Evaluation of Methods for the Application of Preservative Solutions to Saliva on Swabs

Abigail S. Bathrick[1], MFS and Jon M. Davoren[1], MSc

[1] Bode Technology. 10430 Furnace Road, Suite 107, Lorton, VA 22079

This project was supported by Award No. 2015-R2-CX-K037, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the authors and do not necessarily reflect those of the Department of Justice.

Introduction

Proper evidence collection and storage techniques can prevent or minimize DNA degradation in biological samples; however, it may not always be possible to achieve ideal storage conditions during long-term storage of evidence, which can occur for months or years before samples are processed. To preserve the integrity of the DNA, biological evidence should be preserved from environmental damage as soon as it is collected. The ability to apply a preservative solution directly to a swab would minimize the risk of DNA degradation and could allow for the generation of higher quality DNA profiles. The goal of this research was to optimize a method for long-term preservation of DNA evidence by applying an EDTA solution directly to saliva collected on cotton swabs. Six application methods were evaluated for their ease of use and efficacy during preservative application.

Materials

Dropper bottle



Metered dose
40 µl

Nasal spray bottle



Metered dose
120 µl

Oral spray bottle



Metered dose
120 µl

Atomizer



Unmetered

Pretreated swab



Pretreated with EDTA and dried

Moistened swab



EDTA as moistening agent for collection

Ease of Use Evaluation Methods

Ten volunteers were provided written instructions and a verbal overview of each application method. Volunteers performed each method on mock sample swabs. They provided user feedback using a standardized questionnaire, employing seven-point Likert scales to rate their agreement with statements from (1) strongly disagree to (7) strongly agree.

Median Likert score values were determined for each method and questionnaire statement. The Mann-Whitney U Test was used to determine if the Likert scores differed significantly between the application methods (U < 23).

Ease of Use Evaluation Results



Efficacy Evaluation Methods

Sample Preparation

- Saliva (35 µl) applied to the tips of sterile cotton swabs
- Dried at room temperature (RT) for one hour
- Each application method used to deposit a 0.2M EDTA solution on the swabs
- Treated swabs dried at RT overnight and stored
- For each application method and time point, untreated and treated samples prepared in triplicate (n = 147)

Sample Storage Conditions		
Temp (˚C)	**Time (Days)**	**AA* Equivalent (Days)**
RT	0	N/A
RT	183	N/A
	365	N/A
37	183	518
	365	1032
50	183	1274
	365	2542

*Accelerated aging (AA) performed at 37˚C and 50˚C

Sample Processing

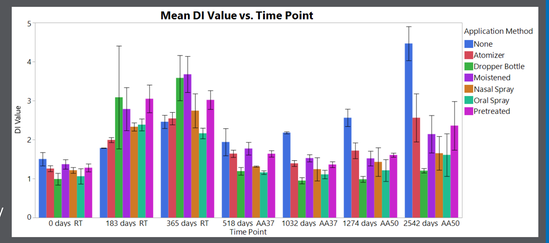
Process	Method
Extraction	QIAsymphony® DNA Investigator®
Quantification	Quantifiler® Trio
Concentration	Microcon® DNA Fast Flow columns
Amplification	GlobalFiler™
CE	3500xL Genetic Analyzer
Data analysis	GeneMapper ID-X® v1.5 Analytical threshold 125 RFU Stochastic threshold 600 RFU
Evaluation metrics	Quantifiler Trio Degradation Index (DI) Forensic DNA profile index (FI)
Statistical analysis	Mean DI ($\overline{DI}$) Mean FI ($\overline{FI}$) Tukey's HSD test - pairwise comparisons (α = 0.01)

DNA Degradation Results

DI < 1
DNA not degraded

1 ≤ DI < 10
DNA slightly to moderately degraded

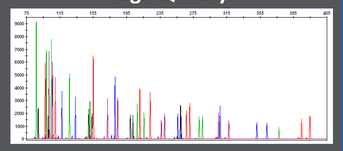
DI ≥ 10
DNA significantly degraded



High Quality

DI 1.31
FI 6.73

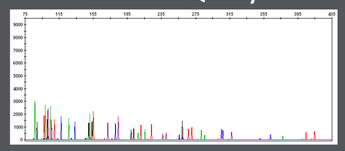
Untreated 0 days RT



Moderate Quality

DI 1.79
FI 4.74

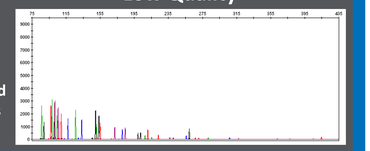
Untreated 183 days RT



Low Quality

DI 4.23
FI 1.34

Untreated 365 days 50˚C

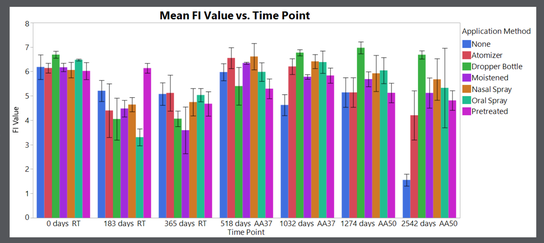


STR Profile Quality Results

FI ≥ 6
High quality profile

4 ≤ FI < 6
Moderate quality profile

FI < 4
Low quality profile



Discussion

Ease of Use

- Pretreated swab method produced the highest median Likert scores
  - Dropper bottle, atomizer, and moistened swab methods each only differed significantly from pretreated swabs at one ease of use statement
- Nasal and oral spray methods produced the lowest median Likert scores
  - Significantly lower than the other application methods for multiple ease of use statements

Researcher observations

- Atomizer method dispersed a substantial amount of EDTA solution into the air and surrounding area

Efficacy

- When compared to the untreated samples, all methods resulted in significantly lower $\overline{DI}$ values and significantly higher $\overline{FI}$ values at the later time points
- Dropper bottles produced $\overline{DI}$ values less than or approximately equal to 1 at every time point except 183- and 365-days RT
  - Increased $\overline{DI}$ values at 183- and 365-days RT may indicate an interaction between the EDTA & storage conditions at RT
  - Profile quality ($\overline{FI}$) maintained between 0-day untreated samples and 2542-day dropper bottle samples

Conclusion

Ease of use results, researcher observations, and efficacy results were used to determine the most effective and practical application methods:

- Dropper bottles
- Pretreated swabs
- Moistened swabs