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Oral fluid and hair in workplace drug testing programs: new technology for immunoassays

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Workplace drug testing programs have embraced both oral fluid and hair as testing matrices. Saliva is popular due to its easy, rapid collection; its non-invasiveness compared to urine or blood; the convenience of collecting a specimen anywhere, anytime; and the difficulty of adulteration. The main advantage of saliva, however, remains its suitability for post-accident or 'for-cause' testing since the presence of a parent drug can assist in the determination of an individual being 'under the influence' of a drug. Hair, on the other hand, is useful for workplace programs, since its ability to provide historical information on drug intake ensures it is an excellent specimen for pre-employment testing. Both technologies have enjoyed collection and laboratory improvements for immunoassay screening over the last few years, and these are discussed in this perspective.

Oral Fluid

As with all specimens, there are certain drawbacks to oral fluid testing: non-standardization of collection devices; low sample volume compared to urine; fewer publications than urine; limited information on drug recovery and stability during storage and transportation; and the inability of some subjects to provide a valid sample – or 'dry-mouth syndrome'. Manufacturers of saliva collection devices along with immunoassay screening companies have begun to address these challenges. The issue of how much sample is collected tends to be device-dependent. The most widely used collection devices are those which incorporate a pad and a buffer rather than the collection of neat oral fluid via expectoration. The use of a pad allows the sample to be collected more quickly, and on some devices indicates when an adequate volume has been absorbed (e.g. Omni-Sal®, Quantisal™). The improved technology associated with composition of the buffer promotes drug stabilization during transportation and efficient drug recovery from the pad, while preventing bacterial growth in biological specimens.

As a rule on-site devices collect less volume than laboratory-based collectors. A recent publication on the Rapid-Stat® on-site device reported a median collection volume of 0.27 mL; the same publication recommended that a minimum of 0.5 mL is necessary.[1] The reasoning behind this volume is that as legal medications are increasingly abused, the test panel will need to expand, without having to alter the collection system in the field. Further, laboratory failures may require additional volume for repeat analysis. In the 2007 National Roadside Survey, a drug test panel of over 50 drugs was utilized from a single collection of 1 mL neat saliva using the Quantisal™ device (Table 1). In many cases, multiple drug positives were screened and confirmed from a single specimen with a volume of 1 mL.

Information about the efficiency of drug recovery from a collection pad (the ability to remove the drug from the pad) as well as stability data and recommendations for storage are becoming more prevalent in publications on oral fluid. It is essential for the validity of a quantitative value to know how much specimen is collected and how efficient drug removal from a collection pad is. Figure 1 shows quantitative percentage recovery of many drug classes from the Quantisal™ collection device.

Laboratory-based analysis generally requires the collected specimen to be transported overnight to a testing facility; therefore the stability of the analytes under these conditions and in long-term storage is an important issue. In 2009, Ventura *et al*. reported the instability of cocaine, 6-acetylmorphine (6-AM) and tetrahydrocannabinol (THC) during transit in two different collection devices.[2] Between 26% and 41% of the cocaine converted to benzoylecgonine (BZE); 9–12% of 6-AM to morphine; and only between 39% and 67% THC was recovered from the devices.[2]

For the Quantisal™, oral fluid specimens may be stored at room temperature for a few days, refrigerated for up to 30 days, and frozen (−20 °C) for long-term storage. The main culprit for adherence to surfaces is marijuana (THC). Twenty-four specimens positive for THC stored in Quantisal™ buffer were re-analyzed after 9 months of storage. Overall, drug loss for THC varied. Three specimens showed significant loss, (>50%) decreasing from 183 to 48 ng/mL in one case; 4.2 ng/mL to less than 1 ng/mL in the second; and 5.5 ng/mL to 2.7 ng/mL in the third. Assessing those as outliers, the mean loss of THC over 9 months was 14.4% (Figure 2). Cocaine was found to be the least stable of the major drug classes in the Quantisal™ device, converting over time to benzoylecgonine. Since both cocaine (COC) and benzoylecgonine (BZE) are detectable in oral fluid, the conversion of COC to BZE is important. While the positivity of the sample remains unchanged, the interpretation of the result will be affected. The presence of parent drug in an oral fluid sample indicates recent use, and potentially a determination of being 'under the influence'. The presence of a metabolite only may indicate use was further back in the metabolic cycle, and the individual may no longer be feeling the effects of the drug.

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Table 1. Drug test profile in oral fluid from National Roadside Survey 2007

Cocaine	Zolpidem
Marijuana	Carisoprodol
Opiates	Oxycodone
Amphetamines	Tramadol
Benzodiazepines	Meperidine
Methadone	Propoxyphene
Fluoxetine	Dextromethorphan
Sertraline	Tricyclic antidepressants
Phencyclidine	Ketamine
Barbiturates	Methylphenidate

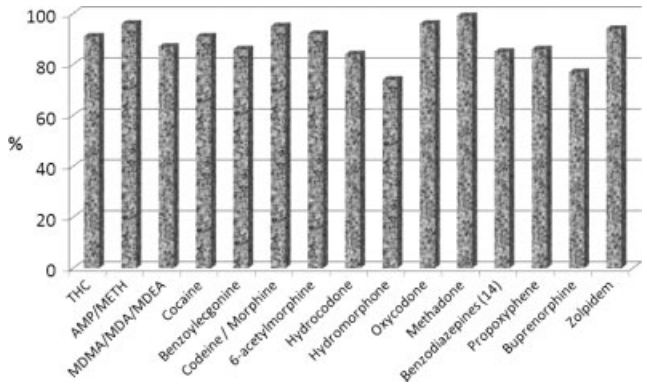

Figure 1. Drug recovery from Quantisal™ oral fluid collection device.

