Oral fluid testing is a promising new matrix for drug-testing programs for drug treatment, the workplace, pain management, and driving under the influence of drugs (DUID). As with any new technology, there are strengths and limitations. We discuss with international experts the role this new alternative matrix will play in diverse drug-monitoring settings, and the research, development, and legislation needed to permit oral fluid testing to best take its place in the modern laboratory armamentarium.

Do you believe oral fluid testing will become the most prevalent matrix tested in these programs and why? What are the strengths and limitations of oral fluid testing for each type of program?

Alain Verstraete: Oral fluid will probably become the most prevalent matrix for DUID, certainly for roadside screening. Legislators and police officers want to rapidly perform DUID testing at the roadside, eliminating transport to hospitals or police stations. Oral fluid is used for DUID in 5 Australian states, Belgium, and France and is described in Swiss and United Kingdom legislation. The main advantages are ease of collection and a generally shorter window of drug detection than urine, hence a better correlation with duration of impairment. Limitations include difficulty of collection following recent drug use and the potential for passive contamination. For DUID confirmation, presently only Belgium and 4 Australian states utilize oral fluid; in other jurisdictions, positive oral fluid presumptive test results are confirmed with blood tests. Oral fluid also could become the most prevalent matrix for drug treatment due to ease of sampling, despite the shorter drug-detection window. In this setting, cost may play a more important role. If additional visits are required for oral fluid testing, urine testing might remain dominant. A strong advantage of oral fluid testing for drug treatment is the greater detection of 6-acetylmorphine, a marker of heroin use. I am unsure that oral fluid will prevail as the most important matrix for workplace drug testing. Although oral fluid collection is simpler and more easily observed, reducing adulteration potential, costs are higher than with urine, with similar detection rates.

Tai Kwong: In clinical settings, particularly drug-treatment and pain-management programs, oral fluid testing will gain popularity. At this time, the preference for oral fluid over urine is primarily due to sample-collection advantages, which include a less invasive collection protocol...
and no need for special collection facilities and a same-sex collector. The migration to oral fluid will accelerate when the following technical issues are resolved: inconsistent oral fluid and elution buffer volume, variable drug recoveries, inadequate oral fluid immunoassay sensitivity and specificity, and lack of homogeneous immunoassays for automated analyzers. The shorter oral fluid detection window following cannabis use is a serious limitation for all but DUID testing, if drug testing is scheduled for once a week or less frequently.

Jorg Morland: For DUID, I suppose oral fluid testing will become prevalent in countries where the legislation is based on “no presence of the drug in any body fluid” because of the convenience of sample collection. In other countries where low drug blood concentrations constitute the legal basis, oral fluid testing might be applied for screening drivers under suspicion, if rapid results covering most drugs of interest are available (e.g., as immunoassays). Workplace drug testing should perhaps focus on more recent drug use to reflect work performance. In that sense, oral fluid appears superior to urine and in my mind could become the most prevalent method for workplace testing.

Michael Vincent: Oral fluid testing will gain a substantial market share in each of the above segments because of the convenience in obtaining a noninvasive, gender-independent, observed specimen. For DUID and pain-management testing, oral fluid offers important interpretation value due to its correlation with plasma concentrations for most drug classes. For workplace and criminal justice testing, the “shy bladder” problem with urine testing could be eliminated. Oral fluid poses analytical challenges, since volume and drug concentrations are much lower than for urine. There could be inadequate specimen for multiple drug confirmations.

Raphael de la Torre: There is no doubt that oral fluid drug testing (especially on-site tests) is the preferred biological matrix for DUID. A positive finding can indicate recent drug consumption and recommend driving discontinuation. Limitations of on-site testing are the reduced number of drug classes that can be tested simultaneously. In drug-treatment programs or methadone-maintenance programs, on-site oral fluid tests may indicate drug use 24 h earlier. A shorter detection window means that signs of intoxication are enough evident that there is no need for drug testing. The main advantage of on-site oral fluid testing is the immediate availability of results and the potential introduction of corrective actions by therapists. For preemployment drug testing, recreational drug users may not be identified because of the limited oral fluid window of detection. Oral fluid drug testing in pain-management programs may check patient compliance and rule out consumption of additional drugs.

