How Can Alternative Matrices or Alternative Analyte Testing Provide Answers to Key Clinical Questions?

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Scope of the lecture:
The value of testing an alternative matrix for drugs of abuse is the unique information gained about the individual's drug intake history, and advantages offered in terms of sample size or less invasive sample collection, and improved drug stability. Examples of the advantages of alternative samples and alternative analytes that answer important clinical questions will be provided for oral fluid, breath, infant plasma, hair and maternal-fetal specimens available at birth.

Learning objectives:
After attending this lecture, attendees will be able to:
1. List potential advantages of testing alternative matrices
2. List markers of recent cannabis intake in blood and oral fluid
3. Describe the disposition of naloxone into infant plasma after maternal Suboxone pharmacotherapy

Extended abstract:

Alternative matrices provide a unique perspective on an individual's drug use history that can greatly improve interpretation of drug test results and may be able to answer important clinical questions. In most clinical settings, plasma and urine are the most common matrices, while in forensic settings, blood, urine, bile and tissues are frequently analyzed. Each matrix offers a different set of available analytes, different drug and metabolite concentrations, and different detection times after drug use. In addition, sample collection varies in required size, invasiveness, and drug stability, as well as in the need for highly sensitive and/or specific instrumentation for analyses. Examples of the added value of results from testing these alternative matrices and those from the maternal-fetal dyad available at birth will be shown.

Unique analytes in Oral fluid and Blood
Oral fluid is one of the most popular alternative matrices. The collection of oral fluid is easy and non-invasive, and gender neutral, meaning that either gender can collect the sample from either gender without the embarrassment of a urine specimen collection that requires close observation and collection site preparation, or the frequent time delay and need for clinical personnel to collect a blood specimen. Oral fluid is routinely utilized now for workplace, pain management, drug treatment and driving under the influence of drugs testing. What clinical and forensic questions can oral fluid answer better than blood and urine? When there is concern about opioid use, and currently the US is in the middle of a serious opioid epidemic, it is important to differentiate heroin use from a positive morphine test that could be due to ingestion of poppy seed foods or codeine use. Identifying 6-acetylmorphine definitely establishes heroin use. 6-acetylmorphine is much more prevalent in oral fluid than urine, providing a better interpretation of the individual's opiate use.

Another major clinical and forensic issue is interpretation of a cannabinoid test. Chronic frequent cannabis users build up a large body burden of Δ9-tetrahydrocannabinol (THC) in

their tissues and slowly release the drug over time. THC could be detected in some chronic frequent cannabis users' blood samples for as long as 33 days at low 0.3 µg/L concentrations. Therefore, in a workplace or home accident investigation or in a driver suspected of impaired driving, it is difficult to know if the individual recently used cannabis? Generally, laboratories only test for THC, its equipotent 11-OH-THC metabolite and the inactive 11-nor-9-carboxy-THC (THCCOOH) metabolite. If additional cannabinoid analytes present in the cannabis plant are quantified in blood, plasma or oral fluid, recent cannabis intake can be identified. The presence of cannabigerol (CBG) and cannabinol (CBD) have the highest detectability in these three matrices to indicate recent cannabis intake. Other markers include THC-glucuronide and tetrahydrocannabivarin (THCV) that also indicate recent cannabis use, but are detected in a lower percentage of cannabis users. Identifying these analytes shows recent cannabis use but their absence does not rule out recent use- finding these analytes is inclusive for recent use, but their absence does not exclude recent use. The most important fact is that these analytes differentiate recent use in chronic frequent cannabis users.

