On-Site Test for Cannabinoids in Oral Fluid

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BACKGROUND: Oral fluid (OF) testing offers noninvasive sample collection for on-site drug testing; however, to date, test performance for Δ9-tetrahydrocannabinol (THC) detection has had unacceptable diagnostic sensitivity. On-site tests must accurately identify cannabis exposure because this drug accounts for the highest prevalence in workplace drug testing and driving under the influence of drugs (DUID) programs.

METHODS: Ten cannabis smokers (9 mol/L, 1 female) provided written informed consent to participate in this institutional review board–approved study and smoked 1 6.8%-THC cigarette ad libitum. OF was collected with the Draeger DrugTest® 5000 test cassette and QuantisalTM device 0.5 h before and up to 22 h after smoking. Test cassettes were analyzed within 15 min (n = 66), and Quantisal GC-MS THC results obtained within 24 h. Final THC detection times and test performances were assessed at different cannabinoid cutoffs.

RESULTS: Diagnostic sensitivity, diagnostic specificity, and efficiency at DrugTest 5000’s 5 μg/L screening cutoff and various THC confirmation cutoffs were 86.2–90.7, 75.0–77.8, and 84.8–87.9%, respectively. Last detection times were >22 h, longer than previously suggested. Confirmation of 11-nor-9-carboxy-THC, absent in THC smoke, minimized the potential for passive OF contamination and still provided 22-h windows of detection, appropriate for workplace drug testing, whereas confirmation of cannabidiol, and/or cannabinol yielded shorter 6-h windows of detection, appropriate for DUID OF testing.

CONCLUSIONS: The DrugTest 5000 on-site device provided high diagnostic sensitivity for detection of cannabinoid exposure, and the selection of OF confirmation analytes and cutoffs provided appropriate windows of detection to meet the goals of different drug testing programs.

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Oral fluid (OF) testing is increasingly used for testing in the setting of drug treatment, workplace, pain management, and driving under the influence of drugs (DUID), but to achieve greater acceptance of this new technology, on-site tests must sensitively detect cannabis (marijuana) exposure. Cannabis is the most commonly abused illegal drug. In 2009, 125–203 million people worldwide smoked cannabis in the past year (1) and in 2010, 17.4 million Americans smoked the drug in the previous month (2).

OF testing is the matrix of choice for roadside screening for DUID, with Δ9-tetrahydrocannabinol (THC) being the most prevalent illicit drug detected in injured drivers in Victoria, Australia (9.8%) (3). OF and blood were collected in a study population for the first time in the 2007 US Roadside Survey, in which cannabinoids were identified in one or both matrices in 8.6% of nighttime drivers (4). To date, no on-site device reproducibly has met the 80% diagnostic sensitivity and specificity, and efficiency criteria proposed by the Driving under the Influence of Drugs, Alcohol and Medicines (DRUID) project for OF drug detection (5–14).

On-site cannabinoid efficiencies in OF were reported as 80% for the OraLine® IV s.a.t. device (7), 57.5%–88% for Drugwipe 5+ (9–12), 60%–71% for Cozart DDSV (11, 13), 66%–86.6% for RapidStat® (11–12, 14), 68%–90% for DrugTest 5000® (11–12), and 78% for OrAlert™ (11). Although the OraLine IV s.a.t. device achieved an 80% efficiency, diagnostic sen-

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3 Nonstandard abbreviations: OF, oral fluid; DUID, driving under the influence of drugs; THC, Δ9-tetrahydrocannabinol; DRUID, Driving under the Influence of Drugs, Alcohol and Medicines; FP, false positive (unconfirmed initial positive test); LC-MS/MS, liquid chromatography–tandem mass spectrometry; DrugTest 5000, Draeger DrugTest 5000; LOQ, limit of quantification; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; CBD, cannabidiol; CBN, cannabinol; 2D-GC-MS, 2-dimensional GC-MS; SAMHSA: Substance Abuse and Mental Health Services Administration; TP, true positive; TN, true negative; FN, false negative (unconfirmed initial negative test).
Situation was only 69.2%. It is difficult to compare reported efficiencies across devices because of frequent device reformulations and differences in device and confirmation cutoffs and matrix evaluations. Poor analyte recovery from the device, which leads to low diagnostic sensitivity, is generally the reason for inadequate performance. Additional variability is generated when visual identification of the presence or absence of a line is needed to determine sample positivity. A high device failure rate due to internal QC failures (typically inadequate lateral flow) has also limited the acceptability of on-site test performance. Since 2004 in Victoria, Australia, on-site OF tests have been successfully used as a deterrent to drugged driving; 2 on-site tests are used to minimize false-positive (FP) results, with any sample initially screening positive for methamphetamine or MDMA (methylendioxymethamphetamine) also confirmed for THC (15).

