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Validation of an Enzyme Immunoassay for Detection and Semiquantification of Cannabinoids in Oral Fluid

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Abstract

BACKGROUND—Oral fluid (OF) is gaining prominence as an alternative matrix for monitoring drugs of abuse in the workplace, criminal justice, and driving under the influence of drugs programs. It is important to characterize assay performance and limitations of screening techniques for Δ^9-tetrahydrocannabinol (THC) in OF.

METHODS—We collected OF specimens by use of the Quantisal™ OF collection device from 13 daily cannabis users after controlled oral cannabinoid administration. All specimens were tested with the Immunalysis Sweat/OF THC Direct ELISA and confirmed by 2-dimensional GC-MS.

RESULTS—The limit of detection was <1 μg/L THC equivalent, and the assay demonstrated linearity from 1 to 50 μg/L, with semiquantification to 200 μg/L. Intraplate imprecision (n = 7) ranged from 2.9% to 7.7% CV, and interplate imprecision (n = 20) was 3.0%–9.1%. Cross-reactivities at 4 μg/L were as follows: 11-hydroxy-THC, 198%; Δ^8-tetrahydrocannabinol (Δ^8-THC), 128%; 11-nor-9-carboxy-THC (THCCOOH), 121%; THC (target), 98%; cannabinol, 87%; THCCOOH-glucuronide, 11%; THC-glucuronide, 10%; and cannabidiol, 2.4%. Of 499 tested OF specimens, 52 confirmed positive (THC 2.0–290 μg/L), with 100% diagnostic sensitivity at the proposed Substance Abuse and Mental Health Services Administration screening cutoff of 4 μg/L cannabinoids and GC-MS cutoff of 2 μg/L THC. Forty-seven specimens screened positive but were not confirmed by 2D-GC-MS, yielding 89.5% diagnostic specificity and 90.6% diagnostic efficiency. Thirty-one of 47 unconfirmed immunoassay positive specimens were from 1 individual and contained >400 ng/L THCCOOH, potentially contributing to cross-reactivity.

CONCLUSIONS—The Immunalysis Sweat/OF THC Direct ELISA is an effective screening procedure for detecting cannabinoids in OF.

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Illicit use of smoked and oral cannabis continues to be a public health concern, as shown in a 2007 Substance Abuse and Mental Health Services Administration (SAMHSA)2 survey, where 5.8% of the US population age 12 years and older smoked cannabis in the past month (1). While potentially detrimental to the individual, cannabis also has a substantial impact on public safety, as many elect to operate motor vehicles soon after self-administration. The National Highway Traffic Safety Administration recently reported that in 2007, 8.6% of nighttime drivers were positive for cannabinoids in blood and/or oral fluid (OF), almost 4 times higher than the percentage of drunk drivers with a blood alcohol concentration ≥0.8 g/L (2). Although finding cannabinoids in blood or OF does not necessarily imply impairment, windows of drug detection in these matrices are short, increasing impairment probability.

Recently, OF was identified as a suitable alternative matrix for monitoring driving under the influence of drugs (DUID) in addition to clinical, workplace, and drug treatment settings. In 2004, SAMHSA proposed revisions to the 1988 mandatory guidelines for federal workplace drug testing programs specifying guidelines for OF testing (3). An OF cannabinoid screening cutoff of 4 μg/L and a Δ^9-tetrahydrocannabinol (THC) confirmation cutoff of 2 μg/L were proposed. At that time, only THC had been detected in OF after cannabis consumption. A potential problem with measurement of the parent drug in OF was whether active cannabis intake could be differentiated from passive exposure to THC-laden cannabis smoke (3). Therefore, simultaneous collection of a urine sample also was proposed, negating the usefulness of OF testing.

Indeed, prior OF research reported no psychoactive 11-hydroxy-THC (11-OH-THC) or inactive 11-nor-9-carboxy-THC (THCCOOH) in OF by GC-MS at a limit of quantification (LOQ) of 0.5 μg/L (4). Additionally, THC and metabolites were not detected in OF after intravenous (IV) administration of radiolabeled THC (5). In 2005, however, Day et al. (6) quantified THCCOOH in authentic OF specimens by a gas chromatography–tandem mass spectrometry procedure. THCCOOH is not present in cannabis smoke; therefore, identification of this metabolite in OF provides conclusive evidence of active cannabis consumption. Moore et al. (7) also verified the presence of THCCOOH in OF and achieved a 2 ng/L LOQ with 2-dimensional (2D) GC-MS. More recently, Milman et al. (8) achieved an empirically determined 7.5 ng/L THCCOOH LOQ and simultaneously quantified THC, 11-OH-THC, cannabidiol (CBD), and cannabinol (CBN) in a single OF extraction.