What do you think are reasonable and achievable performance requirements for oral fluid on-site tests, oral fluid–collection devices, and confirmation testing?

Alain Verstraete: I think the cutoffs proposed in Belgian and French DUID legislation and those used in the Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) project are realistic (Table 1). With further development, lower cutoffs can be reached for Δ⁹-tetrahydrocannabinol (THC), such as the 5-µg/L cutoff now claimed by the Dräger DrugTest 5000. Oral fluid–collection devices must provide good analyte recovery, a sample-adequacy indicator, reasonably low matrix effects in liquid chromatography–tandem mass spectrometry (LC-MS/MS) confirmation, and adequate sample volume. The time to collect and analyze oral fluid is an important variable, especially with DUID testing. A device that reliably collects a small sample volume (200 µL) and an analytical method to measure all targets in this small volume would be ideal and would increase the use of oral fluid testing. Confirmation testing may be performed by state-of-the-art multianalyte LC-MS/MS quantification.

Tai Kwong: First is the improvement of immunoassay (particularly homogeneous immunoassays) low-end sensitivity and consistency in detection, at least at the
proposed Substance Abuse Mental Health Services Administration cutoffs. Second is a broader immunoassay immunospecificity to improve detection of opiates besides morphine and specific and sensitive assays for nonopiate opioids for pain therapy. Third is an improvement of the collection device for consistency in sample volume collected, buffer volume added, and drug recovery. Fourth is the availability of quantitative MS/MS confirmation assays with analytical sensitivity commensurate with screening cutoffs.

**Jorg Morland:** Oral fluid on-site tests should produce easily readable results within 1–2 min, under variable light and climate conditions. Tests should include cannabinoids, opiates, 6-acetylmorphine (separately), amphetamines, cocaine, and as many opioids and benzodiazepines as possible. Oral fluid–collection devices should be easy to handle, be nonbreakable, and minimize the chance of contaminating the surroundings or the person collecting the sample. oral fluid confirmation testing should utilize specific methods, such as LC-MS/MS.

**Michael Vincent:** Oral fluid on-site tests should be able to robustly screen specimens at appropriate concentrations. Additionally, appropriate controls above and below the stated cutoff (±50%) should be discriminated with confidence. Currently, on-site and laboratory-based urine drug tests perform similarly. Unfortunately, there is a great difference in performance between on-site and laboratory oral fluid products. Oral fluid tests are marketed as detecting recent use; if an on-site test screens negative when an individual is visibly impaired or fails a Drug Recognition Experts evaluation, confidence in the matrix is greatly diminished.

**Raphael de la Torre:** Oral fluid on-site tests should provide quick and reliable results, with equivalent performance for all drug classes. Oral fluid devices should collect a reproducible, sufficient (1 mL) sample that is stabilized with buffers and preservatives compatible with immunoassays and LC-MS/MS methods. Currently, there is no standardization of collection devices. For oral fluid testing to grow, the device must collect a known amount of sample within a specified ±10% tolerance to enable concentration determination. The device manufacturer must document drug-extraction efficiency (≥70%) around the screening cutoff and stability in the collection buffer. Lack of adequate THC extraction contributes to false-negative cannabis tests. While drug testing in other biological matrices has “endogenous” biases (e.g., renal function in urine testing), none has a “collection” bias as seen with oral fluid, owing to the lack of standardization in oral fluid collection devices. LC-MS/MS is the most suitable approach for multianalyte confirmation, with much higher sensitivity requirements than for urine drug testing. LC-MS/MS allows laboratories to use small amounts of sample for a large multiple–drug class confirmation panel. Standardized LC-MS/MS performance criteria are needed for ion suppression, signal-to-noise ratios, and ion ratio tolerance limits.

