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Comparison of Fluorescence Polarization Immunoassay, Enzyme Immunoassay, and Thin-layer Chromatography for Urine Cannabinoid Screening

Effects of Analyte Adsorption and Vigorous Mixing of Specimen on Detectability

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ABSTRACT

Four commercial assays for the screening of cannabinoids in urine were compared. Urine specimens from 93 selected subjects were run by fluorescence polarization immunoassay on the Abbott TDx; by enzyme multiplied immunoassay with two Syva EMIT assays; and by thin-layer chromatography with the TOXI-LAB system (Marion Laboratories). The TDx cannabinoid threshold can be set anywhere from 25 to 150 μg per L. Twenty-five μg per L was chosen for this study. The thresholds for EMIT are fixed at 20 μg per L for one assay and 100 μg per L for the other. The detection limit for TOXI-LAB, according to the manufacturer, can be anywhere from 5 to 50 μg/L, depending on the specimen. Urines, positive by at least one method, were further analyzed by gas chromatography with mass spectrometry (GC/MS), The detection limit for the GC/MS method was 10 μg per L. The results showed a few false negatives and unconfirmable positives; in general, correlation was considered acceptable. Dose-response curves comparing TDx and EMIT gave paralell results, with comparable cross-reactivity for the major metabolite, 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid (Δ-9-THC-COOH). A dose-response study of TOXI-LAB using Δ-9-THC-COOH also gave acceptable results. Adsorption to glass was investigated using spiked urine; a 27 percent reduction in concentration was caused by this phenomenon. Foaming of spiked urine caused by vigorous mixing resulted in a reversible 89 percent apparent reduction in concentration.

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Introduction

The reliable detection of 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid (Δ-9-THC-COOH), the major metabolite of Δ-9-THC, presents extraordinary challenges to the clinical toxicology laboratory. While THC metabolite assay still presents some technical problems, the real difficulties lie in the purpose for which the assay is performed, the interpretation and use of the results, and the social cost of making an error.

The objectives of THC metabolite assay or cannabinoid screening are rarely medical. The presence of Δ-9-THC-COOH in urine correlates well with the use of marijuana, at least when the concentration is high enough, but this positive finding is a poor indicator of pharmacologic or toxic effect.[5] Rather, the purposes of THC metabolite assay are usually forensic or administrative. Quite simply one is trying to determine if an individual has broken the law, and perhaps additional regulations, by ingesting THC in some form.

In the case of job-related testing, it is frequently inferred that the subject with a positive test result may have compromised his job performance, and perhaps his safety and the safety of others, through his illicit activity. This conclusion has a thready connection to the data, however.

In spite of continuing debate over the pharmacologic, legal, and ethical issues, urine testing for THC metabolite is here to stay. If a toxicology laboratory is to offer drug screening, it will be expected to provide this analyte as part of its menu. Consideration of the potential for damage to an individual's future which could be caused by a positive result emphasizes two points.

Whatever method or combination of methods used, the prime requirement must be that of specificity. Also, the user of the data must in some effective way be educated as to the true significance of the result. Some concept of predictive value and the possibility of sampling or analytical error should be communicated.

In the absence of GC/MS capability, the minimum recommended requirement for forensic urine cannabinoid assay is the employment of two techniques based on two different scientific principles.[5]

In our laboratory, investigation has been made of the use of either an enzyme immunoassay (EMIT®) or a fluorescence polarization immunoassay (TDx®) in tandem with a thin-layer chromatographic method (TOXI-LAB®) in terms of efficiency of identifying the presence of cannabinoids.

