The Evaluation of a Mini-Vacuum Device for the Collection for Low-Level Samples

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INTRODUCTION

DNA collection is a critical step in the forensic biology workflow, as downstream success heavily depends on the collection method. Common approaches, including swabbing [1], tape lifting [2], and cutting [3], are widely used but often yield variable results. Additionally, DNA recovery is influenced by multiple factors, such as substrate type, collection and extraction methods, and individual "shedder" status [4-5]. These challenges are amplified when low quantities of DNA are present, emphasizing the need for effective collection strategies.

The Squeegee-Aspirator Large Sampling Area (SALSA) is a novel handheld vacuum-based collection system developed by AI Biosciences, Inc. (AIBI, College Station, TX, USA) (**Fig. 1**). The device consists of a 3D-printed, patent-pending manifold paired with a battery-operated aspirator. A wetted probative surface is sampled using combined squeegee action and vacuum suction, directing the collected liquid into a 2 mL tube compatible with existing laboratory workflows. All collection components are designed for single use to minimize contamination.

Originally developed for low-level microbial sampling in National Aeronautics and Space Administration (NASA) Class 100K cleanrooms [6], the SALSA device's portability and ease of use show utility for forensic DNA collection, particularly for in-field sampling.

This study had two objectives: 1) to establish a SALSA workflow suitable for forensic laboratories by comparing direct PCR and extraction and purification-based approaches using the EZ2 Connect FX platform (QIAGEN, Hilden, Germany), and 2) to evaluate DNA recovery from mock casework samples using the optimal workflows.

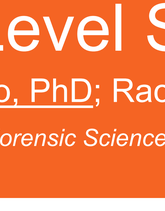

Figure 1: Labelled SALSA device A) Sampling head that functions as a squeegee, B) 3D printed patent-pending manifold, C) Collection tube, and D) Battery-operated aspirator.

MATERIALS & METHODS

Phase 1: A total of 30 glass slides and 30 fabric cuttings were prepared and spiked with known amounts of DNA (10 ng and 1 ng) from buccal cell suspensions collected under IRB approved protocols. The control samples were used to compare two SALSA device processing methods, a SALSA direct PCR workflow and a SALSA EZ2 Large Volume purification workflow (**Fig. 2**), with a traditional cotton swab extraction control using the EZ2 Connect Fx following the Large Volume Protocol as a control.

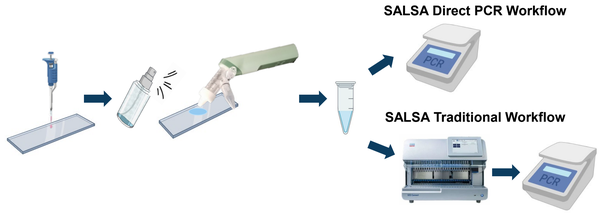

Figure 2: SALSA Direct PCR workflow and SALSA EZ2 workflow were completed with 10 ng and 1 ng samples for both glass and fabric cuttings.

RESULTS & DISCUSSION

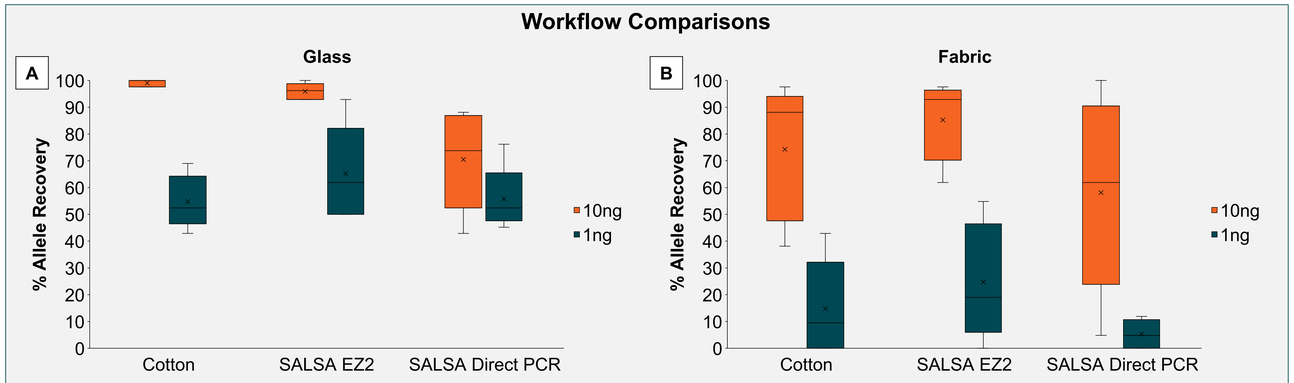

Figure 4: Comparison of percent allele recoveries obtained using cotton swabbing, the SALSA EZ2 method, and the SALSA Direct PCR method for DNA collection from A) glass and B) fabric substrates. Each boxplot summarizes results from five replicate samples per workflow and input amount.

