Biomatrica DNA SampleMatrix® – A New Prospect for Forensic DNA Sample Storage

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Abstract

The purpose of this poster is to address the feasibility of using the Biomatrica DNA SampleMatrix® (SM) as an effective means of stabilizing forensic DNA extracts for long-term, room temperature storage. Many forensic laboratories across the country currently store DNA extracts in -20°C freezers in an attempt to maintain sample integrity. As an alternative method of storage, the Palm Beach County Sheriff's Office began investigating Biomatrica's SM 96-well plates and individual tubes. In this study, the SM 96-well plates were evaluated against current storage methods at six time points ranging from 1 day to 3 months. Matrix samples were assessed for overall sample recovery and quality versus the in-house controls (IHC). Similarly, SM individual tubes were tested at three time points ranging from 2 to 4 weeks and compared to IHC samples. The SM 96-well plate and the SM individual tubes were further evaluated at each time point with respect to two different environmental storage conditions. Samples were stored in identical storage cabinets, one with a humidity controlled environment and the other without humidity control. Sensitivity and mixture series were utilized for the evaluation of the SM. All recovered DNA samples were quantified and compared to determine if DNA stored on the SM was recovered at the same or higher concentrations than those in the IHC condition. The integrity of the samples was evaluated by observing the number of alleles recovered from the samples versus the known number of alleles for each profile.

Figure 1: QIAsafe™ DNA 96-well plate

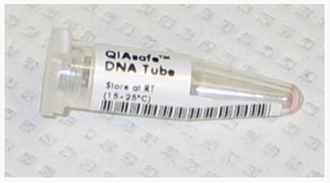


Figure 2: QIAsafe™ DNA Individual Tube

INTRODUCTION

Storing and preserving forensic DNA extracts at -20°C requires cold rooms or freezers and electric generator back-up systems (1). Freezers can consume vast amounts of valuable laboratory space and need to be continuously monitored for temperature fluctuations and potential malfunctions. Freezing samples may sheer and damage DNA, especially with repeated freeze-thaw cycles, in addition to sample evaporation.

Biomatrica, Inc. has developed a pioneering technology for stabilizing DNA at ambient temperatures under anhydrous conditions while still preserving sample integrity (2,3) thereby eliminating the use of freezers. Biomatrica's DNA SampleMatrix® (SM) is a combination of anhydrobiosis and synthetic chemistry to create a proprietary dissolvable matrix. The matrix is formulated to form a thermo-stable protective seal around the DNA as it dries to provide protection against degradation, thus "shrink-wrapping" the sample in a protective coating until ready to use for analysis. Through simple rehydration of the samples, DNA can be recovered without the need for further purification and is ready for immediate use (2).

The Palm Beach County Sheriff's evaluated the recovery and reliability of long-term DNA extract storage of two SM storage formats, the QIAsafe™ DNA 96-well plates and individual tubes, for forensic DNA samples.

Material and Methods

All DNA from the male and female donors was extracted using Promega's DNA IQ™ System on the Maxwell® 16 automation platform and quantified using Applied Biosystems Quantifiler™ Human DNA Quantification kit on the ABI Prism® 7000 under manufacturer's recommendations. All samples were created and studied in duplicate.

The DNA SampleMatrix® (SM) 96-well plates were evaluated at six time points ranging from 1 day to 3 months, while the SM individual tubes were tested at three time points ranging from 2 to 4 weeks and compared against current storage methods. The SM 96-well plate and the SM individual tubes were further evaluated at each time point with respect to two different environmental storage conditions. Samples were stored in identical storage cabinets, one with a humidity controlled (HC) environment and the other without humidity control (HNC). A sensitivity study and a mixture study were evaluated simultaneously. The sensitivity study consisted of DNA concentrations from 4ng to 0.0625ng in a total of 20µL, with a serial dilution factor of 2.0 for both a male and female donor. The mixture study consisted of 1ng total in 20µL of male:female ratios as follows: 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10.

The Beckman Coulter Biomek® NX^P was used to aliquot 20µL of each sample from a stock tube into SM 96-well plates and SM individual tubes and thoroughly mixed according to manufacturer's specification for each time point and condition. The NX^P simultaneously created in-house control (IHC) samples by placing 20µL aliquots of each sample from the same stock into Costar brand dolphin tubes. IHC samples were stored in a -20°C freezer, while the SM samples were dried overnight in a laminar flow hood and then stored in their respective conditions.