As part of ongoing controlled cannabinoid administration research, a reliable, sensitive, and specific immunoassay was needed for screening OF. The Immunalysis Sweat/OF THC Direct ELISA and the Immunalysis Quantisal™ OF collection device were selected owing to a reproducible OF collection volume of mean (SD) 1.0 (0.1) mL (9), THC recovery of 81.3%–91.4% (10), and THC stability in the collection buffer (11). Following immunoassay validation, we screened authentic OF specimens after controlled oral THC administration and used 2D-GC-MS to confirm the presence of THC and several other cannabinoids, including THCCOOH at ng/L concentrations. This enabled us to determine assay diagnostic sensitivity, specificity, and efficiency for authentic OF specimens collected with the Quantisal OF collection device, while also identifying potential contributions from other cross-reacting cannabinoids.

[2]Nonstandard abbreviations: SAMHSA, Substance Abuse and Mental Health Services Administration; OF, oral fluid; DUID, driving under the influence of drugs; THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; LOQ, limit of quantification; IV, intravenous; GC-MS-MS, gas chromatography–tandem mass spectrometry; 2D, 2-dimensional; CBD, cannabidiol; CBN, cannabinol; LOD, limit of detection; Δ^8-THC, Δ^8-tetrahydrocannabinol; TP, true positive; FN, false negative; TN, true negative; FP, unconfirmed positive.

Materials and Methods

SPECIMENS

We studied 13 healthy male participants with a self-reported history of daily cannabis smoking. Participants received oral synthetic THC [Marinol® (dronabinol), Unimed Pharmaceuticals] daily as part of a protocol to study cannabis tolerance and spontaneous and antagonist-elicited cannabis withdrawal. Double-encapsulated 20-mg oral THC doses were administered with increasing frequency (every 4–8 h) for total doses of 40–120 mg/day for 8 days. Participants were admitted to the secure research unit 20 h before the first THC dose and were discharged 24 h after the final dose. Study design was previously described (12). Written informed consent was obtained, and the Institutional Review Boards of the National Institute on Drug Abuse, the University of Maryland, and the State of Maryland Department of Health and Mental Hygiene approved the protocol.

We collected OF specimens 3 times a day with the Quantisal device, which was placed in the mouth until an adequate specimen was collected. The device consists of a cellulose pad affixed to a stem with a 1 (0.1) mL volume adequacy indicator. We placed the pads into tubes containing 3 mL Quantisal transport buffer solution for 24–72 h at room temperature to elute drugs from the pad, simulating transport conditions. Stems were removed, pads squeezed dry, and solutions transferred to Nunc CryoTubes™ for storage at −20 °C until analysis.

We obtained blank OF (n = 9) from healthy volunteers with no self-reported cannabis use in the previous 180 days. The volunteers expectorated into 50-mL polypropylene centrifuge tubes, which were stored at −20 °C for a minimum of 24 h and centrifugated at 7500*g* for 10 min before use. Specimens were confirmed negative by 2D-GC-MS (THC LOQ 0.5 μg/L) and combined in equal parts to create a blank OF pool for preparation of cannabinoid calibrators, QC, and method validation samples. We also obtained OF from these volunteers through direct sampling with the Quantisal device by the procedure detailed above.

REAGENTS AND CONSUMABLES

We obtained all native and deuterated standards from Cerilliant, with the exception of THC glucuronide, which was from ElSohly Laboratories. We purchased HPLC-grade methanol from Fisher Scientific, and water was purified in-house. ELISAs (#224 Sweat/OF THC Direct ELISA) and Quantisal OF collection devices were from Immunalysis.

ELISA SCREENING

We screened all unknown OF specimens singly according to assay instructions provided by Immunalysis (13). Spectrophotometric analysis at 450 nm yielded absorbance that was inversely proportional to the quantity of cannabinoids present. All raw absorbances (B) were normalized to the mean negative control absorbance for the plate (B_0). A qualitative positive sample required a B/B_0 below the mean B/B_0 of the 4 μg/L cutoff calibrator.