So what's new for immunoassay screening?

For many years the sensitivity required for the detection of drugs in oral fluid from an immunoassay point of view was provided only by enzyme-linked immunosorbent assays (ELISA), which are heterogeneous tests, requiring incubation and color-development stages. Developments in technology now allow immunoassay screening of oral fluid samples to be carried out in a homogeneous assay format; liquid reagents are available from several commercial vendors, at least for the main drug classes. The drug panel available for these screening reagents keeps expanding, and means that current urine testing laboratories do not have to buy additional equipment in order to add oral fluid analysis to their workplace testing capability.

Hair

Hair has distinct advantages over urine as a specimen type for pre-employment testing. It is quick and easy to collect, generally not invasive, easily shipped to the test facility, and can be stored at room temperature. The nature of the specimens allows a long-term historical window of the subject's drug intake (usually around 3 months), which is extremely useful for a hiring decision, and certainly preferable to other matrices. The disadvantages of hair testing include higher cost than oral fluid or urine, an inherent color bias, and potential issues with external contamination and adulteration (bleaching, dyeing, etc.) Naturally bald people also are at a disadvantage if required to take a hair test since the sample collection process then becomes somewhat more invasive. Further, drugs which are acidic in nature (e.g. marijuana, barbiturates) do not incorporate well into the hair shaft; in contrast to basic drugs (e.g. cocaine, amphetamine) which are easily accumulated by the hair. Even the drugs which are well incorporated are present at low concentration, but improvements in screening have greatly assisted the laboratory testing process.

So what's new for immunoassay screening?

Hair-testing laboratories which carry out immunoassay screening generally use either ELISA or radioimmunoassay (RIA). For the first time an aqueous extraction for THC has been reported with direct placement of the extract into the ELISA microplate well.[3] The procedure reaches the sensitivity requirements proposed by the European Workplace Drug Testing Society (EWDTS).[4] Since organic solvents, which until now have been necessary to remove drugs from the hair matrix, will degrade the performance of an immunoassay, this was a major breakthrough. An aqueous incubation reduces extraction time and eliminates evaporation and reconstitution stages which are currently used in laboratory hair-testing facilities. Further, the aqueous extraction of a wide range of drugs from hair has caused the panel to expand so

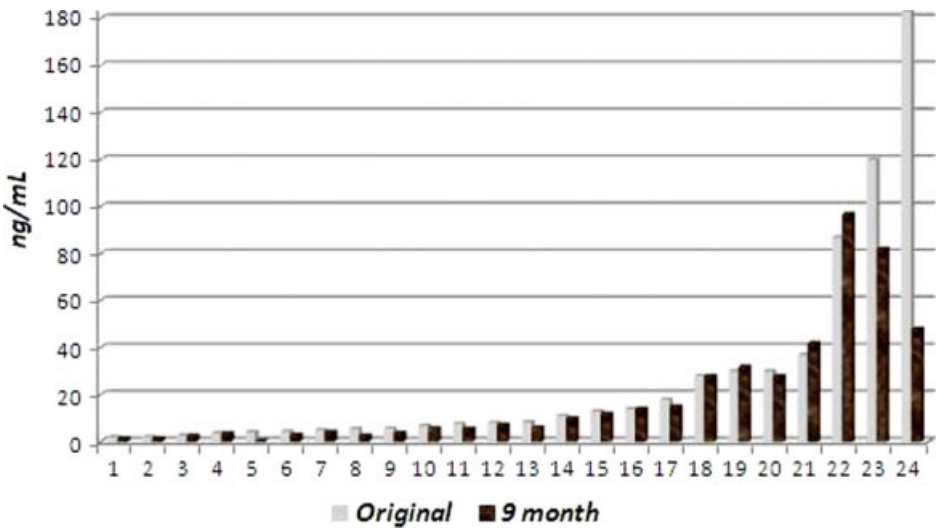

Figure 2. Stability of THC over 9 months.

that testing is no longer limited to a small number of drugs. Pain medications, including carisoprodol, diazepam, fentanyl, methadone, oxycodone, propoxyphene, tramadol, and morphine have all been reported from a single aqueous extract.[5] More recently, Comedical Laboratories commercialized reagents (VMA-T) purposely designed to treat hair samples for compatibility with immunometric methods currently used in urinalysis. Its assays convert 6-MAM and cocaine (which may be present in hair) to their metabolites detected in urine, specifically morphine and benzoylecgonine. The main advantage of the method is the simultaneous extraction of the main drug classes, excluding marijuana, and compatibility with immunological methods applied in urine drug testing.[6]

Summary

Advances in immunochemical techniques for both oral fluid and hair have allowed urine testing laboratories to add the testing of alternative matrices to their existing facilities. Homogeneous liquid reagents for oral fluid are commercially available, and an aqueous hair extract compatible with immunoassays has been developed.

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