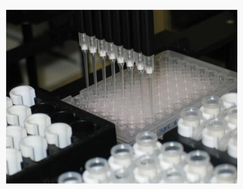


Figure 3: Addition of 20µL of sample to the SM via NX^P, thereby minimizing pipetting errors and saving time

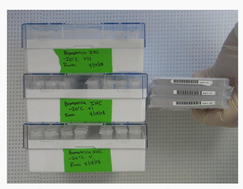


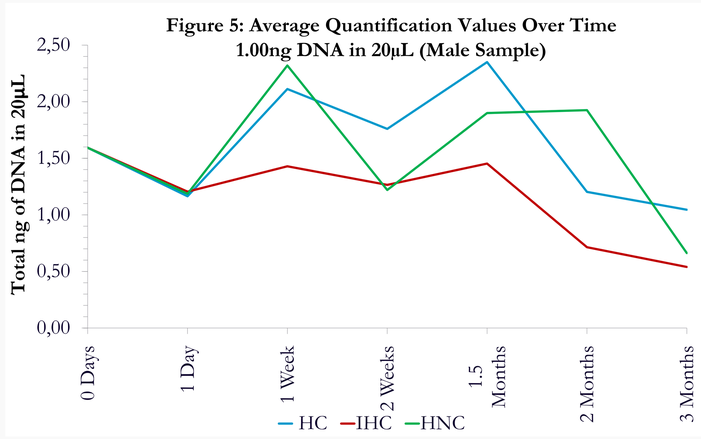
Figure 4: Same number of samples stored in cryoboxes vs. 96 well Qiagen plates

Material and Methods Continued

SM samples were recovered by rehydration with 20µL of autoclaved water. All recovered DNA samples were quantified and compared to determine if DNA stored on the SM was recovered at the same or higher concentrations than those in the IHC condition. All samples were also compared to initial quantification data created from the original DNA stock tube at the time samples were plated (0 Days).

The samples were then amplified using Promega's multiplex STR PowerPlex® 16 system and subsequently run on Applied Biosystems 3130xl Genetic Analyzer to assess the integrity of the DNA after storage on the SM. The overall integrity of the samples was determined by observing complete allele calls at all loci and comparing the average relative florescence unit (RFU) values of each allele at each locus to IHC samples.

Results



Based on quantification values (Figure 5), an overall increase in recovery of DNA from samples on the SM was observed under humidity controlled conditions (HC) when compared to in-house control (IHC) samples stored in dolphin tip tubes.

Table 1: Percentage of Allele Calls (Male Sample)

Time Point/Sample (ng)	0.0625	0.125	0.25	0.5	1.0	2.0	4.0
HC_1 day	72.4	96.6	100	100	100	100	100
IHC_1 day	0.0	62.1	58.6	62.1	100	100	100
HNC_1 day	3.4	69.0	79.3	69.0	100	100	100
HC_1 week	58.6	75.9	100	100	100	100	100
IHC_1 week	72.4	100	100	100	100	100	100
HNC_1 week	41.4	62.1	96.6	100	100	100	100
HC_2 weeks	37.9	100	100	100	100	100	100
IHC_2 weeks	72.4	100	100	100	100	100	100
HNC_2 weeks	48.3	93.1	100	100	100	100	100
HC_1.5 months	44.8	89.7	100	100	100	100	100
IHC_1.5 months	51.7	96.6	100	100	100	100	100
HNC_1.5 months	41.4	86.2	100	100	100	100	100
HC_2 months	31.0	96.6	100	100	100	100	100
IHC_2 months	10.3	96.6	100	100	100	100	100
HNC_2 months	31.0	96.6	100	100	100	100	100
HC_3 months	55.2	86.2	100	100	100	100	100
IHC_3 months	41.4	100	100	100	100	100	100
HNC_3 months	31.0	55.2	96.6	100	100	100	100

Based on percentage of allele calls (Table 1), integrity of the DNA samples on the SM were not compromised compared to the IHC samples. HNC samples did not perform as well as HC samples.

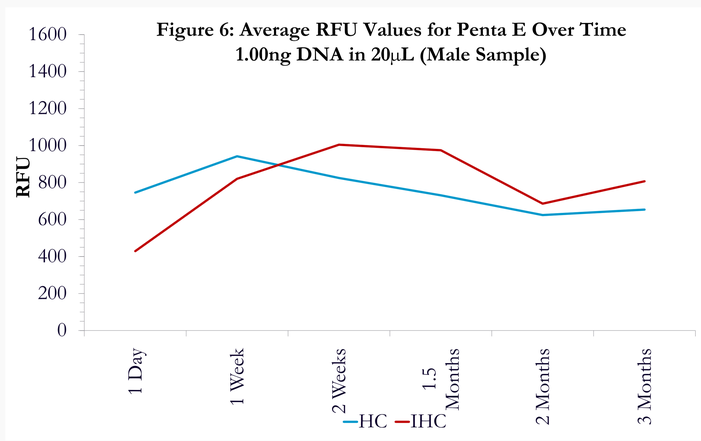


Figure 6 illustrates that the average RFU values for the Penta E locus of HC on the SM 96-well plate samples are relatively the same as the IHC samples. Fewer RFU fluctuations are observed with the HC samples when compared to IHC samples throughout the time points.