Materials and Methods

Reagents

Manufacturer's instructions were followed for each of the assays. The two Syva assays were EMIT® d.a.u.™ Cannabinoid 20 ng Assay and the EMIT® d.a.u.™ Cannabinoid 100 ng Assay. Calibrators are not included in the reagent kits and must be purchased separately for each of the two assays. The instrumentation for EMIT® was a Gilford Stasar III spectrophotometer with a Syva pipettor-dilutor 1500, a Gilford 3021 vacuum pump and a Syva 1000 computer printer. Cannabinoid "reagent packs" and a set of calibrators were obtained from Abbott Diagnostics and were used on a TDx® analyzer with software revision 11.2. The TOXI-LAB® Cannabinoid Initial System was purchased from Analytical Systems. Chemicals required but not supplied by Analytical Systems were reagent grade or better. Diethylamine was purchased from Sigma Chemical Co., dichloromethane from EM Science, and acetone from J. T. Baker Chemical Co. The following were purchased from

Fisher Scientific: hydrochloric acid, ethyl acetate, n-hexane, glacial acetic acid, and n-heptane. The GC/MS analysis was done by Damon Clinical Laboratories using a Perkin-Elmer gas chromatograph with a Finnegan ITD mass spectrometer. Ion monitoring was used for the detection of Δ-9-THC-COOH. The Δ-9-THC-COOH was obtained from Altech Applied Science. Bovine albumin was Sigma product number A4503.

Three systems, EMIT®, TDx® and TOXI-LAB®, use the isomer Δ-8-THC-COOH as calibrator or standard. Besides detecting Δ-9-THC-COOH, the EMIT® and TDx® assays also detect 11-hydroxy-Δ-9-THC, 8-β-hydroxy-Δ-9-THC and 8-β-11-dihydroxy-Δ-9-THC. In addition, Δ-9-THC is detected by the EMIT® 100 ng assay and cannabinol by TDx®[6,7] With TOXI-LAB®, Δ-9-THC-COOH and 11-hydroxy-Δ-9-THC display the same detection characteristics as Δ-8-THC-COOH.[7]

Specimen Collection and Preparation

A total of 93 urine specimens were obtained for the comparative study. Damon Clinical Laboratories* provided 39 specimens from a methadone clinic. Most of those urines tested positive by their screening method. The determinations were made by the EMIT® d.a.u.™ 100 ng assay using a COBAS-BIO® instrument.† The rest of the urines were obtained from 54 volunteers, most of whom were expected to give negative results.

The TOXI-LAB® dose-response study was done with drug-free urine spiked in glass tubes with Δ-9-THC-COOH to concentrations of 4, 8, 15, 38, 56, 75, 150, and 375 μg per L. The TDx® and EMIT® dose-response curves were done with the same Δ-9-THC-COOH standards and with the assay calibrators. The TDx® assay calibrators consist of a negative sample and five standards at 25, 40, 60, 80, and 150 μg per L. The two EMIT® assay calibrator sets each consist of a negative sample and two standards, one at the threshold (20 and 100 μg per L) and the other at a higher concentration (75 and 400 μg per L). Standards were prepared of additional concentrations of the Δ-8 isomer for EMIT® by diluting the 750 μg per L TOXI-LAB® cannabinoid control with drug-free urine to concentrations of 5, 10, 20, 50, 75, 100, 200, and 500 μg per L.

Adsorption Study

Adsorption of Δ-9-THC-COOH to glass was investigated in two ways. First, drug-free urine was spiked with Δ-9-THC-COOH in both a TOXI-LAB® glass extraction tube and a 5 oz. plastic container ordinarily used for clinical urine collections.* In a second experiment, bovine albumin was added to urine in the glass tube at a concentration of 10.0 g per L before spiking with the metabolite. Spiking in both cases was done using a Hamilton 50 μL syringe. Fifty microliters of a 7.5 mg per L methanolic solution of the metabolite was added directly into 5.0 mL of urine and mixed gently to give a final concentration of 75 μg per L. Concentrations were determined on the TDx® three times over a period of at least three hours in both experiments.

In another experiment, using the spiked urines from the second glass-adsorption experiment, an attempt was made to free the glass-adsorbed Δ-9-THC-COOH by sonicating, freezing, and shaking the urines. Concentrations

* Westwood, MA.