- All methods yielded comparable DNA concentrations (Data not shown).
- Lower allele recovery was shown with the SALSA Direct method for glass and fabric samples compared to the SALSA EZ2 method and traditional cotton swabbing (**Fig. 4**). Therefore, the SALSA EZ2 method will be applied to mock casework samples.

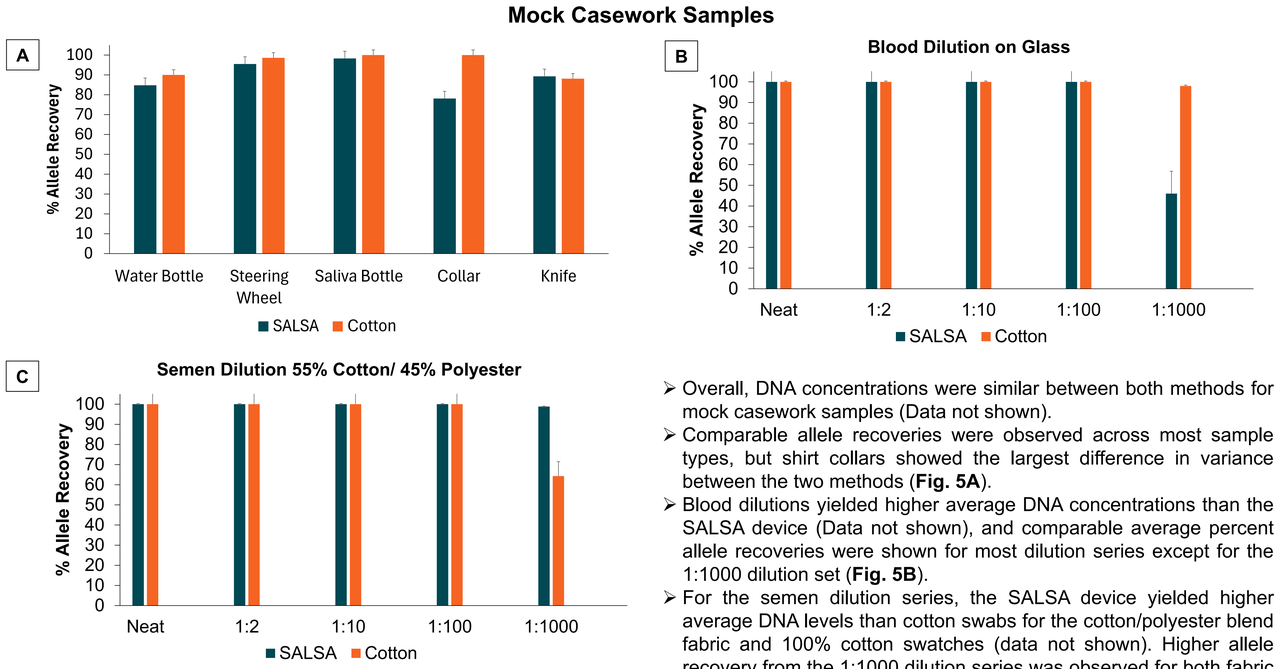

Figure 5: Comparison of percent allele recoveries obtained using cotton swabbing and the SALSA device for A) mock casework samples, B) semen dilutions, and C) blood dilutions. Errors bars indicate standard error.

- Overall, DNA concentrations were similar between both methods for mock casework samples (Data not shown).
- Comparable allele recoveries were observed across most sample types, but shirt collars showed the largest difference in variance between the two methods (**Fig. 5A**).
- Blood dilutions yielded higher average DNA concentrations than the SALSA device (Data not shown), and comparable average percent allele recoveries were shown for most dilution series except for the 1:1000 dilution set (**Fig. 5B**).
- For the semen dilution series, the SALSA device yielded higher average DNA levels than cotton swabs for the cotton/polyester blend fabric and 100% cotton swatches (data not shown). Higher allele recovery from the 1:1000 dilution series was observed for both fabric types (100% cotton not shown). An example is shown in **Fig. 5C**.

