30th CONGRESS OF THE INTERNATIONAL SOCIETY FOR FORENSIC GENETICS
SANTIAGO DE COMPOSTELA
9-13 SEPTEMBER 2024

iSFG 2024

POSTER NR. 468

Optimizing the Recovery of DNA from Exhaled Breath Devices for 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*

Introduction

The recovery of DNA from exhaled breath presents significant challenges. While exhaled breath is a unique source of DNA, its potential for forensic applications remains largely unexplored. Traditionally, exhaled breath is commonly used for diagnosing lung disease or identifying abuse of volatile illicit drugs[2]. 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. However, extraction from exhaled breath is complicated by the high degree of dilution with water vapor[3].

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, typically used for drug detection, are sent to laboratories for further analysis. Therefore, it is essential that the chain of custody be maintained to ensure sample integrity; hence the suggestion of processing these breath devices for drugs of concern and DNA to confirm the identity of the user. 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.

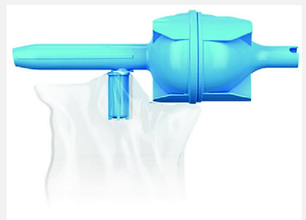

Figure 1: SensAbues® Device

Figure 2: Inside SensAbues® Device

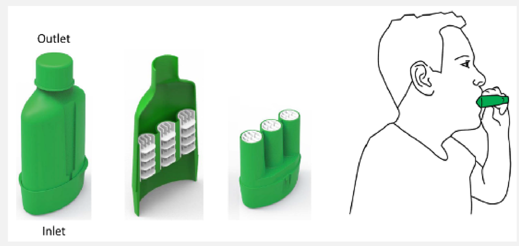

Figure 3: Breath Explor® Device

Materials and Methods

Phase 1: The mouthpieces and filters of SensAbues® and Breath Explor® were sampled (Fig. 1 & 3). Ten participants were asked to breathe into each device. The mouthpieces of both devices were swabbed with cotton and microFLOQ® swabs. Filters were swabbed with a microFLOQ® swab and two soaking methods (Fig. 4). N = 10 donors, n = 100 total samples.

