Postmortem oral fluid toxicology testing

The following is an editorial contribution from SteelFusion Clinical Toxicology Laboratory LLC. It was authored by Amy J. Reisinger, Autumn C. Miller, Melissa R. Baldwin, Jana L. Champion, and Terra L. Shuma, all of SteelFusion.

January 2022—Death investigators are tasked with the responsibility of providing irrefutable and accurate determinations in the cause and manner of death in a timely fashion for ongoing investigations. Outcomes of any death may be revealed by the specimens submitted for toxicology analysis.[1] The conundrum of ample opinions is that there is no "one size fits all" when selecting appropriate specimens. Human nature tends to rely on traditional approaches for the collection, processing, and reporting of results because it's easier to stay status quo. However, with drug overdose deaths increasing, reports are further delayed, causing an influx of backlogged cases. To assist with these issues, our laboratory developed an economical, time-efficient, safe, and less-invasive collection method that delivers real-time results within 24 to 48 hours, accelerating the autopsy process and the ability to close cases expeditiously.

SteelFusion Clinical Toxicology Laboratory LLC's patented rapid oral fluid technology has streamlined the toxicology process by collecting one matrix from postmortem decedents for the detection and quantification of medicinal and illicit drugs and alcohol.[2-5] William James said, "The aim of science is always to reduce complexity to simplicity"; however, motivating people to adopt new matrices and procedures is easier said than done. It has been stated that the use of oral fluid drug testing for postmortem toxicology has not been extensively researched.[6]

On the contrary, postmortem oral fluid has been developed and validated to meet the ongoing quality assurance as outlined by accreditation bodies such as the ANSI National Accreditation Board and the American Board of Forensic Toxicology and adopted by medical examiners and coroners since 2016. To date, thousands of cases have been conducted using this patented technology. Prior controlled studies were designed to document the efficacy, accuracy, and rapidity of using oral fluid to detect the presence of drugs and quantify drug concentrations compared with conventional collection modalities used in forensic autopsies. Samples of oral fluid were collected from sublingual and submandibular locations. These studies revealed that drugs collected after death from the sublingual location were preserved in the salivary glands, which served as intact reservoirs[2-5] even in cases where blood, purge, or fluids that have seeped from mucosal tissues and capillaries have been observed in the oral cavity. Pharmacokinetics of the drugs in oral fluid are described in literature as being similar to those in blood concentrations, which signifies recent use of the drug. Mathematical models have been developed to predict saliva-plasma drug concentration ratios.[7,8] The passive diffusion of drugs from blood to oral fluid is influenced greatly by five factors: the drug's pKa, protein binding of the drug, lipophilicity, spatial configuration, and molecular size.[9-12]

The collection process is performed by placing a cellulose collection pad into the sublingual area, adjacent to the second bicuspid and first molar, for approximately 15 minutes. The collection pad is then removed, observed for pad saturation (a minimum of 1 mL is required), placed into the collection device, and shipped to the laboratory. Analysis is performed using an enzyme-linked immunosorbent assay (ELISA) and/or liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) instrumentation.[7] The drug classes tested consisted of nonsteroidal anti-inflammatory drugs, alcohol, alcohol metabolites, barbiturates, benzodiazepines, synthetic cannabinoids, cathinones, general anesthetics, muscle relaxants, neuroleptics, opiates, semisynthetic opioids, opioid antagonists/analgesics, stimulants, hypnotics, antitussives, antidepressants, cannabinoids, antipsychotics, anticonvulsants, antihistamines, and illicit drugs.

To demonstrate the prevalence of drugs detected in paired samples, participating medical examiners and coroners simultaneously collected oral fluid, blood, urine, vitreous fluid, and tissue from five postmortem decedents. Oral fluid was collected and analyzed per our laboratory procedures, whereas the remaining matrices were sent to outside reference laboratories for analyses (lab SF and labs A–D, respectively). These cases demonstrate the matrix, number of drugs quantified, and turnaround times (**Fig. 1**). In some instances, several matrices were collected but not tested, and not all presumptive positives were quantified, resulting in lengthy turnaround times compared with oral fluid. For example, in case No. 1, four of the eight presumptive positive results were not quantified, even with the analysis of an additional matrix. However, 11 drugs were quantified within seven hours using oral fluid. The importance of evaluating drug concentrations is to determine if drugs are within the range of being toxic or lethal, which is impossible to do if relying only on qualitative findings.

