Biomatrica DNAstable® Supplemental Validation and Retro-Analysis of Long Term Storage Effects

Amber Hiranaka, B.S.[1]; Julie Conover Sikorsky, M.S.[2]; Catherine Cothran, M.S. [2]; Cecelia Crouse, Ph.D. [2]; Pamela Staton, Ph.D.[1]

[1]Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701
[2]Palm Beach County Sheriff's Office, 3228 Gun Club Road, West Palm Beach, FL 33406

Abstract

The Palm Beach County Sheriff's Office completed an initial validation of DNAstable® in 2011 and concluded that DNA extracts could be successfully stored at room-temperature on Biomatrica® plates in a humidity controlled environment. This initial validation did not examine DNA profile allelic peak height quality.

Therefore, the original validation data was reexamined to assess profile signal intensities as representative of profile quality. During this secondary re-examination, allele peak height signal intensity change over a year was calculated.

In order to further investigate these observations, additional testing was performed on case-like samples stored for approximately two years. The profile quality produced upon reanalysis was compared to the original profile and the percent signal intensity change was calculated.

A comparison of Cq values was also performed during the reevaluation and reanalysis to examine the effects of storage on DNA quantity.

Introduction

As a result of the rising demands for DNA analysis, the field of forensic science is facing challenges in the ability to store purified DNA extracts for future retesting. Currently, the generally accepted method of storage is as liquid extracts retained in a -20˚C to -80˚C freezer. However, space is limited when an increasing number of samples require storage and the energy cost of freezers is problematic. Therefore, a more efficient DNA storage method is desired so extracts can be preserved at room-temperature. The molecular biology company Biomatrica Incorporated has developed a synthetic polymer to aid in the cost-effective, room-temperature preservation of DNA. The polymer, DNAstable® (Biomatrica, Inc., San Diego, CA), imitates trehalose, a naturally produced sugar involved in the process of anhydrobiosis.

The purpose of this study was to evaluate DNAstable® as a room-temperature, DNA storage option to ensure the quality of DNA is not compromised after long periods of storage (up to two years).

Methods and Materials

Retro-analysis
- *Quantitation*: Applied Biosystems® Quantifiler®
- *Amplification*: Promega® PowerPlex® 16 System
- *Capillary electrophoresis:* Applied Biosystems® 3130xl Genetic Analyzer

Supplemental Validation: reanalysis of case-like samples
- Biomatrica® plates containing DNA analyst proficiency samples stored for approximately two years
  - Rehydrated with sterile water; the volume of which was equal to the extract volume originally plated
- *Quantitation*: Promega® Plexor® HY System
- *Amplification*: Promega® PowerPlex® 16 System
  - Amplification was performed using the same volume of sample as had been amplified during the initial analysis.
- *Capillary electrophoresis:* Applied Biosystems® 3130xl Genetic Analyzer

Long-term storage study preparation
- *Extraction*: QIAGEN® DNA Investigator® Kit on the QIAGEN® EZ1® Advanced XL
- Biomatrica® plates and foil seals
- *Quantitation*: Promega® PowerQuant™
- *Amplification*: Promega® Fusion System
- *Capillary electrophoresis:* Applied Biosystems® 3500xl Genetic Analyzer

Results and Discussion

During the retro-analysis of the original validation, the DNA profile quality produced by samples stored in a -20˚C freezer (in-house control, IHC) was compared to the DNA profile quality produced by corresponding samples stored in a room-temperature, humidity controlled environment (HC) on Biomatrica® plates which utilize the DNAstable® technology.

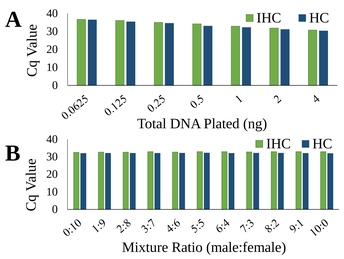

Figure 1: Cq value comparison averaged for all quantitations performed over a period of one year for the IHC and HC samples in the sensitivity (A) and mixture (B) study

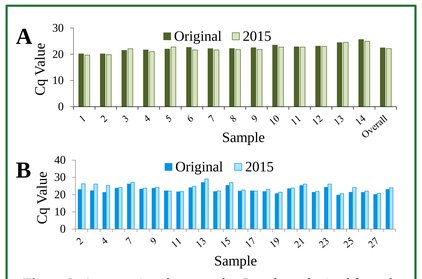

Figure 2: A comparison between the Cq values obtained from the original quantitation and the Cq values obtained from the 2015 reanalysis of the frozen proficiency samples (**A**) and Biomatrica® stored proficiency samples (**B**)

DNA Quantity

Retro-analysis (Figure 1)

On average, there was less than a 5% difference in Cq value between the IHC and corresponding HC samples for both the sensitivity and mixture studies.