In addition to the 4 μg/L cutoff calibrator, each assay included a negative synthetic OF and OF fortified at 2 μg/L THC (low control) and 8 μg/L THC (high control) that was prediluted 1:4 with Quantisal extraction buffer. We assayed all calibrators and controls at the beginning and end of the plate to evaluate assay performance. In-house OF controls at 1.0, 3.0, 5.0, and 6.0 μg/L THC were prepared weekly, diluted 1:4 with Quantisal buffer, and run in duplicate interspersed throughout specimens.

VALIDATION PROCEDURES

Blank volunteer expectorant samples (n = 9) were fortified with THC at 2.0 and 8.0 μg/L, sampled with the Quantisal device, and analyzed in triplicate to determine effects of matrix on quantification.

Limits of detection—We calculated limit of detection (LOD) absorbance values with negative buffered expectorant and Quantisal OF samples (n = 9 for each matrix) by subtracting 3 times absorbance SD from mean absorbance of the 9 negative OF samples (A_0) for each matrix. Thus, LOD is a concentration of THC that yields an absorbance of A_0 – 3SD.

Linearity—We investigated response linearity with regression line calculation by the method of least squares, expressed as the coefficient of determination (r^2). We evaluated linearity with the following THC concentrations: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 15, 20, 25, 30, 50, 75, 100, 125, 150, and 200 μg/L in pooled negative OF. Samples (diluted 1:4 in Quantisal buffer) were run in triplicate, and mean B/B_0 values were plotted against concentration on a log-logit plot to characterize linearity. Logit values (L) were calculated as $L = \ln[(B/B_0)/(1 – (B/B_0))]$.

Imprecision—We measured imprecision with in-house controls at 1.0, 3.0, 5.0, and 6.0 μg/L THC. For intraplate imprecision, we assayed 7 replicates at each concentration on a single plate. This plate was read 5 times over 25 min to determine any variation in absorbance after addition of stop solution (interread imprecision). For interplate imprecision, we assayed 2 replicates of controls at each concentration on 10 plates (n = 20) over 6 weeks.

Plate drift and control charting—We determined variation in absorbance as a function of physical location on the 96-well plate (drift). Imprecision was monitored across all plates for calibrators and controls through control charting of individual and mean B/B_0. A priori reanalysis cutoffs were 25% CV (intraplate) or ±25% of the running mean at any control level (interplate).

Cross-reactivity—We characterized cross-reactivity by analyzing 11-OH-THC, CBD, CBN, Δ^8-tetrahydrocannabinol (Δ^8-THC), THCCOOH, THCCOOH-glucuronide, and THC-glucuronide in triplicate at 0.1, 1.0, 4.0, 400, and 1000 μg/L. Other commonly encountered drugs that were evaluated included acetaminophen, 6-acetylmorphine, acetylsalicylic acid, alprazolam, amphetamine, benzoylecgonine, caffeine, cathinone, cocaethylene, cocaine, codeine, dextromethorphan, diazepam, diazepam, flunitrazepam, flurazpam, hydrocodone, hydromorphone, ibuprofen, imipramine, lorazepam, methadone, methamphetamine, 3,4-methylenedioxy-methamphetamine, morphine, nicotine, nitrazepam, oxazepam, pentazocine, phencyclidine, and temazepam at 1000 μg/L. Cross-reactivity (%) was determined as 100 × (apparent THC concentration from the calibration curve)/(analyte concentration).

Interferences—We evaluated several common products for cross-reactivity or matrix effects including Listerine® mouthwash, orange juice, toothpaste, brewed coffee, soy milk, and tap water (control). For all products other than toothpaste, 50 mL was swirled in the mouth of a volunteer for 15 s and then swallowed (orange juice, soy milk, coffee, and water) or expectorated (mouthwash). In addition, potential interference from toothpaste was evaluated by brushing the teeth for 60 s with 500 mg toothpaste, rinsing the mouth once with 50 mL tap water, and expectoration to waste. OF specimens were collected with the Quantisal device 5 and 30 min after product exposure. One replicate was analyzed as-is, the other fortified at 32 μg/L THC to evaluate signal enhancement or suppression.