Fig. 1.

			Matrix and number of drugs quantified							
Case number	Lab	Number of presumptive positive detections	Oral fluid	Heart blood	Urine	Iliac blood	Vitreous fluid	Femoral blood	Kidney, bile, brain, liver, vitreous fluid	Turnaround time
1	SF		11							7 hours
	B	8 Urine		4						14 days
2	SF		6							5 hours
	D	1 Iliac blood				3				12 days
3	SF		4							6 hours
	A						2			40 hours
4	SF		3							6 hours
	C		2	Collected not analyzed				2	Collected not analyzed	29 days
5	SF		9							4 hours
	C							4		21 days

Oral fluid collected, processed, and analyzed from a postmortem decedent is a novel, alternative matrix in forensic toxicology for detecting and quantifying drugs, generating results comparable to the historical gold standards and/or sometimes even detecting more drugs than the gold standards. Furthermore, in instances where cases would be considered indeterminant due to insufficient and/or unavailable matrices, such as with decomposition (up to 174 days postmortem), drowning, charred bodies, embalming, homicide, suicide, motor vehicle accidents, factory and train accidents, or in stillborn babies, oral fluid has proved otherwise.

1. Flanagan RJ, Connally G, Evans JM. Analytical toxicology: guidelines for sample collection postmortem. *Toxicol Rev*. 2005;24(1):63–71.
2. Reisinger AJ. Rapid and sensitive method of forensic toxicology in post-mortem subjects using oral fluid testing. U.S. patent 9,366,685 B1. June 14, 2016.
3. Reisinger AJ. Rapid and sensitive method of forensic toxicology in post-mortem subjects using oral fluid testing. U.S. patent 9,817,006 B2. Nov. 14, 2017.
4. Reisinger AJ. Rapid and sensitive method of forensic toxicology in post-mortem subjects using oral fluid testing. U.S. patent 10,267,811 B2. April 23, 2019.
5. Reisinger AJ. Rapid and sensitive method of forensic toxicology in post-mortem subjects and in living and post-mortem animals using oral fluid

testing. U.S. patent 10,753,952 B2. Aug. 25, 2020.
6. Harper C, Lockwood R, Maxwell J. Postmortem oral fluid drug testing in Alabama. Abstract presented at: Annual Meeting of the Society of Forensic Toxicologists; Sept. 25–Oct. 1, 2021; Nashville, Tenn. Abstract S-038.
7. Reisinger AJ, Miller AC, Shaw LA, Champion JL, Neiswonger MA. Oral cavity fluid as an investigative approach for qualitative and quantitative evaluations of drugs in postmortem subjects. *J Anal Toxicol*. 2019;43(6):444–451.
8. Busardo FP, Pichini S, Pellegrini M, et al. Correlation between blood and oral fluid psychoactive drug concentrations and cognitive impairment in driving under the influence of drugs. *Curr Neuropharmacol*. 2018;16(1):84–96.
9. Heiskanen T, Langel K, Gunnar T, Lillsunde P, Kalso EA. Opioid concentrations in oral fluid and plasma in cancer patients with pain. *J Pain Symptom Manage*. 2015;50(4):524–532.
10. Scheidweiler KB, Kolbrich Spargo EA, Kelly TL, Cone EJ, Barnes AJ, Huestis MA. Pharmacokinetics of cocaine and metabolites in human oral fluid and correlation with plasma concentrations after controlled administration. *Ther Drug Monit*. 2010;32(5):628–637.
11. Lee D. Oral fluid testing. In: Levine BS, Kerrigan S, eds. *Principles of Forensic Toxicology*. Springer; 2020:632–633.
12. Bakke E, Høiseth G, Arnestad M, Gjerde H. Detection of drugs in simultaneously collected samples of oral fluid and blood. *J Anal Toxicol*. 2019;43(3):228–232.

SteelFusion Labs, 724-691-0263