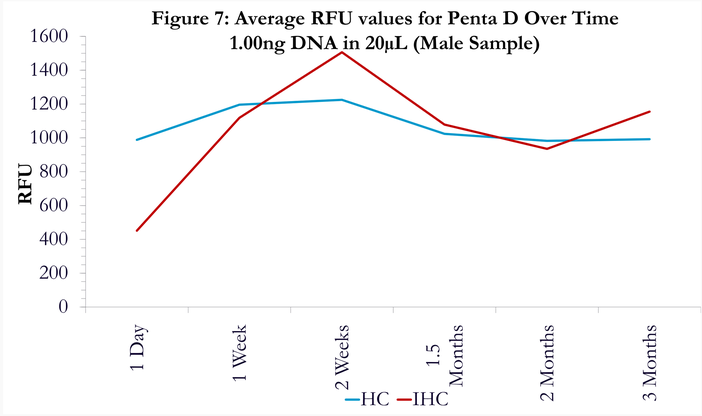


Figure 7 illustrates that the average RFU values for the Penta D locus of HC samples on the SM 96-well plate are relatively the same as the IHC samples. RFU fluctuations are less extreme with HC samples when compared to IHC samples throughout the time points.

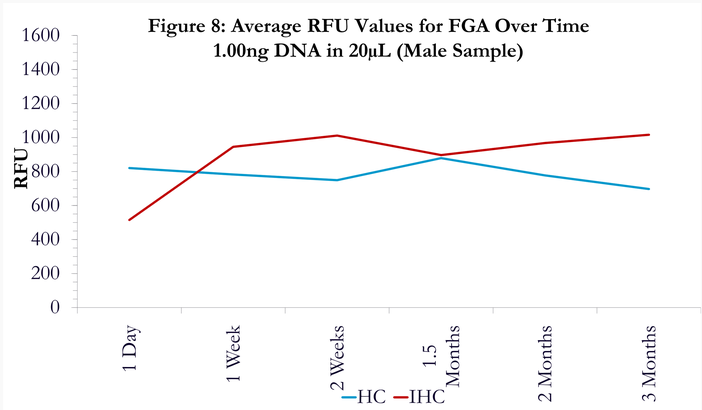


Figure 8 illustrates that the average RFU values for the FGA locus of HC samples on the SM 96-well plate are relatively the same as the IHC samples. Overall RFU values remained more stable throughout the time points for both the HC and IHC samples for this locus.

Discussion and Conclusion

Based on quantification values, an increase in recovery of DNA from all samples on the SampleMatrix® (SM) 96-well plate under humidity control (HC) conditions was observed when compared to in-house control (IHC) samples stored in dolphin tubes. Full profiles were observed for both the SM and IHC samples up to three months for DNA samples as low as 0.25ng in 20µL. The lower end of the sample concentrations (0.0625ng) exhibited allele drop out in both SM and IHC samples throughout the time points. All RFU values of HC samples were relatively the same when compared to IHC samples. As expected, the samples without humidity control (HNC) did not perform as well as the HC samples.

Though the data is not shown, the DNA samples in SM individual tubes were evaluated. However, the SM individual tubes did not readily dry in the laminar flow hood as per protocol, compromising the samples and adversely affecting the data collected.

Overall, the data presented show that the integrity of the single source samples was not compromised when added to the SM 96-well plate over a 3 month time period. Mixture samples on the 96-well plate and the individual tubes are still being evaluated. Thus far, the Biomatrica DNA SampleMatrix® 96-well plate is a prospective storage option for forensic DNA samples.

Future Studies

Further studies need to be conducted to evaluate if DNA can be stabilized at periods longer than 3 months, on samples volumes less than 20µL, in SM individual tubes, and on the effects of repeated cycles of rehydration and drying of the SM (similar to freeze-thawing of sample extracts).

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Acknowledgements

The author would like to acknowledge the Palm Beach County Sheriff's Office for supplying all the materials, equipment, and instruments required to complete this study as well as their endless assistance throughout the project. The author would also like to thank the Marshall University Forensic Science Program for the training, support, and encouragement prior to the internship and to the National Institute of Justice for supplying the funds for the project.

This project was supported by Cooperative Agreement Numbers 2005-MU-BX-K020 or 2001-RC-CX-K002 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings and conclusions or recommendations expressed in this publication/exhibition are those of the author(s) and do not necessarily reflect the views of the US Department of Justice.