† Roche Diagnostic Systems, Montclair, NJ.

* Superior Plastic Products, Providence, RI.

TABLE I

Comparison of Urinary Cannabinoid Assay Results

Method	*Threshold or Detection Limit*	*Number of Urines* 53	32	3	1	1	1	1	1
TDx®	25	-	+	+	-	+	+	+	+
EMIT®20ng	20	-	+	+	-	-	+	-	+
EMIT®100ng	100	-	+	-	-	-	+	+	+
TOXI-LAB®	5 - 50	-	+	+	+	+	-	+	+
GC/MS (μg/L)	10		32-2523	25-41	-	381	406	648	-

were determined on the TDx® immediately after a 30 minute sonication, after which the urines were frozen overnight and assayed again. Then they were shaken by hand for about 20 seconds, which produced a foam "head" on the specimen. After measuring concentration again, an attempt was made to eliminate the foam through various methods. Sonication, freezing, application of a stream of nitrogen and vacuum failed to produce satisfactory results. Application of four drops of 1:100 aqueous suspension of 2-octanol to the urine samples was found to be effective in dissipating the foam.

Results and Discussion

The results of the comparative study are shown in table I. For the purposes of this study, a positive result with a screening method is considered a confirmed positive finding if Δ-9-THC-COOH was found by GC/MS assay at a concentration of at least 10 μg per L. Fifty-three urines were negative by all four screening assays. Thirty-two gave consistently positive results by the screening methods and were confirmed positive by GC/MS with results ranging from 32 to 2,523 μg per L. All four screening assays gave uncomfirmable positive results on one of the specimens. TOXI-LAB® gave one additional unconfirmable positive result and one false negative. EMIT® 100 ng gave one false negative and EMIT® 20 ng gave two. One of the latter was contradicted by EMIT® 100 ng which gave a positive result. This specimen was sent to an independent laboratory which confirmed our findings. The concentrations of Δ-9-THC-COOH obtained by GC/MS on 38 specimens ranged from 25 to 2,523 μg/L with a mean of 423.

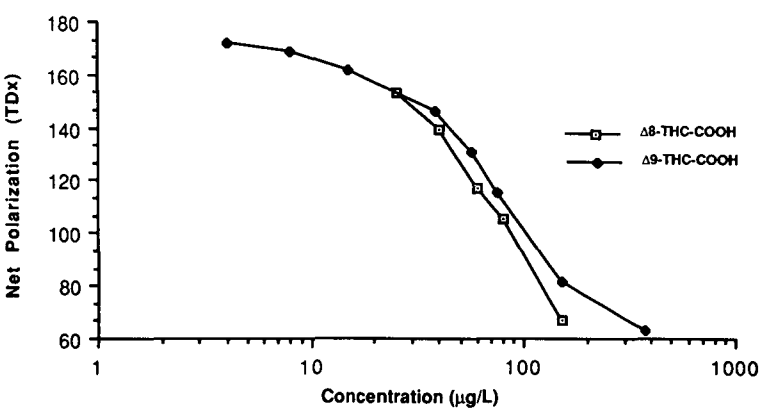

FIGURE 1. TDx cannabinoid assay dose-response study.

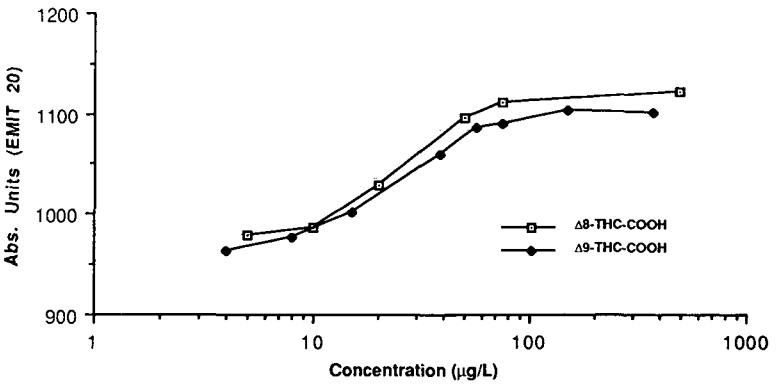

FIGURE 2. EMIT 20 ng cannabinoid assay dose-response study.

