Optimized Recovery of DNA from Exhaled Breath Devices: from Drug Detection to Human Identification

Kayli Carrillo, BS[1]; Tim Kalafut, PhD[1]; Sheree Hughes, PhD[1]

[1]Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

INTRODUCTION

Recovery of DNA from exhaled breath is challenging. Exhaled breath is a unique DNA matrix that has yet to be explored but is commonly used for diagnosing lung disease or identifying abuse of volatile illicit drugs[1]. The composition of exhaled breath consists of mediators and nucleic acids, which is explained by apoptosis, necrosis, and spontaneous cell death in the respiratory tract due to oxidative stresses. DNA may also be recovered from sloughed epithelial cells from the respiratory buccal mucus membranes. However, extraction from exhaled breath is complicated by the high degree of dilution with water vapor[2].

This study explored whether DNA could be captured from exhaled breath using two different collection devices SensAbues® (Fig. 1) and Breath Explor® (Fig. 3). These devices are traditionally used for drug detection and are sent to laboratories for further analysis. As it is essential that the chain of custody be maintained to ensure sample integrity, processing these breath devices for both drugs of concern and DNA to confirm the identity of the user could be beneficial.

SensAbues® contains a thin electret polymer air filter that captures and retains bioaerosol particles from the airway lining fluid of lungs (Fig 2)[3]. Breath Explor® collects aerosol products of surfactant from the distal areas of the lungs through impaction of the three internal filters[4].

Several DNA collection methods using cotton and microFLOQ® swabs and soaking method[5] were compared to determine if useable STR profiles could be attained from the mouthpiece and/or internal filters of the devices. As an alternate method for capturing and preserving DNA in breath, wet or dry FTA® card punches were placed into the mouthpiece of the Breath Explor® device.

Diamond™ Nucleic Acid Dye is a fluorescent dye that has been applied to evidence to visualize where DNA is located[6]. We briefly explored this method with both breath devices to establish if cells or cell-free DNA is being transferred from exhaled breath to the device.

Optimizing DNA recovery from exhaled breath devices could potentially offer an alternate approach to collecting DNA for forensic purposes, and possibly also assist in improving the recovery of DNA from other trace evidence.

Figure 1: SensAbues® Device

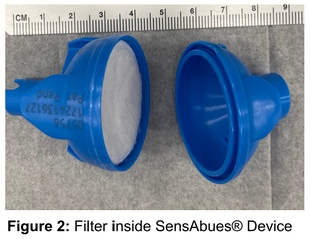

Figure 2: Filter inside SensAbues® Device

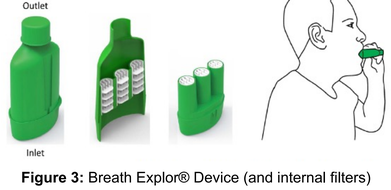

Figure 3: Breath Explor® Device (and internal filters)

RESULTS AND DISCUSSION

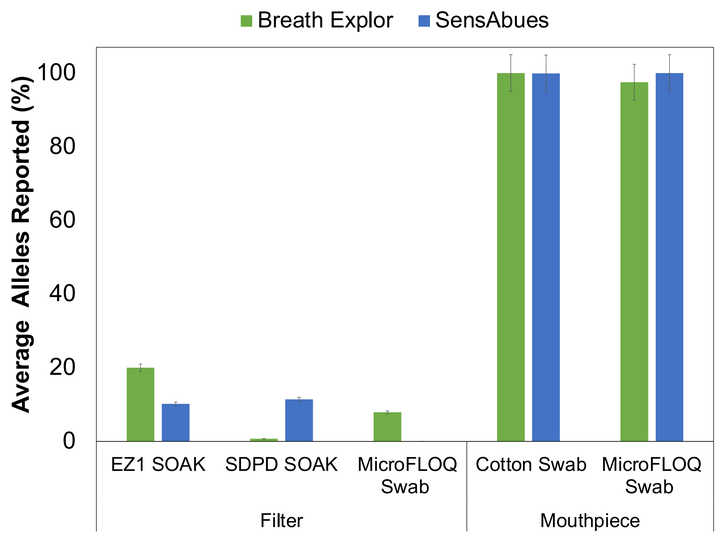

Figure 4: Average reportable alleles (%) obtained when the mouthpiece or internal filters of Breath Explor® and SensAbues® devices were processed via swabbing or soaking methods for DNA recovery (n=100). Error bars represent ± standard error of the mean (SEM)