Hook effect—We assayed high THC concentrations to ensure lack of a "hook effect." A hook effect (so named for the characteristic shape of the analytical dilution curve) has been reported for some (primarily single-step) immunoassays when an extraordinarily high antigen concentration is present, yielding an artificially high absorbance (i.e., negative result) (14). To test for this effect, pooled negative OF was fortified to 1000 μg/L THC and assayed in triplicate.

2D-GC-MS CONFIRMATION

We quantified cannabinoids in oral fluid specimens by a previously validated 2D-GC-MS method (8). LOQs were 0.5, 0.5, 0.5, 1.0, and 0.0075 μg/L for THC, 11-OH-THC, CBD, CBN, and THCCOOH, respectively.

DIAGNOSTIC EFFICIENCY

We evaluated diagnostic sensitivity and specificity by ELISA screening (cutoff 4 μg/L) of 499 authentic OF specimens collected with the Quantisal device followed by 2D-GC-MS confirmation. We determined diagnostic sensitivity, the calculated true-positive rate, from total true positives (TP) and false negatives (FN), with sensitivity = 100 × TP/(TP + FN). Diagnostic specificity was based on the number of true negatives (TN) and unconfirmed positives (FP), with specificity = 100 × TN/(TN + FP). Diagnostic efficiency was 100 × (TP + TN)/total specimens. To evaluate plate performance at various GC-MS cutoffs, we calculated efficiency at 0.5, 1, 2, 3, 4, 5, and 6 μg/L THC cutoffs. Plate performance was also evaluated at THCCOOH GC-MS cutoffs of 20, 50, 100, 200, 300, 400, and 500 ng/L.

STATISTICAL ANALYSIS

Statistical calculations were performed with SPSS 15.0 for Windows (SPSS Inc.). A paired *t*-test determined potential differences ($P < 0.05$) between matrix responses at specific concentrations (n = 9). As variances were unequal, we used the Welch test for evaluating omnibus differences ($P < 0.05$) during interference characterization. Mean (n = 2) calibrator B/B_0 values were used for cutoff determinations and diagnostic specificity and sensitivity calculations. Individual cross-reactivity samples quantifying less than the negative control were included in the means as having 0% cross-reactivity.

Results

SPECIMENS

We collected 499 authentic OF specimens with the Quantisal device, including 184 duplicate OF samples obtained simultaneously (both devices in the mouth at the same time) to investigate interdevice variability. At the time of collection, negative control OF samples had variable viscosity, color, and mucus content. After frozen storage and centrifugation, however, a slightly viscous, clear, homogeneous solution was obtained.

VALIDATION

At 2 μg/L, no differences in absorbance were observed between fortified OF diluted directly with buffer and fortified OF sampled with the Quantisal device ($t_{52} = 1.00$, $P = 0.322$, $r = 0.14$). However, a statistical difference was observed with samples prepared in the same manner at 8 μg/L ($t_{52} = -4.845$, $P < 0.001$, $r = 0.56$). Despite the difference, both of the 8 μg/L fortified samples quantified greater than the manufacturer's control at 8 μg/L and were qualitatively positive.

Limits of detection—On 5 different days, LODs of 0.30, 0.23, 0.24, 0.27, and 0.23 μg/L were obtained for directly buffered expectorant and 0.88, 0.77, 0.82, 0.91, and 0.77 for

negative samples collected with the Quantisal device. Thus, LODs were 0.30 μg/L for centrifuged, buffered expectorant and 0.91 μg/L for samples collected with the Quantisal device.

Linearity—Evaluation of immunoassay linearity from 1 to 50 μg/L THC (Fig. 1) yielded a logarithmic regression line of $y = -1.552 \log(x) + 0.277$ ($r^2 = 0.9936$). The limit of quantification was 1 μg/L based on the correlation coefficient and triplicate concentrations within 26.2% of target. Further testing demonstrated acceptable linearity ($r^2 = 0.9835$) up to 200 μg/L (data not shown) for estimating required dilutions for GC-MS confirmation.

Imprecision—We assessed imprecision at 1, 3, 5, and 6 μg/L THC. Intraplate, interplate, and interread imprecision are detailed in Table 1. Intraplate imprecision (n = 7) was <7.7% CV for all concentrations. Interplate (n = 20) and interread imprecisions (n = 5) were <9.1% and 1% CV, respectively.

