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Technical Note

Assessment of the stability of DNA in specimens collected under conditions for drug testing—A pilot study

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ABSTRACT

For forensic biological sample collections, the specimen donor is linked solidly to his or her specimen through a chain of custody (CoC) sometimes referenced as a chain of evidence. Rarely, a donor may deny that a urine or oral fluid (OF) specimen is his or her specimen even with a patent CoC. The goal of this pilot study was to determine the potential effects of short-term storage on the quality and quantity of DNA in both types of specimen under conditions that may be encountered with employment-related drug testing specimens. Fresh urine and freshly collected oral fluid all produced complete STR profiles. For the "pad" type OF collectors, acceptable DNA was extractable both from the buffer/preservative and the pad. Although fresh urine and OF produced complete STR profiles, partial profiles were obtained after storage for most samples. An exception was the DNA in the Quantisal OF collector, from which a complete profile was obtained for both freshly collected OF and stored OF.

1. Introduction

The only currently allowed specimen under the SAMHSA (Substance Abuse and Mental Health Services Administration) Mandatory Guidelines for employment-related drug testing is urine [1]. Oral fluid (OF), which is commonly mis-referenced as saliva, may be usable for employment-related drug testing in the near future [2]. Although hair drug testing may become a useful specimen for Federal employment-related drug testing in the future, it was not considered in this brief pilot study [3].

Forensic specimens sent to a laboratory for drug/drug metabolite analyses are accompanied by an external chain of custody. A properly executed external chain of custody documents donor identity and sample collection, security, and transfer to the laboratory. However, in extremely rare instances, despite appropriate documentation, a sample donor may deny that a specimen was produced by them at the place, time and date specified on the external chain of custody. In such instances, deoxyribonucleic acid (DNA) is a logical choice to demonstrate unequivocally that a given specimen is linked to a given specimen donor. For DNA to suffice as a marker of identity, DNA of acceptable quality must exist in sufficient quantity with the appropriate analyses of inclusion or exclusion applied. Although several approaches [4] to using DNA for identity have been used for human samples, the best system currently appears to be use of short tandem repeats or STRs [5] (microsatellites, simple sequence repeats or SSRs [6]).

In urine, with exception to sperm cells that might be present in a woman's specimen and microbial cells/cell remnants, all nucleated cells present contain the urine donor's DNA. Using HLA-DQA1 (Human Leukocyte Antigen-DQA1) genotyping and amelogenin for sex determination, Yokota et al. [7] found that the sediment from 10 mL of human urine voided within 24 h prior to analysis was adequate for DNA extraction. DNA from urine stored 2 weeks at 4 °C, 25 °C and 30 °C was found to be equivalent to fresh urine for DNA extraction. If the sediment was fixed with ethanol, the sediment could be stored for 2 weeks at 4–30 °C without

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evidence of degeneration or fermentation. DNA from microbial sources did not appear to present any problems with human DNA analysis. Using frozen (−20 °C) urine samples (50 mL aliquots) from a cohort of female patients enrolled in the Dutch DOM (Diagnostische Onderzoek Mammacarcinogen) study, van der Hel et al. [8] obtained an 89.3% genotype success rate with MTHFR (methylene tetrahydrofolate reductase gene) polymorphisms. By separating human cells from contaminating organisms prior to DNA extraction, a practice not necessarily applicable to a high-production testing laboratory, Prinz et al. [9] were able to improve successful typing (HVR region 3′ of the apolipoprotein B gene) from 20% to 35% in males and from 35% to 77% in females. Due primarily to the presence of increased epithelial cells in human female urine, more extractable DNA was found in female urine than in male urine. Although not acceptable for employment-related urine toxicology under the Federal Mandatory Guidelines [1] the addition of EDTA [10] or sodium azide [11,12] to urine may stabilize human DNA.

Saliva, more correctly referenced as oral fluid (OF), has been compared to blood for genotyping and found to be acceptable [13]. Oral fluid usually contains large numbers of buccal epithelial cells and it is thus not surprising that it is a good sample from which to extract DNA. DNA extracted from OF is frequently used for paternity testing, criminal identification, identification of human remains, and other identification purposes.