Another critical clinical question is whether Suboxone® should be given to pregnant opioid dependent women receiving buprenorphine-assisted treatment. Suboxone contains buprenorphine and naloxone, while Subutex® is buprenorphine alone. The advantage of Suboxone is that the bioavailability of naloxone is low due to poor absorption when taken orally as prescribed, but when administered intravenously during abuse of buprenorphine, it acts as an opioid antagonist diminishing buprenorphine's effects. Thus, it has advantages in a drug abusing population. But nothing is known about the disposition of naloxone in infants exposed in utero to Suboxone. We monitored buprenorphine (LOQ=0.05 µg/L), buprenorphine-glucuronide (LOQ=0.025) µg/L), norbuprenorphine (LOQ=0.25 µg/L), norbuprenorphine-glucuronide (LOQ=0.1 µg/L), naloxone (LOQ=0.05 µg/L), naloxone-glucuronide (LOQ=0.025 µg/L), nornaloxone (LOQ=0.1 µg/L), and naloxone-N-oxide (LOQ=0.025 µg/L) in maternal and infant plasma after mothers received Suboxone throughout pregnancy. Mother's received a median (range) of 16 mg (4-20 mg) buprenorphine and 4 mg (1-5) naloxone each day. Median maternal plasma buprenorphine concentrations were 1.0 (0.1-2.8) µg/L, buprenorphine-glucuronide 0.5 (0.1-5.2) µg/L, norbuprenorphine 0.7 (<LOQ-6.4, 1<LOQ) µg/L, norbuprenorphine- glucuronide 11.8 (1.4-53.6), and in infants' buprenorphine 0.5 (0.1-1.0) µg/L, buprenorphine-glucuronide 1.4 (0.1-5.3) µg/L, norbuprenorphine 0.5 (<LOQ-3.5, 2<LOQ) µg/L, norbuprenorphine-glucuronide 12.6 (1.2-51.0). Median maternal plasma naloxone concentrations were 0.07 (<LOQ-0.3 µg/L, 6<LOQ), naloxone-glucuronide 3.8 (<LOQ-25.7, 1<LOQ) µg/L and nornaloxone 0.45 (<LOQ-3.3, 3<LOQ) µg/L. For infant cord blood naloxone concentrations were 0.16 (<LOQ to 0.3, 5<LOQ) µg/L, naloxone-glucuronide 4.4 (<LOQ-26.3, 1<LOQ) µg/L and nornaloxone 0.5 (<LOQ-2.3, 2<LOQ) µg/L. No naloxone-N-oxide was detected in any specimen. By measuring the naloxone and metabolite concentrations in maternal and infant plasma, we documented for the first time the low concentrations in maternal and infant plasma from naloxone in Suboxone pharmacotherapy. These data contribute to the safety data supporting the use of Suboxone in pregnant women.

Breath

Breath is also an easily collected alternative matrix, most commonly used for breath alcohol testing. There is tremendous interest in on-site breath testing for other drugs including cannabis due to the medicalization (29 US states, and many countries around the world) and legalization (Uruguay, Canada, 8 US States) and decriminalization in many other countries. Many research groups investing in the development of roadside breathalyzers for drugs, but none has yet reached commercialization. We conducted the only controlled smoked cannabis

administrations study to chronic frequent and occasional cannabis users that monitored the presence of THC and THCCOOH in breath. After smoking a 6.8% THC cigarette, THC was detected in the breath of frequent cannabis users for up to 4 h and in the breath of occasional users for a shorter period of time. One individual had no THC-positive breath sample, so finding THC in breath documents recent use, but its absence does not exclude recent cannabis use. THCCOOH was not identified in any breath sample.

We also conducted the only controlled administered cocaine study investigating the disposition of cocaine and benzoylecgonine in cocaine users' breath. Breath was collected from 10 healthy adult cocaine users by asking them to breathe into a SensAbues device for 3 min before and up to 22 h following 25 mg intravenous (IV) cocaine dosing on days 1, 5, and 10, and assayed with a validated liquid chromatography-high resolution mass spectrometry (LC-HRMS) method to quantify breath cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), and norcocaine. The assay was linear from 25 to 1,000 pg/filter, extraction efficiencies were 83.6–126 %, intra- and inter-assay imprecision was <10.6 %, and bias was between −8.5 and 16.8 %. Of breath specimens collected up to 22 h after controlled cocaine administration, 2.6% were positive for cocaine, 0.72 % for BE and 0.72% for EME. Cocaine marker concentrations and detection times were cocaine 26.1 – 66 pg/filter from 1–9.5 h, BE 83.3 – 151 pg/filter for 6.5–12.5 h, and EME 50–69.1 pg/filter for 6.5–12.5 h. Norcocaine was not detected in any breath sample. Thus, cocaine, BE or EME detection in breath could be a marker of recent cocaine use. Due to the high concentrations of cocaine and metabolites in oral fluid, it is important to preclude oral fluid from reaching the filter in the SensAbues device.

Hair

Hair is a specimen that is easily collected and drugs are generally more stable in hair than other biological specimens. Hair can also be stored and shipped at room temperature. Hair offers a much longer monitoring period from about 7 days, the time necessary for drugs ingested and incorporated in the hair follicle to reach the scalp, and years depending upon the length of the hair. Hair is highly useful to determine the pattern of an individual's drug use over time. Hair test results can answer important questions about whether someone uses drugs on a regular basis, or whether a sexual assault may have been drug facilitated. In other cases, biological specimens may not have been available at the time of an event, but hair tests can provide some information about drug use in the past.

These data provide answers to important clinical and forensic questions about an individual's drug use history and the question of safety of naloxone dosing in pregnancy. This shows the value of the information provided by alternative matrices and alternative markers during analytical testing.