Plate drift and control charting—We calculated mean intraplate drift (n = 20) at each control concentration. Statistically significant drift was observed for the 2 μg/L control, with controls at the end of the plate having an overall higher mean (SD) B/B_0 [52.2 (3.5)] than at the beginning [49.4 (3.3)], $t_{40} = -2.66$, $P = 0.011$, $r = 0.39$. Additionally, a significant similar plate drift was observed for the 8 μg/L control, with a higher B/B_0 [28.5 (2.6)] at the end compared to the beginning [25.3 (2.4)], $t_{40} = -4.00$, $P < 0.001$, $r = 0.53$. However, all 2 and 8 μg/L controls produced appropriate negative and positive qualitative results. No significant drift was observed for the 4 μg/L calibrator, $t_{40} = -1.08$, $P = 0.288$, $r = 0.17$.

Control charting within a single assay lot generally demonstrated an increase in mean B/B_0 absorbance over time within the lot, similar to other commercial immunoassays. However, the test continued to perform within qualitative and quantitative a priori specifications, and no reanalyses were required.

Cross-reactivity—Immunoassay cross-reactivities at 4 μg/L were 11-hydroxy-THC, 198%; Δ^8-THC, 128%; THCCOOH, 121%; THC (target), 98%; cannabinol, 87%;THCCOOH-glucuronide, 11%; THC-glucuronide, 10%; and cannabidiol, 2.4%. As detailed in Fig. 2, cannabinoid cross-reactivities varied substantially across concentrations. Because of loss of linearity at higher concentrations, cross-reactivities typically decreased with increasing cannabinoid concentration. Large ranges were often observed; however, this variability is expected when samples quantify below LOD (1 μg/L) or beyond the assay's upper limit of linearity (200 μg/L). Cross-reactivities for the 30 common drugs or chemicals tested at 1000 μg/L were <0.05%, and samples were qualitatively negative in all cases. The 1000 μg/L hook effect sample quantified at approximately 260 μg/L THC.

Interferences—Using a Welch test, we assayed blank OF after product exposure, and it demonstrated no significant differences from control (OF after water rinse) at 5 or 30 min after exposure ($P = 0.274$ and 0.073, respectively). In all cases, samples quantified below the LOQ (1 μg/L); thus, these product exposures did not affect assay performance.

We also fortified OF at 32 μg/L THC after product exposure to characterize any effects of these products on semiquantification. Product-exposed OF at 32 μg/L THC was not significantly different from control 5 min ($P = 0.233$) or 30 min ($P = 0.331$) after exposure.

DIAGNOSTIC EFFICIENCY

According to the SAMHSA-proposed 4 μg/L cannabinoids screening cutoff and THC GC-MS 2 μg/L confirmation cutoff, 52 of 499 authentic OF specimens confirmed positive (THC

concentrations 2.0–293 μg/L). No false negatives were reported, yielding 100% diagnostic sensitivity. However, 47 samples screened positive above the 4 μg/L cutoff, but were not confirmed by 2D-GC-MS, yielding an overall diagnostic specificity of 400/477 = 89.5% and diagnostic efficiency of 452/499 = 90.6%. Median (range) THC concentrations of these unconfirmed positive specimens were 5.8 (4.1–9.9) μg/L by immunoassay and 0.5 (none detected–1.8) μg/L by 2D electron impact GC-MS. Interestingly, THCCOOH concentrations ranged from 7.5–1118 ng/L by 2D negative chemical ionization GC-MS. Table 2 details a comparison of diagnostic efficiencies with various GC-MS THC confirmation cutoffs. Generally, diagnostic sensitivity increased and specificity decreased as the GC-MS cutoff concentration increased. Diagnostic efficiency peaked at a confirmation cutoff of 1 μg/L THC, although false-negative samples increased at (and below) this cutoff.

With the recent identification of THCCOOH in OF (6–7), THCCOOH immunoassay detection could become important if this analyte is selected as conclusive evidence of cannabis intake, removing the possibility of passive contamination. Table 3 highlights the capability of this immunoassay to screen for OF THCCOOH. With a manufacturer-recommended 4 μg/L cannabinoid screening cutoff, employing a 400 ng/L THCCOOH confirmation cutoff provided acceptable diagnostic sensitivity, specificity, and efficiency. However, the assay currently provides better performance for THC than THCCOOH, with 65 true positives at maximum diagnostic efficiency (1 μg/L) for THC vs only 44 at maximum THCCOOH diagnostic efficiency (400 ng/L). These data suggest that an antibody with greater THCCOOH cross-reactivity could improve THCCOOH sensitivity in OF.