**Table 1.** Screening and confirmation drug cutoffs according to Belgian, French, and Australian (Victoria) legislation, and proposed Substance Abuse Mental Health Services Administration (SAMHSA) oral fluid testing guidelines.

<table>
<thead>
<tr>
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<th>Belgium screen</th>
<th>Belgium confirm</th>
<th>France screen</th>
<th>Victoria confirm</th>
<th>SAMHSA confirm</th>
<th>SAMHSA confirm</th>
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</thead>
<tbody>
<tr>
<td>Amphetamines, μg/L</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>5*</td>
<td>50</td>
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</tr>
<tr>
<td>MDMA,a μg/L</td>
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<td>*</td>
<td>5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>THC, μg/L</td>
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<td>10</td>
<td>15</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cocaine/BE, μg/L</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>NA</td>
<td>20</td>
<td>8f</td>
</tr>
<tr>
<td>Morphine, μg/L</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>NA</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>6-AM, μg/L</td>
<td>9</td>
<td>5</td>
<td>10</td>
<td>NA</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Phencyclidine, μg/L</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
</tbody>
</table>

* Methamphetamine.
* Methamphetamine is the target analyte.
* Methamphetamine, amphetamine, N-methyl-3,4-methylenedioxyamphetamine (MDMA), 3,4-methylenedioxyamphetamine, and N-ethyl-3,4-methylenedioxyamphetamine.
* BE, benzoylecgonine; NA, data not available; 6-AM, 6-acetylmorphine.
* MDMA included in amphetamines screen.
* Cocaine or BE.
* 6-AM included in morphine screen.

Now that 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THCCOOH) has been shown to be present in oral fluid, albeit in nanogram-per-liter concentrations,
do you think that this would be a better biomarker than THC for detection of cannabis smoking? And why? Do you think that immunoassays could achieve acceptable detection at nanogram-per-liter concentrations of THCCOOH? Or should the screen be directed toward the parent, THC?

Alain Verstraete: THCCOOH will not become the biomarker of choice in the coming years, because of low concentrations. Presently, only a few laboratories can detect oral fluid THCCOOH. Maybe in 10 years with more sensitive techniques it will become more routine, but the future I see is that THC testing will be the standard and that THCCOOH testing will be reserved for a few cases where the result is challenged, as, for example, in the detection of amphetamine enantiomers, which is not routine but is used in disputed cases.

Tai Kwong: THCCOOH is a marker of systemic exposure and can be useful in ruling out environmental contamination. An impediment to general THCCOOH screening is the lack of assays with the requisite limits of detection. Until adequate routine THC- COOH screening and confirmation assays are available, general screening for cannabis use should be directed toward THC.

Jorg Morland: THCCOOH is a nonpsychoactive metabolite of psychoactive THC. In my mind, there is no reason to measure this metabolite for DUID- and workplace-testing purposes. For treatment of patients with cannabis dependence to monitor therapeutic goals, THCCOOH measurement in oral fluid could possibly make sense, provided it had an extended detection window. Other approaches for such monitoring would probably be better.

Michael Vincent: THCCOOH would be a better marker for cannabis detection since it is not a pyrolysis byproduct; hence, its presence is a good indicator of cannabis use and not passive inhalation. ELISA products for THCCOOH detection in hair can be modified to detect THCCOOH in oral fluids in the nanogram-per-liter range. It has been demonstrated that a commercial immunoassay can screen cannabinoids in oral fluid at 20 ng/L.

Raphael de la Torre: On-site oral fluid devices have multiple limitations in the detection of cannabis consumption. In the near future, it is difficult to foresee detection of nanogram-per-liter THCCOOH. Therefore, screening should be directed to THC detection. Instrument-based immunoassays/confirmation methods have the potential to detect nanogram-per-liter THCCOOH.

Pain-management and DUID-treatment programs, among others, require identification of a wide spectrum of psychoactive compounds. Do you think it is feasible to screen oral fluid for a sufficient number of compounds at low-enough concentrations to efficiently identify drug abuse in this population?