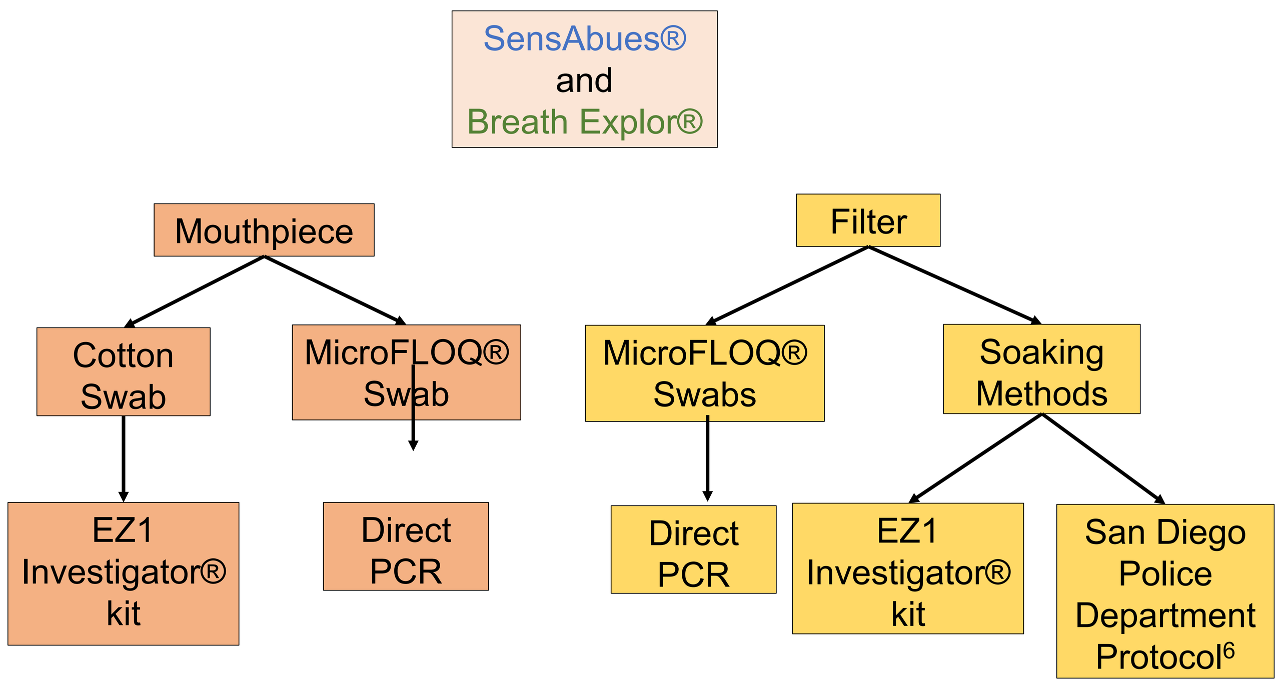

Figure 4: Phase 1 workflow

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

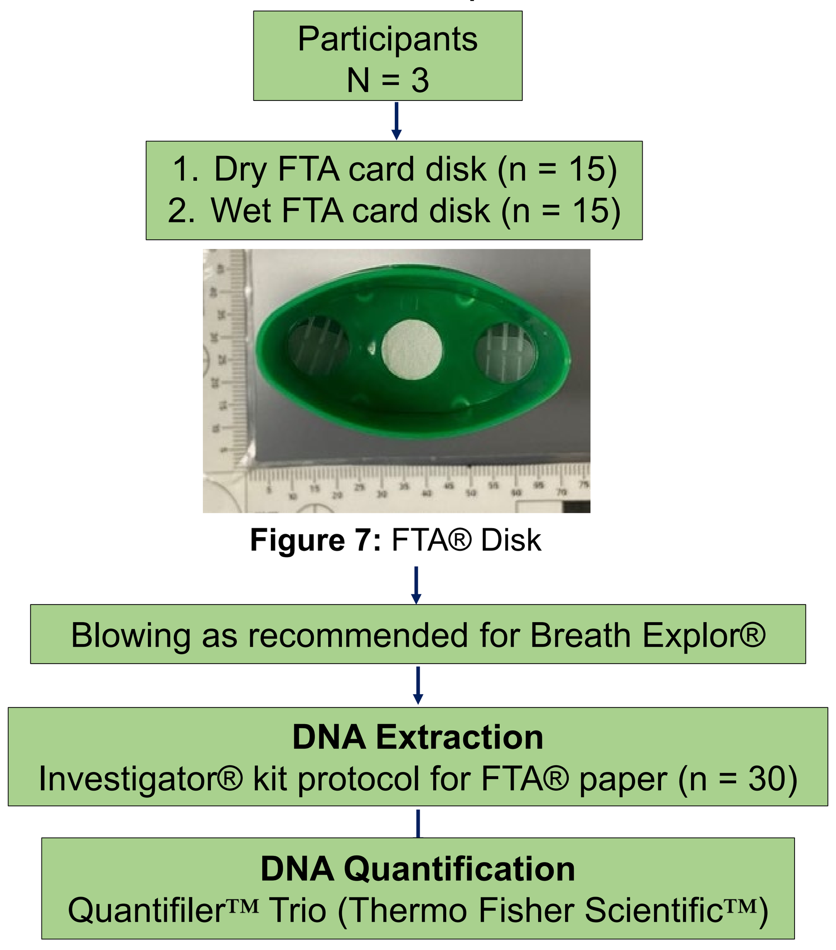

Figure 7: FTA® Disk

Figure 5: Phase 2 workflow

Phase 3: Diamond Dye® was tested for its ability to visualize DNA. As a positive control, 5µl of saliva was spiked on one slide, while 5µl of water was used as a negative control on a separate slide. A solution of 20X Diamond Dye® in 75% ethanol was sprayed onto the substrates to determine if cells could be visualized. Substrates were examined with an excitation wavelength of 494 nm and an emission wavelength of 555nm with a Keyence VHX-7000 microscope.