Discussion

As OF testing gains acceptance in DUID, workplace, and clinical monitoring scenarios, validation of robust, sensitive, and cost-effective screening assays will allow detection of illicit drug use while reducing confirmatory demands on personnel and instrumentation. Extensive validation of the Immunalysis Sweat/OF THC Direct ELISA documented sensitive, precise, and accurate screening for THC in OF.

As this validation demonstrates, control B/B_0 values vary as a function of physical location on the plate, yielding intraplate drift. Although drift had no effect on qualitative results, it can be moderated with use of automated equipment (multichannel pipettes, automatic plate washers, and dispensers). Additionally, mean values for calibration curves and cutoff absorbance determinations could be applied if 1 set is assayed at the beginning and another at the end of the plate. This does not appear to be necessary or cost-effective, as all controls below and above the cutoff calibrator assayed appropriately.

The 1000 μg/L hook effect sample quantified lower than expected (approximately 260 μg/L), suggesting deviation from linearity above 200 μg/L. Linearity failure is expected as absorbance approaches 0 and should have little effect on qualitative analysis at a 4 μg/L cutoff, although estimating dilutions for GC-MS confirmation may not be accurate in specimens with these high concentrations.

Metabolite cross-reactivity in some authentic specimens may have contributed to positive immunoassay tests that were not confirmed by 2D-GC-MS, reducing specificity to 89.5%. This is similar to other reported THC ELISA specificities (15, 16). Further analysis of metabolite quantifications indicated that 31 of 47 unconfirmed positives (GC-MS cutoff 2 μg/L THC) were from a single participant and had remarkably high (>400 ng/L) THCCOOH concentrations. In these cases, THCCOOH (and potentially less than μg/L 11-OH-THC) cross-reactivity likely contributed to positive B/B_0 absorbance.

Diagnostic specificity, sensitivity, and efficiency are markedly influenced by changes in the GC-MS confirmation cutoff. Using the SAMHSA-proposed 2 μg/L confirmation cutoff yields improved plate performance over a 4 μg/L cutoff with an expected increase in TP and decrease in FP. Lowering the cutoff to 1 μg/L THC further increases TP; however, increased FN results are observed with this cutoff. Generally, screening cutoffs greater than confirmation cutoffs are desired when multiple metabolites may cross-react in the assay. On the other hand, performing unnecessary, expensive, and time-consuming confirmation testing is not efficient and delays reporting of negative results. The current Immunalysis cannabinoid ELISA provides acceptable diagnostic sensitivity, specificity, and efficiency for a SAMHSA GC-MS cutoff of 1 or 2 μg/L THC.

With 198% cross-reactivity at 4 μg/L, 11-OH-THC presence could contribute to unconfirmed positive specimens for this study. First-pass metabolism of orally administered cannabinoids (as in this study) yields higher relative plasma 11-OH-THC concentrations compared with smoked administration (17), although based on previous research (4), we did not expect to observe OF 11-OH-THC concentrations >0.5 μg/L. For this study, OF also was analyzed for 11-OH-THC by 2D-GC-MS (LOQ 0.5 μg/L); all specimens were below LOQ for this analyte. Further research is needed, as detection of 11-OH-THC at ng/L concentrations may provide additional evidence of active cannabis consumption.

This immunological test is a diagnostically sensitive screening procedure to detect cannabinoids in OF using a screening cutoff of 4 μg/L cannabinoids and a confirmation cutoff of 2 μg/L THC. The test requires about 2.5 h for 80 unknowns assayed singly with manual pipetting. The assay is effective for measuring cannabinoids in expectorated OF as well, although dilution of expectorated OF with buffer before analysis is required. As OF testing is accepted as a reliable means of detecting cannabis consumption, this OF screening assay should prove useful in clinical and forensic applications. However, if approval of THCCOOH in OF as evidence of active cannabis intake occurs, improved performance should be achieved with an antibody directly targeting THCCOOH.