An earlier version of the Draeger DrugTest® 5000 (DrugTest 5000) on-site test had a 25 µg/L THC screening cutoff, and diagnostic sensitivity failed to meet DRUID criteria. When evaluated against OF collected with the Intercept® device with a 0.5-µg/L LC-MS/MS THC limit of quantification (LOQ), the earlier version of the DrugTest 5000 device had 49.5% diagnostic sensitivity, 100% diagnostic specificity, and 55% efficiency (6). Compared to THC in simultaneously collected plasma with a 2 µg/L THC confirmation cutoff, OF diagnostic sensitivity, diagnostic specificity, and efficiency were 72%, 50%, and 68%, respectively (12). However, with the most recent DrugTest 5000 version (5 µg/L THC screening cutoff), 93% diagnostic sensitivity, 71% diagnostic specificity, and 90% efficiency for cannabinoids in suspected drugged drivers was observed, with confirmation in simultaneously collected plasma (2 µg/L THC cutoff) (12). When evaluated against expectorated OF with a 1-µg/L confirmation cutoff, diagnostic sensitivity, and diagnostic specificity, and efficiency of the older DrugTest 5000 version were 56%–76%, 89%, and 80%, whereas with the new version they were 53%, 99%, and 84%, respectively, evaluated against OF collected with the StatSure Saliva Sampler (1 µg/L confirmation cutoff) (11). Even with this low diagnostic sensitivity, the authors suggested that the DrugTest 5000 had the best cannabinoid diagnostic sensitivity among the on-site drug testing devices currently available in the market.

We administered smoked cannabis to individuals with a current history of cannabis smoking to evaluate performance characteristics and windows of OF detection with the on-site DrugTest 5000. THC, 11-nor-9-carboxy-THC (THCCOOH), 11-hydroxy-THC (11-OH-THC), cannabidiol (CBD), and cannabiol (CBN) were quantified by 2-dimensional GC-MS (2D-GC-MS) to investigate different cannabinoid markers and windows of cannabinoid detection to meet the goals of diverse drug testing programs.

Methods

Participants
Healthy men and women provided written informed consent to participate in this National Institute on Drug Abuse Intramural Research Program Institutional Review Board–approved study. Individuals were recruited by television, radio, and newspaper advertisements; flyers; and participant referrals. Participants received a comprehensive medical and psychological evaluation to verify compliance with eligibility criteria. Inclusion criteria were ages 18–45 years and a minimum cannabis intake frequency of at least twice per month during the 3 months before study screening. History of cannabis use was confirmed by a positive urine cannabinoid test. Exclusion criteria included: breastfeeding or pregnant women; current medical condition or history of neurological illness; history of a clinically significant adverse event associated with cannabis intoxication; donation of >450 mL blood within 30 days of drug administration; presence of clinically significant anemia; increased systolic or diastolic blood pressure or heart rate >100 bpm after 5-min rest; clinically significant electrocardiogram abnormality; or interested in drug abuse treatment, currently or within 60 days of study screening. Pregnancy tests were administered at screening and on the morning of drug administration to women with reproductive potential.

Study Design
Participants entered the secure research unit 16–19 h before smoking to preclude intoxication at the time of cannabis dosing. Participants smoked one 6.8% THC (approximately 54 mg THC) cannabis cigarette ad libitum for up to 10 min; OF was collected before (−0.5) and 1, 2, 3, 4, and 6 h after smoking. Participants could stay on the secure residential unit the evening after smoking, providing an OF sample 22 h after dosing. OF was collected with the Quantisal™ device (Immunalysis), followed by the DrugTest 5000 (Draeger Safety Diagnostics). OF collected with the Quantisal device was immersed in the extraction buffer and stored at 4 °C for 19–24 h before 2D-GC-MS analysis; DrugTest 5000 on-site samples were analyzed with the DrugTest 5000 analyzer within 15 min of collection (THC cutoff
5 μg/L). Participants were discharged no earlier than 6 h after cannabis smoking.

OF SAMPLE COLLECTION
The DrugTest 5000 test cassette is equipped with a polymeric noncompressible pad for OF collection. OF was collected by swiping the test cassette on the tongue and sides of the cheeks. The Quantisal device consists of an absorbent pad on a plastic stick that is placed under the tongue for collection of 1 (0.1) mL OF. The test cassette collects 270 μL ±15% OF in approximately 70 s, with volume adequacy confirmed with a blue ring at the tip of the collection pad. An independent OF collection is required for confirmation. All OF samples were collected a minimum of 10 min after eating or drinking.