Supplemental Validation (Figure 2)

Samples after 2 years of storage on Biomatrica® plates or in a -20˚C freezer showed on average, less than a 10% difference in Cq value.

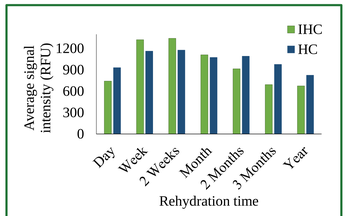

Figure 3: Representative results of the signal intensity comparison between IHC and HC samples for the sensitivity study. Results portrayed are specifically from the female sensitivity sample plated with a total DNA concentration of 0.5 ng.

No distinct trend in signal intensity corresponding to time was observed from the sensitivity study results (**Figure 3**) and similar signal intensities were observed for the day and year mixture study samples (data not shown).

The signal intensities for the IHC and HC sensitivity samples also remained comparable (**Figure 3**) and consistent results were obtained from the mixture samples also.

The loss of HC profile signal intensity was below 20% for both the sensitivity and mixture studies and did not change the interpretation of the DNA profile produced (**Figure 4**). Sometimes sister alleles can demonstrate an imbalance as low as 50%.

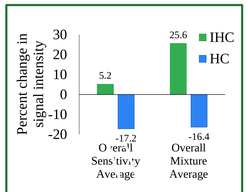

Figure 4: Percent signal intensity change after a year-long storage period averaged over all DNA concentrations of the male and female sensitivity study and over all mixture ratios of the mixture study

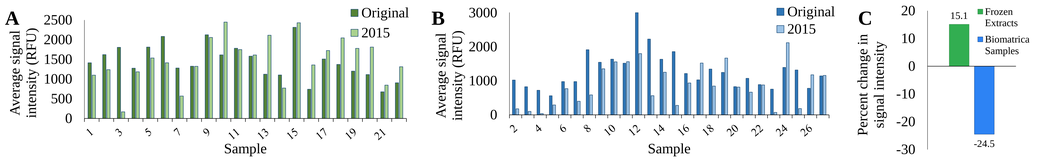

Figure 5: A comparison between the original signal intensities and the signal intensities obtained from the 2015 reanalysis of the frozen proficiency samples (**A**) and Biomatrica® plated proficiency samples (**B**); Sample 23 showed signs of degradation. Sample 25 showed signs of inhibition). A comparison of the average percent signal intensity change after approximately two years of storage (**C**) within a -20˚C freezer or at room-temperature on Biomatrica® plates.

Similar results were obtained from the reanalysis of case-like (**Figure 5C**). Considering peak height thresholds for a DNA profile, interpretation of the produced profile was unaffected.

It is important to note that for the IHC samples in the retro-analysis, some samples did lose signal intensity while some HC samples gained signal intensity (data not shown). For the reanalysis of case-like samples, some of the frozen extracts also lost signal intensity (**Figure 5A**) while some Biomatrica® stored samples gained signal intensity **(Figure 5B).**

For the mixture study, the change in signal intensity was equivalent for the entire profile, not dependent on whether the DNA originated from the minor or major mixture component (**Figure 6**). This gives evidence that mixture components are not affected differently with storage on Biomatrica® plates.

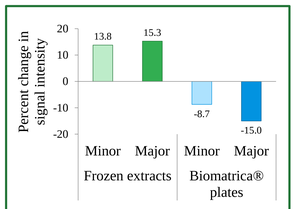

Figure 6: Percent signal intensity change after a year-long storage period for the loci of the major and minor contributors of the mixture study samples.

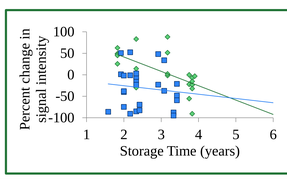

Figure 7: The correlation of signal intensity change for frozen extracts and Biomatrica stored samples with storage time (green y = -33.4x + 108.15; blue y = -9.9x – 5.6)

The change in signal intensity correlates strongly with storage time in a -20˚C freezer and to a lesser degree with storage time on Biomatrica® plates (**Figure 7**). Because the signal intensity change for Biomatrica® stored samples in relation to storage time is more constant than that for the frozen extracts, if the trend continues, after five years of storage samples on Biomatrica® plates are expected to portray a less negative change in signal intensity than samples stored in the freezer.

Conclusion

DNA storage on Biomatrica® plates can be considered as a possible alternative to extract storage in a -20˚C freezer. The Cq value comparison indicates DNA quantity remains unaffected after storage on Biomatrica® plates.