MATERIALS & METHODS

Phase 2: After identifying the most suitable SALSA workflow (EZ2 purification), 8 mock forensic sample types collected under IRB approved protocols were tested in 10 replicates (**Fig. 3**). Mock substrates included personal water bottles, steering wheels, knife handles, saliva–spiked plastic water bottles, shirt collars, knife handles and blood and semen dilutions on glass and fabric substrates, respectively. All samples were sprayed with nuclease-free water before sampling with the SALSA device. A total of 160 samples were processed, half tested with the SALSA device (n = 80) and the other half with wetted cotton swabs (n = 80).

Sample Processing: Each personal water bottle was divided along the midline, and each half was sampled using one of the two collection methods. For steering wheels, clothing collars, and knife handles, one half of each surface was sampled with a swab, while the other half was sampled with the SALSA device. For saliva-spiked bottles, 30 µL of neat saliva was applied to the mouthpiece to simulate drinking. Control blood and semen samples (Innovative Research, Inc.) were used to prepare dilutions. Due to the surface area of the steering wheel and clothing, additional sprays were required to adequately cover the entire surface, resulting in approximately 125 – 150 µL of collected liquid.

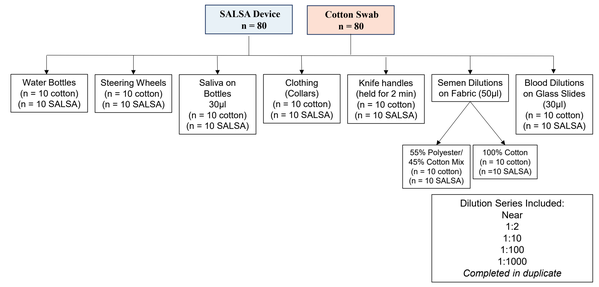

Figure 3: Mock casework samples tested with cotton swabbing and the SALSA device.

DNA quantification and STR amplification: All samples were quantified using the Investigator® Quantiplex Pro Kit (QIAGEN) following the manufacturer's guidelines. Quantification was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems™, Waltham, USA).

STR amplification was performed using the Investigator® 24plex QS Kit (QIAGEN) with 30 cycles on a ProFlex™ 96-well PCR System (Applied Biosystems™). PCR products were separated and detected using the ABI 3500 Genetic Analyzer and analyzed using GeneMapper™ IDX v1.6 software.

CONCLUSIONS

- SALSA workflow amenable to forensic laboratories was developed.
- SALSA device was tested on a variety of substrates.
- Comparable quantification results and STR recovery were observed in most samples, but traditional swabbing remains a reliable method.
- More testing will be needed to determine the optimal amount of wetting agent to be applied to each sample type.
- SALSA device shows potential for sexual assault samples (**Fig. 5C**).

ACKNOWLEDGEMENTS

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REFERENCES

[1] Pang BCM, Cheung BKK (2007) Double swab technique for collecting touched evidence. Leg Med 9:. https://doi.org/10.1016/j.legalmed.2006.12.003
[2] Verdon TJ, Mitchell RJ, Van Oorschot RAH (2014) Evaluation of tapelifting as a collection method for touch DNA. Forensic Sci Int Genet 8:. https://doi.org/10.1016/j.fsigen.2013.09.005
[3] Petricevic SF, Bright JA, Cockerton SL (2006) DNA profiling of trace DNA recovered from bedding. Forensic Sci Int 159:. https://doi.org/10.1016/j.forsciint.2005.06.004
[4] Verdon TJ, Mitchell RJ, van Oorschot RAH (2014) Swabs as DNA collection devices for sampling different biological materials from different substrates. J Forensic Sci 59:. https://doi.org/10.1111/1556-4029.12427
[5] Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW (2014) Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PLoS One 9:. https://doi.org/10.1371/journal.pone.0116351
[6] Simpson AC, Tighe S, Wong S, et al (2023) Analysis of Microbiomes from Ultra-Low Biomass Surfaces Using Novel Surface Sampling and Nanopore Sequencing. Journal of Biomolecular Techniques 34:. https://doi.org/10.7171/3FC1F5FE.BAC4A5B3

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