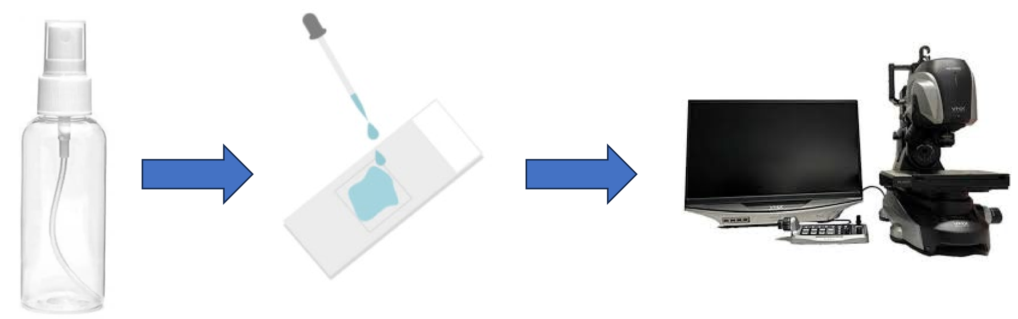

Figure 6: Phase 3 workflow

Results and Discussion

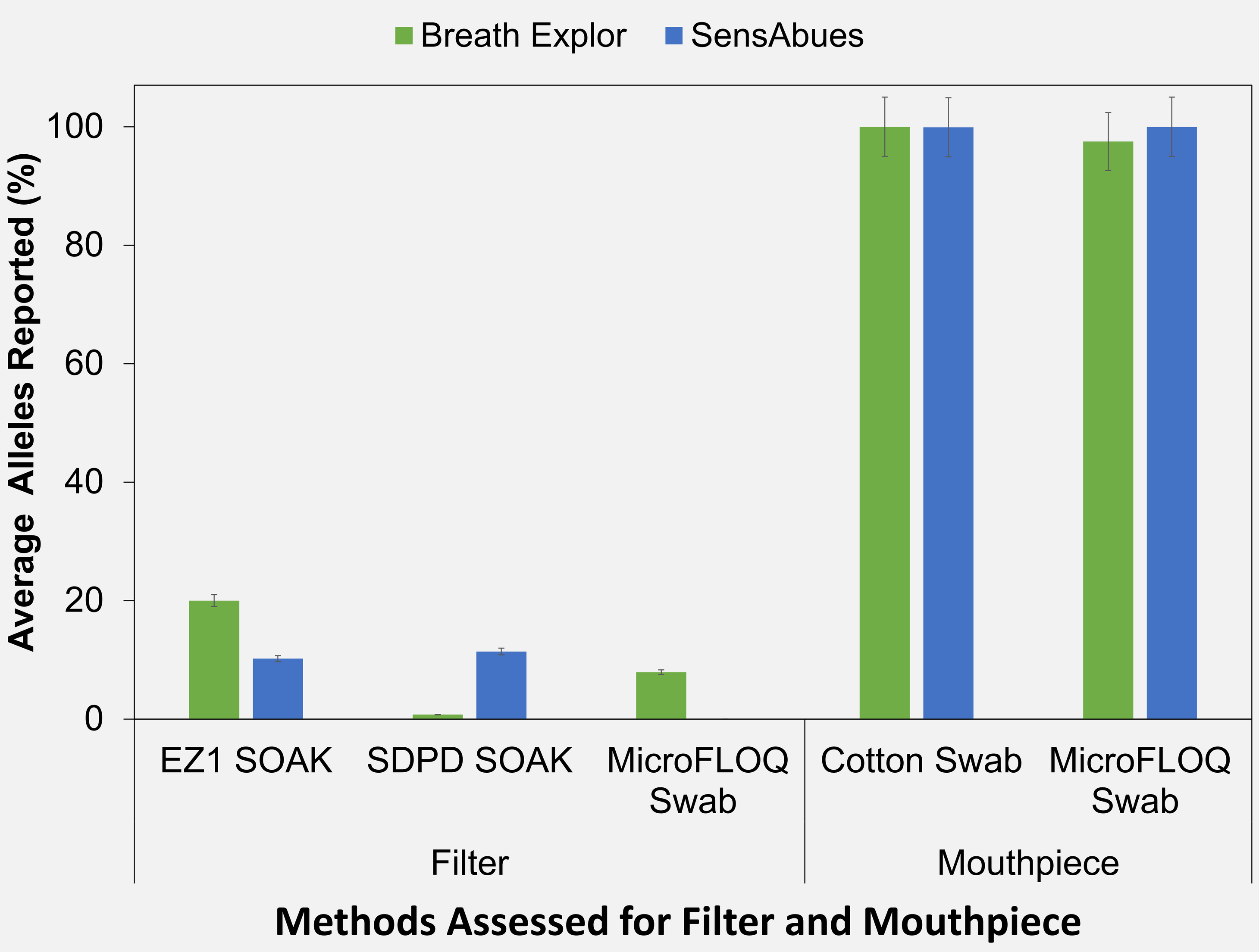

Figure 7: Average reportable alleles (%) versus each extraction and collection method for