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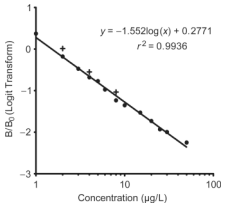

Fig. 1. Immunalysis Sweat/Oral Fluid Δ^9-THC Direct ELISA linearity

Linearity determined with in-house calibrators (●) prepared by fortifying centrifuged, buffered OF with THC. Evaluation from 1–50 µg/L THC yielded a logarithmic regression line in good accordance with manufacturer-provided calibrators and controls (+). Logit values (*L*) were calculated as $L = \ln[(B/B_0)/(1 - (B/B_0))]$.

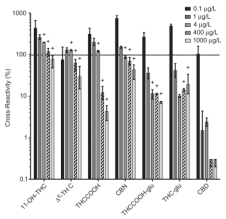

Fig. 2. Mean (n = 3) (range) cannabinoid cross-reactivity in fortified authentic OF in the Immunalysis Sweat/Oral Fluid Δ^9-THC Direct ELISA

Dashed line represents 4 µg/L THC (target) cross-reactivity (98%). Individual cross-reactivity samples quantifying less than negative control were included in means as having 0% cross-reactivity. +, sample qualitatively positive.

Table 1

Assay imprecision around the 4 µg/L screening cutoff for Immunalysis Sweat/Oral Fluid Δ^9-THC Direct ELISA.

Control level (µg/L THC)	Intraplate (n = 5), %		Interplate (n = 20), %		Interread[a] (n = 5), %	
	Mean (SD) B/B_0[b]	CV	Mean B/B_0	CV	Mean B/B_0	CV
1	56.4 (1.6)	2.9	60.3 (1.8)	3.0	56.6 (0.3)	0.5
3	37.2 (2.9)	7.7	38.5 (2.0)	5.1	37.4 (0.3)	0.9
5	30.0 (1.1)	3.6	30.6 (2.8)	9.1	30.1 (0.2)	0.8
6	27.1 (2.0)	7.4	27.8 (1.3)	4.7	27.3 (0.2)	0.9

[a] Variation in absorbance after addition of stop solution.

[b] Raw absorbance values (B) normalized to the mean negative control absorbance value (B_0).

Table 2

Immunoassay performance (4 μg/L screening cutoff) in 499 authentic OF specimens at varying Δ^9-THC GC-MS confirmation cutoffs.

	GC-MS THC cutoff, μg/L						
	0.5	**1**	**2**	**3**	**4**	**5**	**6**
True positive[a]	73	65	52	44	32	21	15
True negative	362	389	400	400	400	400	400
Unconfirmed positive	26	34	47	55	67	78	84
False negative	38	11	0	0	0	0	0
Diagnostic sensitivity, %	65.8	85.5	100	100	100	100	100
Diagnostic specificity, %	93.3	92.0	89.5	87.9	85.7	83.7	82.6
Diagnostic efficiency, %	87.2	91.0	90.6	89.0	86.6	84.4	83.2

[a]True positive, immunoassay positive and confirmed positive by GC-MS; true negative, immunoassay negative and confirmed negative by GC-MS; unconfirmed positive, immunoassay positive but confirmed negative by GC-MS; false negative, immunoassay negative but confirmed positive by GC-MS.

Table 3

Immunoassay performance (4 μg/L screen cutoff) in 499 authentic OF specimens at varying THCCOOH GC-MS confirmation cutoffs.

	GC-MS THCCOOH cutoff, ng/L						
	20	**50**	**100**	**200**	**300**	**400**	**500**
True positive[a]	90	75	63	57	54	44	35
True negative	75	155	249	350	375	392	397
Unconfirmed positive	9	24	36	42	45	55	64
False negative	325	245	151	50	25	8	3
Diagnostic sensitivity, %	21.7	23.4	29.4	53.3	68.4	84.6	92.1
Diagnostic specificity, %	89.3	86.6	87.4	89.3	89.3	87.7	86.1
Diagnostic efficiency, %	33.1	46.1	62.5	81.6	86.0	87.4	86.6

[a]True positive, immunoassay positive and confirmed positive by GC-MS; true negative, immunoassay negative and confirmed negative by GC-MS; unconfirmed positive, immunoassay positive but confirmed negative by GC-MS; false negative, immunoassay negative but confirmed positive by GC-MS.