OF DRUGTEST 5000 ANALYSIS
The DrugTest 5000 consists of an analyzer, a test cassette, and a buffer cartridge to determine if cocaine, opiates, benzodiazepines, cannabinoids, amphetamines, or methamphetamine are present in OF above specified cutoffs. After the buffer cartridge and test cassette are inserted into the instrument, the analysis is automated and the cartridge is pushed onto the tip of the test cassette for the lateral flow immunoassay. Test cassettes were calibrated during production with fortified native OF and the threshold for a positive result was set at 5 μg/L. Delta-9-THC, 2 μg/L; THCCOOH, 10 μg/L; 11-OH-THC, 90 μg/L; CBD, 350 μg/L; CBN, 11 000 μg/L; cis-tramadol HCl, 45 000 μg/L; amisulpride, 80 000 μg/L; diphenhydramine HCl, and 80 000 μg/L dopamine HCl.

OF CANNABINOID GC-MS ANALYSIS
OF samples collected with the Quantisal device were analyzed for THC, 11-OH-THC, THCCOOH, CBD, and CBN according to a previously published method (16). Linear ranges were 0.5–50 μg/L for THC, 11-OH-THC, and CBD; 1–50 μg/L for CBN; and 7.5–500 ng/L for THCCOOH. Analytical bias was 99.1%–113.8%, and intra- and interassay imprecision were <6.6% CV.

DATA ANALYSIS
Qualitative OF DrugTest 5000 cannabinoid results used a preprogrammed 5 μg/L THC cutoff. These results were evaluated against the quantitative Quantisal OF THC 2D-GC-MS results. In addition, raw absorbance data from the device were evaluated at a higher 10 μg/L cutoff. True-positive (TP) (DrugTest 5000 and GC-MS positive), true negative (TN) (DrugTest 5000 and GC-MS negative), false positive (FP) (positive DrugTest 5000, but less than GC-MS–specified cutoff), and false negative (FN) (negative DrugTest 5000, but positive GC-MS at specified cutoff) results were calculated at DrugTest 5000 screening cutoffs of 5 and 10 μg/L THC and GC-MS THC confirmation cutoffs of 0.5 (method LOQ), 1 (DRUID), 2 [Substance Abuse and Mental Health Services Administration (SAMHSA)], and 10 μg/L (Australian Standards, AS 4760–2006 (17)). Diagnostic sensitivity, 100 × [TP/(TP + FN)]; diagnostic specificity, 100 × [TN/(TN + FP)]; and efficiency, 100 × [(TP + TN)/(TP + TN + FP + FN)] were calculated at multiple screening and confirmation cutoffs. Rates of detection and windows of detection were evaluated with the DrugTest 5000 screen and different confirmation analytes and cutoffs.

Results
HUMAN PARTICIPANTS
Ten healthy volunteers (9 males, 1 female) ages 18–45 years completed the protocol (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue10). Participants were chronic daily cannabis smokers [median (range) lifetime cannabis use 9.0 (2–25) years], with median cannabis smoking of 11.0 (8.5–14) days in the last 14 and 5.5 (1–12) joints/blunts smoked per day. Self-reported last cannabis intake was 2 (1–4) days before dosing. Six of 10 participants spent a second night on the secure unit and provided samples 22 h after dosing. A total of 66 DrugTest 5000 and 66 Quantisal samples were collected.

DRUGTEST 5000 CANNABINOID SCREENING
With a 5-μg/L DrugTest 5000 screening cutoff, 52 of 66 (78.8%) of OF samples were positive. When the cutoff was raised to 10 μg/L, 44 (66.7%) were positive. At baseline, 4 participants’ OF samples were positive at 5 μg/L and 3 at 10 μg/L (Fig. 1). All OF samples collected 1 h after cannabis smoking were positive at both cutoffs, and all were positive for at least 3 h at 5 μg/L and at least 2 h at 10 μg/L. Times of last THC detection ranged from 4 to >22 h (Fig. 1 and 2) and 2 to >22 h with the 5- and 10-μg/L cutoffs, respectively. One sample collected 1 h after smoking did not fulfill the volume adequacy criterion (due to dry mouth); nevertheless, the result was positive. One sample collected at 22 h had an invalid result and collection was repeated.
with a 5-μg/L THC screening cutoff and a 2-μg/L THC confirmation cutoff, yielding 90.7% diagnostic sensitivity, 75.0% diagnostic specificity, and 87.9% efficiency. Detection rates (Fig. 1) and times of last detection (Fig. 2) also were evaluated with the DrugTest 5000 drug screen and various confirmation cutoffs.