As with frozen samples, loss of DNA profile quality is a possibility. However, the loss in signal intensity for samples stored on Biomatrica® plates did not change the interpretation of the profile produced. During the reexamination of the prior validation, drop-out for both IHC and HC samples only occurred when the initial profile was below the stochastic threshold. During the reanalysis of case-like samples, only three out of the twenty-six profiles obtained from the case-like samples stored on Biomatrica® plates showed a significant loss in signal intensity, where the entire initial profile was above the stochastic threshold and then drop-out occurred in the reanalysis. One out of the twenty-two profiles obtained from frozen extracts also showed a significant loss in signal intensity.

Results indicate that Biomatrica® plate storage is comparable to storing extracts in a -20˚C freezer and the signal intensity.

To further research the effects of DNA storage on Biomatrica® plates, additional sensitivity and mixture study samples were plated in replicate (**Figure 8**) to be analyzed over three years.

More information on how the DNA profile quality may be affected by longer storage times on DNAstable® is hoped to be obtained.

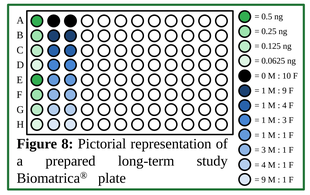

Figure 8: Pictorial representation of a prepared long-term study Biomatrica® plate

References

Applied Biosystems®. Applied Biosystems® 3500/3500xL Genetic Analyzers User Guide. June 2010.

B. Budowle, A. van Daal, Extracting evidence from forensic DNA analyses: future molecular biology directions, BioTechniques 46 (2009) 339-350.

C. Frippiat, F. Noel, Efficiency of a novel forensic room-temperature DNA storage medium, Forensic Sci. Int. Genet. 9 (2014) 81-84.

C. Frippiat, S. Zorbo, D. Leonard, A. Marcotte, M. Chaput, C. Aelbrecht, F. Noel, Evaluation of novel forensic DNA storage methodologies, Forensic Sci. Int. Genet. 5 (2011) 386-392.

E. Wan, M. Akana, J. Pons, J. Chen, S. Musone, P. Kwok, W. Liao, Green Technologies for Room-temperature Nucleic Acid Storage, Curr. Issues Mol. Biol. 12 (2010) 135-142.

J. Bonnet, M. Colotte, D. Coudy, V. Couallier, J. Portier, B. Morin, S. Tuffet, Chain and conformation stability of solid-state DNA: implications for room-temperature storage, Nucleic Acids Res. 38 (2010) 1531-1546.

Promega®. Plexor® HY System for the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems Technical Manual. May 2013.

Promega®. PowerPlex® 16 System Technical Manual. June 2013.

Promega®. PowerPlex® Fusion System Technical Manual. March 2015.

Promega®. PowerQuantTM System Technical Manual. March 2015.

QIAGEN®. QIAsafe™ DNA Tube and 96-Well Plate Handbook. October 2010.

S.B. Lee, C.A. Crouse, M.C. Kline, Optimizing storage and handling of DNA extracts, Forensic Science Review 22 (2010) 131-144.

S.B. Lee, K.C. Clabaugh, B. Silva, K.O. Odigie, M.D. Coble, O. Loreille, M. Scheible, R.M. Fourney, J. Stevens, G.R. Carmody, T.J. Parsons, A. Pozder, A.J. Eisenberg, B. Budowle, T. Ahmad, R.W. Miller, C.A. Crouse, Assessing a novel room-temperature DNA storage medium for forensic biological samples, Forensic Sci. Int. Genet. 6 (2012) 31-40.

S.E. Howlett, H.S. Castillo, L.J. Gioeni, J.M. Robertson, J. Donfack, Evaluation of DNAstableTM for DNA storage at ambient temperature, Forensic Sci. Int. Genet. 8 (2014) 170-178.

Acknowledgements

This project could not have been possible without the collaboration between the Palm Beach County Sheriff's Office Forensic Biology Unit (PBSO FBU) and Steve Lee. I thank Catherine Cothran for her encouraging supervision and guidance as well as Julie Conover Sikorsky, Dr. Cecelia Crouse and Dr. Pamela Staton who all provided feedback and assistance with the presentation of the results. I also thank Amy McGuckian and the training DNA analysts of the PBSO FBU, Stacie Brenner, Brandy Cook, Todd Cohen and Alyse Yacovone-Margetts, for their involvement in the preparation of the future studies. I thank the rest of the PBSO FBU analysts for their continual encouragement and support as this project took place within their laboratory.