Alain Verstraete: I am not convinced that DUID for medicinal drugs taken in normal, prescribed doses significantly increases accident risk. Recently, odds ratios (ORs) were determined for 72 685 injured French drivers on medicines classified into 4 risk levels, from 0 (no risk) to 3 (high risk). Drivers on level 2 (OR, 1.31; 95% CI, 1.24–1.40) and level 3 (OR, 1.25; 95% CI, 1.12–1.40) prescription medicines were at higher risk of being responsible for a crash, but the ORs were quite low. However, the illicit use of medicines (e.g., benzodiazepines) is a problem. Presently, the number of target drugs is small and feasible for one LC-MS/MS method; however, for the high number of benzodiazepines available, LC-MS/MS might not be the ideal technique, and a screening immunoassay might be warranted.

Tai Kwong: Screening a large number of drugs/metabolites in a limited volume of oral fluid is technically challenging; however, recently published assays for detecting a broad spectrum of drugs at low concentrations are promising. It will be interesting to see the inevitable adaptation of LC-MS-TOF to oral fluid testing.

Jorg Morland: For these populations, it is hard to see that immunoassays would yield results with the required sensitivity and specificity. Therefore, screening in such cases would be difficult. LC-MS/MS confirmation would yield acceptable results, but estimating doses and resulting drug effects (which require blood drug concentrations) would not be possible.

Michael Vincent: Pain-management and DUID programs want to avoid false-negative results. Current commercial immunoassays and confirmation methods can utilize lower cutoffs to demonstrate feasibility. Correlations with simultaneously collected blood specimens can be high for most drug classes. Great care should be taken in the analysis of compounds with low saliva-to-plasma ratios, such as benzodiazepines, to ensure that screening cutoffs and immunoassay cross-reactivities are relevant to oral fluid drug concentrations.

Raphael de la Torre: The number of drug classes screened in a single run limits on-site drug testing. In
many situations, adequate testing can be accomplished. Alternatively, oral fluid could be collected and analyzed by instrument-based methods for a larger drug panel.

**There is a move away from immunoassay screening for a large number of drugs/drug classes to multianalyte LC-MS/MS. What do you think is the best, most analytically sensitive approach to screening for drugs in oral fluid? Can LC-MS/MS handle the volume of testing required at a reasonable cost? Does biochip array technology offer advantages for oral fluid testing due to the low volume required and simultaneous analysis of multiple drug classes?**

**Alain Verstraete:** In DRUID, LC-MS/MS (or GC-MS) was used, while in the US roadside survey an immunoassay screen was performed first, demonstrating that both strategies are possible. A disadvantage of LC-MS/MS screening is that it was based on multtarget screening; thus, not all benzodiazepines or opiates were detected. In the DRUID study in our laboratory, the cost for analysis of 1 oral fluid sample was approximately €50 (approximately $70), with the ability of analyzing only about 80 samples per day per technician. Biochip technology could be useful for high-volume testing if the process were fully automated. In the future, LC-TOF methods may offer broad-spectrum oral fluid drug screening.

**Tai Kwong:** Testing for compliance and the use of nonprescribed medications and illicit drugs in pain-management and drug-treatment programs may require analyte identification at low concentrations, which cannot be achieved by immunoassays. From a clinical laboratory standpoint, sensitive multianalyte LC-MS/MS screening is more efficient than immunoassays. But LC-MS/MS is too costly and technologically prohibitive for most clinical laboratories, and it may not have the capacity and throughput necessary to replace immunoassays as the primary mode for screening. Among current immunoassays, ELISA assays are more analytically sensitive, but they cannot be integrated efficiently and cost-effectively into existing automated instrumentation in clinical laboratories, as would be possible with homogeneous immunoassays. Biochip array is a new technology with the potential advantage of reducing sample volume requirements and increasing throughput, but it must allow specific identification of structurally similar opioids.