The primary purpose of the pilot study described below was to determine the approximate amount of degradation that DNA might experience under routine short-term storage conditions typical of a drug testing laboratory. The main exception was that donors collected their own urine specimen without the intervention of a designated collection site person. OF specimens were self-collected, transported and stored using manufacturer's instructions for drug testing. As was true for urine specimens, a collection site person was not used for collection of oral fluid samples. In a departure from routine oral fluid employment-related drug testing laboratory processing of so-called "pad" device (Intercept i2™, Oral-EZE™, and Quantisal™), the pad itself was retained and any DNA eluted and analyzed as described below. Drug/drug metabolite testing was not a part of this pilot study.

2. Materials and methods

2.1. Donor recruitment

The pilot study was approved by the RTI Institutional Review Board August 17, 2016. Participants provided written, signed, informed consent that was witnessed. Five donors, consisting of three males and two females, self-collected at least 45 mL of urine, four buccal swabs, and four duplicate oral fluid specimens over a period of approximately four hours.

2.2. Sample collection materials

Sealed urine split kits were obtained from ThermoFisher Scientific (Waltham, MA). Quantisal™ collection devices were obtained from Immunalysis Corporation (Pomona, CA). Intercept® i2 collection devices were obtained from OraSure (Bethlehem, PA). Oral-EZE® collection devices were obtained from Quest Diagnostics (Madison, NJ). RapidEASE® oral fluid collection devices were obtained from Biophor (Redwood City, CA). Buccal swabs and containment envelopes were provided by Laboratory Corporation of America Holdings (LabCorp, Burlington, NC).

2.3. Collection methods

Four buccal swabs were collected by each donor using the four quadrants of the oral cavity between the cheek and the gum. Collected buccal swabs were air-dried and placed in the paper envelope provided with each set of four swabs. Roman numerals were used for specimen identification to avoid confusion with the designation of a bottle as "A" or "B" in urine and oral fluid collections.

In the four-hour period following buccal swab collection, the following oral fluid collections were performed:

1. Quantisal™ "A" and "B".
2. Oral-EZE® "A" and "B".
3. Intercept® i2 "A" and "B".
4. RapidEASE® "A" and "B".

Collection using the Quantisal™, Oral-EZE® and Intercept® i2 systems involves placing the collection pad, which is attached to a plastic holder, into the oral cavity and collecting oral fluid until collection sufficiency is shown by an indicator window on the plastic holder. Post-oral fluid collection the pad and placed into a tube containing an exact amount of buffer/preservative and the tube containing the buffer/preservative and pad is securely capped. Each type of collector provides the equivalent of approximately 1 mL of neat oral fluid. Collection using the RapidEASE® system involves expectoration into the glass tube using a plastic funnel provided by the tube manufacturer. Each tube contains approximately 2 mL of neat oral fluid.

A period of 30 min separated each oral fluid collection. Identification of oral fluid was the same as that used for urine specimens.

On the same day as the collection of buccal swabs and oral fluid samples, donors self-collected a split ("A" = ∼30 mL; "B" = ∼15 mL) urine specimen using standard protocol [1].

Specimens were retained at the point of collection at ambient temperature (22 + 2 °C) for two days; samples were then transported to the testing laboratory. Samples were exposed to fluorescent overhead lighting for most of the post-collection time period but only briefly exposed to sunlight during transport to the testing laboratory.

2.4. Sample handling

Upon receipt by the testing laboratory all samples were assigned a unique laboratory identification number.

2.4.1. Buccal swabs

Of the four (4) buccal swabs collected from each donor, one was randomly designated "A" and the other was randomly designated "B". Two swabs from each donor were processed for DNA extraction and two swabs were retained as backups and not used in this study. Swabs were stored in their original paper envelope at ambient laboratory temperature.

2.4.2. Urine

"A" bottles were stored at 4 °C while "B" bottles were frozen at −20 °C. Prior to initial testing, the "A"samples were brought to room temperature, mixed several times by inversion and a 5 mL aliquot was centrifuged at 6000 × *g* (8000 rpm) for 5 min to provide a cell pellet for DNA extraction. The supernatant from each centrifuged sample was discarded appropriately as biohazardous medical waste.