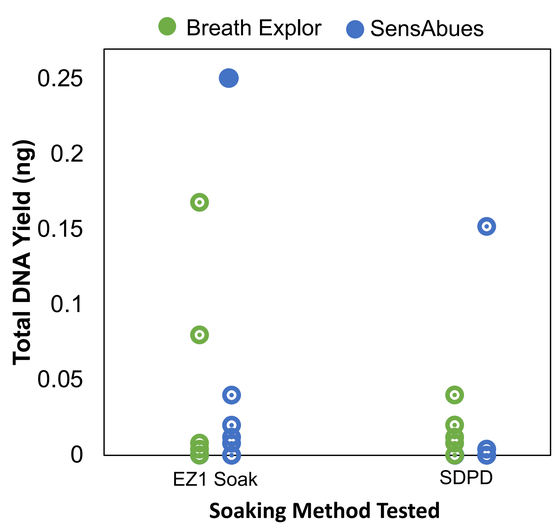

Figure 5: Comparison of total DNA yield (ng) from Breath Explor® and SensAbues® devices using both soaking methods (n=40).

- Near complete STR profiles were recovered from the mouthpieces of both breath devices using cotton and microFLOQ® swabs (average of 99.9% and 97.5% of allele recovery, respectively) (Fig. 4).
- Although no STR profile was obtained for 76% of filter types, 6% of samples yielded a full profile (n=60) (data not shown).
- Overall, the observed average percentage of reportable alleles was less than 20% from the filters of both breath devices regardless of the methods used (Fig 4).
- Both filter types yielded picogram or sub picogram amounts of DNA. The highest yielding sample was a SensAbues® device using the SDPD soaking method with 0.26 ng. (Fig. 5). No statistical difference was observed in DNA yield between the two methods for Breath Explor® (p = 0.2) and SensAbues® (0.68).
- Initial testing of Diamond™ Nucleic Acid Dye sprayed onto the Breath Explor®, SensAbues® devices, and FTA® disks was not successful due to lack of contrast between substrate and DNA sample.

CONCLUSIONS

- Poor DNA recovery and incomplete STR profiles were observed from both filter types of SensAbues® and Breath Explor®.
- The incorporation of a pre-wet or dry FTA® card punch into the Breath Explor® did not improve DNA collection. Less than 10% of samples yielded detectable amounts of DNA.
- Laboratories are recommended to swab the mouthpiece of the breath devices to confirm the identify of the user.
- Further testing of Diamond™ Nucleic Acid Dye to visualize cells on Breath Explor®, SensAbues®, and FTA® disks substrates will be investigated.

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MATERIALS AND METHODS

Phase 1: The mouthpiece and filters of SensAbues® and Breath Explor® were sampled (Fig. 1 & 2). Ten participants were asked to breathe into each device. Mouthpieces of both devices were swabbed with cotton and microFLOQ® swabs. Filters were subjected to swabbing with a microFLOQ® swab and two soaking methods (Fig. 6) N= 10 donors, n = 100 total samples.

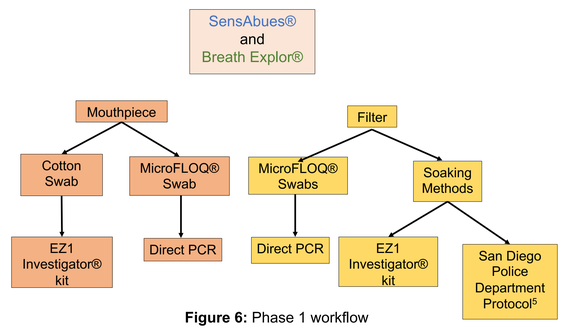

Figure 6: Phase 1 workflow

Phase 2: Pre-wet or dry FTA® punches were placed inside the Breath Explor® device to investigate an alternative approach to capture and preserve DNA from exhaled breath samples (Fig. 7).

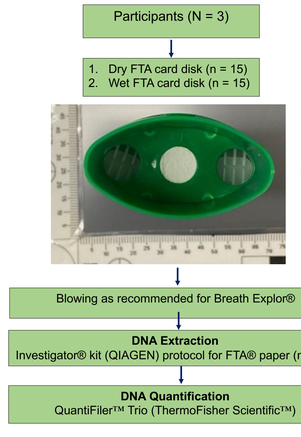

Figure 7: Phase 2 workflow

FTA® Disk (white circle) placed inside device

Phase 3: Breath Explor®, SensAbues®, and FTA card punches were spiked with a buccal cell suspension, cell-free DNA (lysed buccal suspension), or sterile water (control). In this preliminary study, a solution of 20X Diamond™ Nucleic Acid Dye (Promega) in 75% ethanol was sprayed on the substrates to determine if cells or cell-free DNA could be visualized. Substrates were examined with an excitation wavelength of 494 nm and an emission wavelength of 555 nm using a Leica EZ4 stereo microscope at 35X magnification.

ACKNOWLEDGEMENTS

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