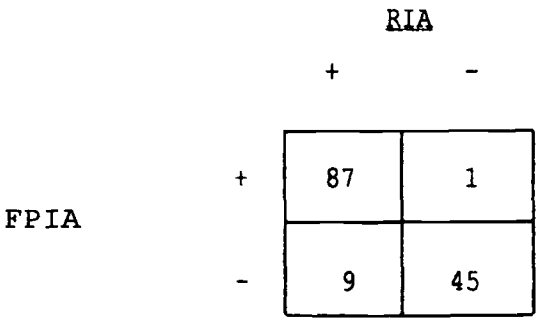

FIG. 1—*Comparison of FPIA with RIA using the 142 specimens analyzed where + = number of specimens with concentrations greater than or equal to 100 ng/mL and − = number of specimens with concentrations less than 100 ng/mL.*

distinguish these from positive urine specimens [*1,2*]. Eighty-seven tested positive by both methods, one screened positive by FPIA and negative by RIA, and nine screened positive by RIA and negative by FPIA. Forty-five specimens screened negative by both immunoassays. This information is displayed in Fig. 1. The data from specimens giving different results between FPIA and RIA are given in Table 1.

The two immunoassays produced similar screening results. Of the 142 specimens tested, 132 generated the same qualitative results. All specimens negative by FPIA and positive by RIA were within 20% of the cut-off, and 7 of the 9 specimens were within 10% of the cut-off. Similarly, the RIA counts per minute of the specimen negative by RIA and positive by FPIA were within 10% of the counts per minute produced by the cut-off calibrator. These data suggest that the RIA antibody might have slightly broader specificity in screening for cannabinoids. However, this slight difference is not deemed significant. Note that FPIA as originally established by Abbott recommended a cut-off of 25 ng/mL [*3*]. This is consistent with the more specific antibody used in FPIA. Using a 25-ng/mL cut-off, 141 of the 142 specimens analyzed screened positive by FPIA. Eight specimens failed to confirm for carboxy-THC at the 15-ng/mL confirmation cut-off, but all confirmed at the method limit of detection of 1 ng/mL. We therefore conclude that either of these immunoassays could be used singularly in a mass drug-screening laboratory and generate comparable results.

TABLE 1—*Specimens with discrepent immunoassay results.*

FPIA, ng/mL	RIA, +/−	GC/MS, ng/mL
110	−	47
81	+	40
89	+	25
92	+	35
95	+	15
95	+	43
96	+	36
98	+	22
98	+	29
99	+	45

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