FN and FP results occurred when THC concentrations were close to cutoff thresholds. At the 5-μg/L screening and 2-μg/L THC confirmation cutoffs, OF THC concentrations in DrugTest 5000 FP screened samples were <LOQ, 1.5 μg/L; DrugTest 5000 FN screened samples occurred when THC was confirmed at 2.1–7.8 μg/L. At the same screening cutoff but with a lower confirmation cutoff (1 μg/L), 2 FP samples had unconfirmed THC concentrations (<LOQ), and 7 FN samples had THC concentrations of 1.3–7.8 μg/L. With the DrugTest 5000 10-μg/L cutoff, there were no FP results; FN concentrations were 2.1–20.7 μg/L at the LOQ, and 1.3–20.7 and 2.1–20.7 μg/L at the 2- and 1-μg/L confirmation cutoffs, respectively.

CONTRIBUTIONS FROM OTHER CROSS-REACTING CANNABINOIDs
Cross-reactivity data from the manufacturer indicated that THCCOOH, 11-OH-THC, CBD, and CBN produced positive results at 2, 10, 90 000, and 350 μg/L, respectively. THCCOOH, 11-OH-THC, and CBD did not exceed the specified cross-reacting concentrations in any OF sample. CBN OF concentrations exceeded the cross-reacting concentration in only 1 sample: a 1-h sample with 365 μg/L CBN. However, the THC concentration in this sample was 2440 μg/L. Concentrations of CBD and CBN ≥20 μg/L were found only in Quantisal samples collected within 2 h of smoking. The low cross-reactivity for these other cannabinoids is also evidenced in the higher number of FP observed when considering other cannabinoids (Tables 1 and 2).

Discussion
To our knowledge, this report is the first to document detection rates and windows of THC detection with the DrugTest 5000 and GLC-MS cannabinoid confirmation following controlled smoked cannabis. Participants in this study were primarily chronic, daily cannabis smokers, accounting for the positive baseline OF samples in 4 participants (who self-reported last cannabis use 1–3 days before dosing). There was no apparent correlation between a positive baseline screen and time since last cannabis smoking. THC detection windows with the DrugTest 5000 ranged from 4 to >22 h and 2 to >22 h with the 5- and 10-μg/L cutoffs, respectively. Last detection times could not be conclusively determined; the >22-h detection window was unexpectedly much longer than previously reported.
Insufficient OF sample volume was uncommon, occurring in only 1.5% (1 of 66) of DrugTest 5000 samples. An additional 1.5% of DrugTest 5000 samples tested invalid, possibly because of inadequate lateral flow or interfering compounds. Fewer DrugTest 5000 samples (6 of 66 or 9.1%) had insufficient OF volume than did Quantisal samples, most likely owing to the smaller required collection volume (0.27 mL vs 1.0 mL for Quantisal).

DrugTest 5000 screening results were evaluated against Quantisal confirmation data to determine TP, TN, FP, FN, diagnostic sensitivity and specificity, and efficiency at various cutoffs (Tables 1 and 2). When compared to THC alone, the diagnostic sensitivity and specificity and efficiency were 86.2%–90.7%, 75.0%–77.8%, and 84.8%–87.9% at the 5-μg/L cutoff and 75.9%–92.7%, 76.0%–100.0%, and 78.8%–86.4% at the 10-μg/L DrugTest 5000 cutoffs. Overall, the