Breath Explor® and SensAbues® (n=100). Error bars represent percent error (5%).

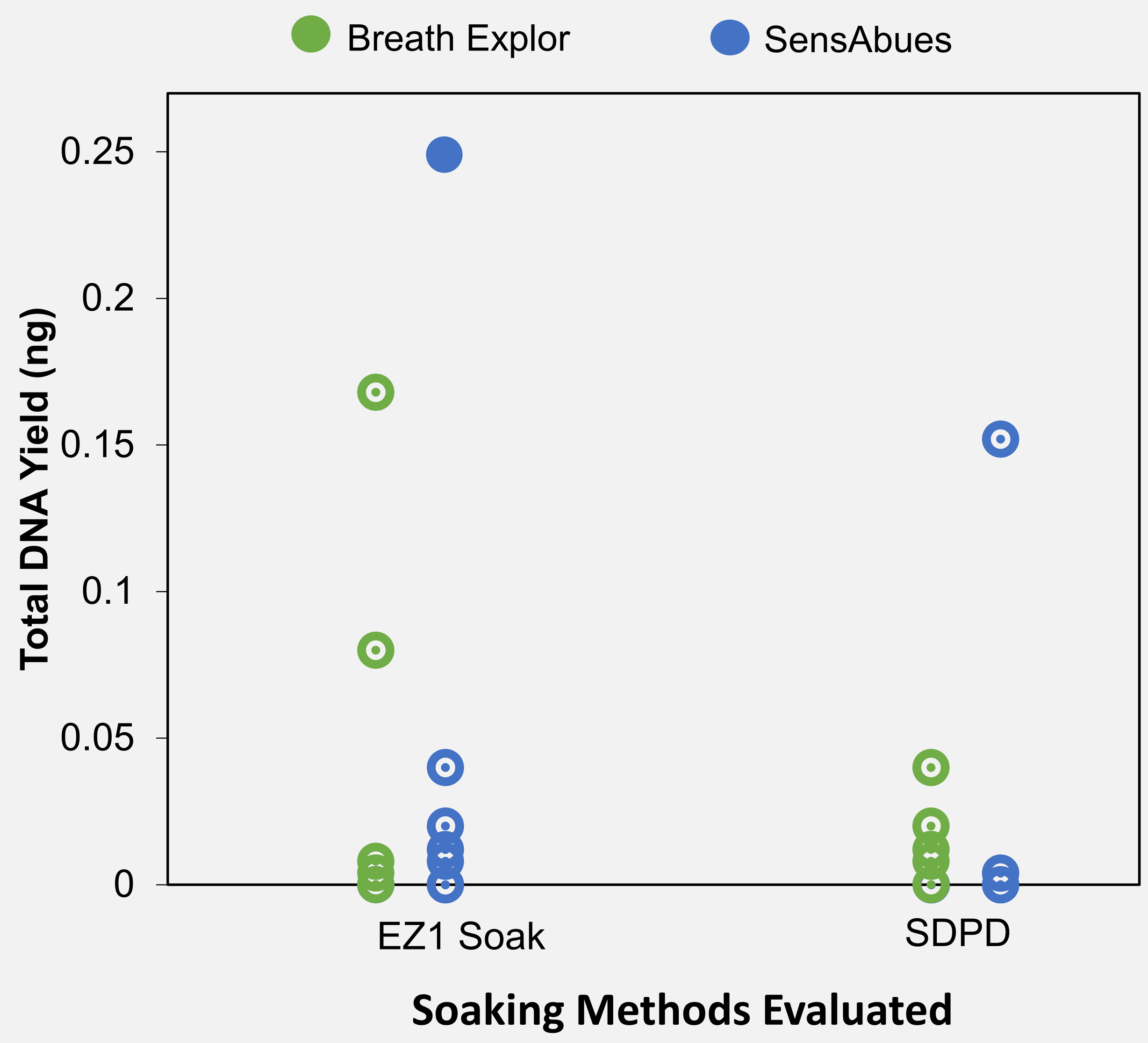

Figure 8: Soaking methods comparison of total DNA yield (ng) between Breath Explor® and SensAbues® (n=40).