**Jorg Morland:** LC-MS/MS multianalyte approaches have the advantage of including many psychoactive drugs present at low concentrations that cannot be adequately screened by immunoassays. Our experience is that this is feasible as long as the volume of samples submitted for analysis is limited. Robots and automation coupled to LC-MS/MS might (hopefully) increase throughput substantially, but we are not there yet. We have so far no experience with biochip array technology.

**Michael Vincent:** ELISA methodology has a faster throughput and is far more sensitive than current commercially available LC-MS/MS assays. However, care should be taken to ensure that the appropriate cross-reactivity and cutoffs are employed. Workload is important for selecting the appropriate primary screen. LC-MS/MS requires a high capital expenditure and high operating costs due to the need for highly skilled operators, service contracts, columns, and solvents. In the US commercial market, if a laboratory encounters few specimens, screening is performed by LC-MS/MS, but as the number of specimens increases, immunoassay screening followed by LC-MS/MS confirmation becomes necessary. Biochip array technology requires lower specimen amounts than conventional ELISA testing. However, confirmation analysis is currently the major limiting factor on throughput.

**Raphael de la Torre:** New-generation LC-MS/MS instruments have the potential to analyze large drug panels with adequate sensitivity and specificity. Equipment costs are decreasing, and costs per run are competitive if the sample number is large. Additionally, LC-MS/MS is amenable to high-throughput analysis, and the turnaround time per sample is similar to automated immunoassays (excluding sample preparation). Alternative approaches, such as ELISA tests or biochip arrays, suffer in that further identification and confirmation are needed.

**What is needed to improve interpretation of oral fluid drug test results?**

**Alain Verstraete:** Further research on: (1) passive smoking and external contamination; (2) adulteration and THC washout from the mouth; (3) development of on-site devices with a small sample volume (like the DrugWipe) and results in <5 min (the DrugWipe in Belgium requires 12 min, but 5 min in Australia for only 2 analytes and with lower THC sensitivity); (4) reproducibility of multiple sampling; (5) finding a marker for concentration normalization, similar to creatinine measurements in urine; (6) more toxicokinetic controlled-administration studies to provide concentration–time data and detection windows (concentrations are sampling dependent, with results from one sampling method not necessarily representative for another method); and (7) more studies on the relationship between oral fluid drug concentrations and impairment, or crash risk.
Tai Kwong: Our ability to interpret urine drug test results is based on published controlled drug-administration studies. We need similar studies on oral fluid before we can interpret oral fluid drug test results properly.

Jorg Morland: An oral fluid test tells us that a particular drug was recently used, but no interpretation of blood or brain concentrations can be made. Oral fluid pH and secretion rate markedly influence drug concentrations. A reference substance (similar to creatinine in urine) for normalization of oral fluid results is needed. This could at least be helpful when serial samples from a single individual are evaluated over time to detect new drug intake.

Michael Vincent: Currently, most work has focused on analytical-method development, rather than result interpretation. Pain-management and DUID testing are areas where drug and metabolite concentrations can provide valuable information. Further research is needed for oral fluid and blood concentration correlations and for correlations with motor skill impairment and brain activity. Another area of concern with interpretation is the presence of multiple drugs in numerous cases. Most research involves single-drug administration in a controlled environment, making it difficult to predict effects when multiple drugs are present with and without alcohol.

Raphael de la Torre: Oral fluid drug testing was approached as urine drug testing, with some cosmetic changes. Most companies forgot that there is strong science and experience behind urine drug testing. In this context, it has been possible over time to define new target biomarkers and change cutoff concentrations based on scientific evidence. In the case of oral fluid testing, we need to follow the same approach. Manufacturers developed oral fluid analytical devices (particularly for on-site analysis) without knowing sensitivity and performance requirements. As the market was not yet mature, many initiatives were halted. There is a need for well-designed controlled clinical studies to guide selection of target biomarkers and cutoff concentrations (in the context of a given application). This is relevant not only for diagnostic companies, but also for law-enforcement authorities and clinicians. Interpretation of results is at an early stage.

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