![Table 1. Performance characteristics for the Draeger DrugTest 5000 on-site test with a 5-μg/L THC screening cutoff in OF with different confirmation cutoffs (N = 66).](image)
DrugTest 5000 performed better with the 5-μg/L screening cutoff, with diagnostic sensitivity and efficiency above the DRUID-recommended 80%. There were few FP and FN tests, and when they occurred, concentrations were at or near the confirmation cutoff. A limitation of this study was the inclusion of a small number of TN samples, only 6–12 with the 5-μg/L DrugTest 5000 and 1- and 2-μg/L confirmation cutoffs, to adequately evaluate diagnostic specificity. On the basis of previous reports, more TN samples were expected over the 22-h collection period. Detection rates were highest and windows of detection were longest when we confirmed for THC alone (Fig. 1 and 2). However, consideration of only THCCOOH approximated the 10 min between eating/drinking and sample collection. Interestingly, THCCOOH concentrations increased between 3 and 4 h (Fig. 1). In all participants after eating lunch 3 h after smoking, THCCOOH concentrations decreased 29.2%–77.7% compared to 2-h THCCOOH concentrations. This decrease occurred even though participants waited the recommended 10 min between eating/drinking and sample collection. Interestingly, THCCOOH concentrations increased between 3 and 4 h (Fig. 1). In contrast, THC, CBD, and CBN concentrations decreased and then increased at 4 h in only 3–5 samples. This result was unexpected because THC, CBD, and CBN, but not THCCOOH, are present in cannabis smoke. Hence, it might be expected that the cannabinoids in smoke that contaminate the oral mucosa would be the analytes that decreased with exposure to food and drink, but the effect was more consistently observed with THCCOOH.

Combination of THC ≥1 and CBD ≥0.5 or CBN ≥1 μg/L also had lower diagnostic sensitivity than confirmation of THC alone. These combinations provided a shorter window of detection (1–6 h) that might be appropriate for DUID, because this time frame is similar to the acute intoxication impairment window.

Our data are consistent with those of Wille et al. (12), who reported 93% diagnostic sensitivity, 71%

<table>
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<tr>
<th>Cutoff</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Diagnostic sensitivity</th>
<th>Diagnostic specificity</th>
<th>Efficiency</th>
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<td>THC ≥10 μg/L (Australian Standard AS4760–2006)</td>
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<td>19</td>
<td>6</td>
<td>3</td>
<td>92.7</td>
<td>76.0</td>
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<td>12</td>
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<td>10</td>
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<td>100.0</td>
<td>84.8</td>
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<td>100.0</td>
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<td>THC ≥2 μg/L and THCCOOH ≥20 ng/L</td>
<td>37</td>
<td>16</td>
<td>7</td>
<td>6</td>
<td>86.0</td>
<td>69.6</td>
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<tr>
<td>THC ≥1 μg/L and THCCOOH ≥20 ng/L</td>
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<td>15</td>
<td>7</td>
<td>7</td>
<td>84.1</td>
<td>68.2</td>
<td>80.3</td>
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<tr>
<td>THCCOOH ≥20 ng/L</td>
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<td>7</td>
<td>12</td>
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<td>97.3</td>
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<tr>
<td>THC ≥1 μg/L, CBN ≥1 μg/L</td>
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<td>21</td>
<td>11</td>
<td>1</td>
<td>97.1</td>
<td>65.6</td>
<td>81.8</td>
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</tbody>
</table>

Table 2. Performance characteristics for the Draeger DrugTest 5000 on-site test with a 10-μg/L THC screening cutoff in OF with different confirmation cutoffs (N = 66).
diagnostic specificity, and 90% efficiency for THC detection in suspected drugged drivers, employing the 5-µg/L DrugTest 5000 and 2-µg/L plasma THC confirmation cutoffs in simultaneously collected samples (n = 48). Our results documented better diagnostic sensitivity for the DrugTest 5000 device than Blencowe et al. (11), who reported diagnostic sensitivity, specificity, and efficiency of 53%, 99%, and 84%, respectively, when the newer version of the DrugTest 5000 was evaluated against OF collected with the StatSure Saliva Sampler. It is unclear why we observed better diagnostic sensitivity, but recent controlled cannabis administration and collection in a research setting reduced FN results compared to those obtained in the drug addiction center and roadside settings used in the Blencowe et al. study. In fact, Blencowe et al. stated that samples obtained near the coffee shop, where individuals were thought to have recently smoked cannabis, had a diagnostic sensitivity of 76%, even with the older version of the DrugTest 5000.

In the new DrugTest 5000 on-site test with a 5-µg/L cutoff, a longer elution and lateral flow time (8.5 min for THC vs 5 min for other drugs) enhanced analyte recovery. The lower cutoff provided higher diagnostic sensitivity compared to previous versions and other currently available POC OF tests (5–14). The DrugTest 5000 offered the highest diagnostic sensitivity achieved to date compared to other point-of-care devices for THC OF detection. In addition, the Analyzer provided an objective digital readout, reducing subjectivity. The DrugTest 5000 appears highly promising as a diagnostically sensitive on-site device for workplace, pain management, drug treatment, and roadside drugged driving monitoring, although further research is required to ensure that field performance results are similar to those obtained in a controlled setting.

### Author Contributions
All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

### Authors' Disclosures or Potential Conflicts of Interest
Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

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