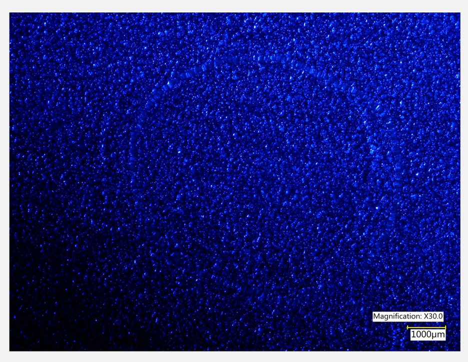

Figure 9: Positive control with Diamond Dye® at 30X to view entire saliva droplet

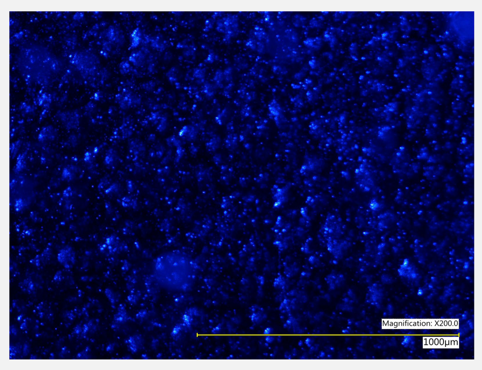

Figure 10: Negative control with Diamond Dye®

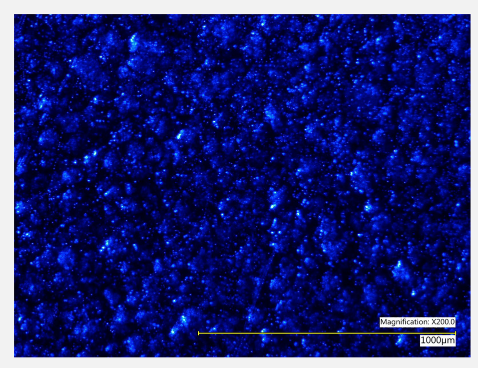

Figure 11: Glass slide blank with Diamond Dye®

- 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. 7).
- The observed percentage of reportable alleles was less than 20% from the filters of both breath devices (Fig 7). No profile was obtained for 76% of filter types, and only 6% of samples yielded a full profile (n=60) (data not shown).
- 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. 8). No statistical difference was observed between the two methods for Breath Explor® (p = 0.2) and SensAbues® (p = 0.68).
- Diamond Dye® was not deemed suitable for the application of Breath Explor®, SensAbue®, and FTA® disks due to lack of contrast between substrate and sample and autofluorescence (Fig. 9-11).

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 identity of the user.
- Further testing of Diamond Dye® to visualize cells on substrates should be investigated on Breath Explor®, SensAbues®, and FTA® disks.

Acknowledgments

The authors would like to acknowledge the Department of Forensic Sciences, the volunteers, and SensAbues® for providing the devices free of charge.

References

1. O.Beck, K. Leine, G. Palmskog, J. Franck, Amphetamines detected in exhaled breath from drug addicts: A new possible method for drugs-of-abuse testing, J. Anal.Tox. 34 (2010) https://doi.org/10.1093/jat/34.5.233.
2. S. kazeminasab, B. Emamalizadeh, V. Jouyban-Gharamaleki, A. Taghizadieh, M. Khoubnasabjafari, A. Jouyban, Tips for improving the quality and quantity of the extracted DNA from exhaled breath condensate samples, Nucleosides, Nucleotides and Nucleic Acids. 39 (2020). http://doi.org/10.1080/15257770.2019.1677910.
3. S. Montpetit, P. O'Donnell. An Optimized Procedure for Obtaining DNA from Fired and Unfired Ammunition. Forensic Science International: Genetics. 17 (2015). https://doi.org/10.1016