The TOXI-LAB® dose-response study indicated a detection limit of about 20 µg per L for Δ-9-THC-COOH in the specimen which was used for this experiment. Migration distance was constant throughout the 5 to 500 µg per L range. Band color and intensity varied consistently with concentration.

The dose-response curves for the TDx® and the two EMIT® assays for both Δ-9-THC-COOH and the Δ-8 isomer are shown in figures 1, 2, and 3. In each case, the two isomers gave a parallel response.

In table II are given the results of the glass-adsorption experiments The average measured concentration of Δ-9-THC-COOH is shown for samples in untreated glass, in plastic, and in glass with albumin added to the sample. The concentration found was 27 percent lower in untreated glass-stored samples vs. both the plastic and albumin-containing specimens. Measured concentrations remained the same over a three to four hour period at room temperature.

The urines contained in glass were then used to determine if sonication, freezing, or vigorous mixing would free adsorbed Δ-9-THC-COOH from the glass. Sonication and freezing caused no change in the measured concentrations. Shaking the urines had a dramatic negative effect, especially on the albumin-free urine. The measured concentrations after shaking were 89 percent lower in the shaken albumin-free urine, and 55 percent lower in the urine which contained the added protein.

The reduction of measured concentration which results from shaking could be reversed by defoaming the sample with

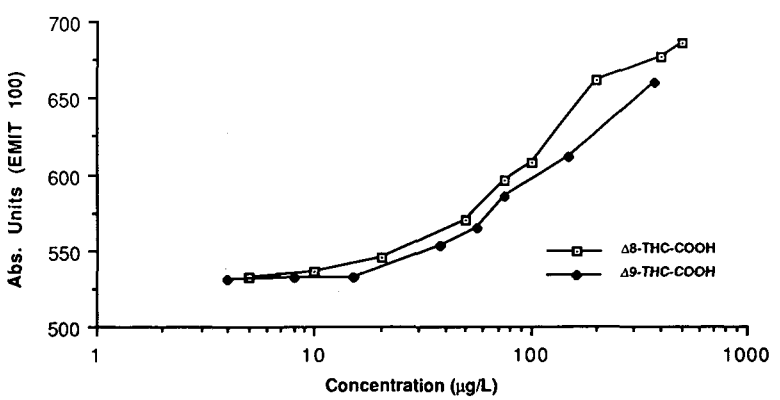

FIGURE 3. EMIT 100 ng cannabinoid assay dose-response study.

TABLE II

Adsorption of Cannabinoids to Glass

	Container	*Contains Albumin*	*Mean TDx® Result (μg/L)*
Experiment 1	Plastic	No	73.9
	Glass	No	58.1
Experiment 2	Glass	Yes	65.8
	Glass	No	51.9

dilute 2-octanol, or by allowing the urine sample to sit for 90 minutes while the foam subsided.

The TDx®, TOXI-LAB®, and EMIT® systems demonstrate adequate sensitivity and specificity for a qualitative assay for a Δ-9-THC-COOH in urine, although our data indicate false negative results seem possible at least with the EMIT® and TOXI-LAB® systems. Rapid preliminary screening can be done on the TDx® or on an automated EMIT® system, followed by confirmation of positive results with the more labor-intensive TOXI-LAB® system. This use of two systems in series fulfills the characteristics of a forensic urine cannabinoid assay recommended in the work of Sutheimer et al.[5]

Our results also demonstrate the sometimes overlooked importance of careful collection and handling of specimens for cannabinoid screening. This factor is more significant when the concentration of the sample is close to the threshold or sensitivity of the assay.

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