DNAstable® LD VALIDATION PROJECT

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ABSTRACT

DNAstable ® LD (liquid-to-dry) from Biomatrica, Inc. was validated for the long-term storage of DNA extracts from criminal cases in Alaska. Historically, DNA extracts at the Alaska Scientific Crime Detection Laboratory were stored at -80°C and currently at -20°C. DNAstable ® LD was investigated as an alternative to freezer storage of DNA extracts to save costs on purchasing, running and maintaining additional freezers. DNAstable ® LD is a proprietary liquid that is added to DNA extracts, which are then dried and stored at room temperature. DNAstable® LD protects the DNA by forming a protective seal around the DNA during the drying process (Biomatrica, 2012). Results from this validation study support the claim that DNAstable ® LD acts to protect DNA from degradation when exposed to simulated harsh environments (54°C), and proved to be beneficial for maintaining the quality and quantity of low-level DNA samples (< 0.05 ng/µl DNA). Samples with sufficient quantities of DNA commonly encountered in forensics (≥ 0.20 ng/µl) performed similarly in DNA recovery and amplification success using Promega's PowerPlex ®16 with or without DNAstable ® LD stored dry at room temperature or stored as a liquid frozen at -20°C.

INTRODUCTION

In casework at the Alaska Scientific Crime Detection Laboratory (ASCDL), an item of evidence may be consumed during the extraction process, and a minimum of half the extract shall be retained as evidence for possible future testing. Historically at the ASCDL, retained DNA extracts were stored at -80°C, and are currently stored at -20°C. Over time, a substantial number of extracts (>13,000) have accumulated and continue to accumulate. Frozen storage of DNA extracts is expensive, and frozen liquids (DNA extracts) require special attention and increased shipping costs if transported. Additionally, literature and practical experience indicate that long-term storage of frozen extracts leads to a gradual decrease in the amount and quality of recoverable DNA (Frippiat et al, 2010; Lee et al, 2011).

In this study, we compared the quantity and quality of DNA stored with DNAstable ® LD at room temperature, without DNAstable ® LD at room temperature and stored as a liquid frozen at -20°C. This experiment was repeated by re-dehydrating samples with and without DNAstable ® LD and re-freezing samples at -20°C. We also assessed DNA quality and quantity of extracts stored with DNAstable ® LD and without DNAstable ® LD in a simulated stress environment by exposing dried extracts to 54°C. Previously frozen extracts, created mixtures and low concentration DNA samples were also assessed.

MATERIALS and METHODS

Extraction, Quantification, Amplification and Electrophoresis

Known buccal samples and reagent blanks were extracted on the Qiagen BioRobot EZ-1 Advanced XL using the EZ-1 Investigator Kit. Previously frozen extracts and reagent blanks were extracted approximately 10 years ago using Chelex. Quantification was done using Quantifiler (Applied Biosystems) on the MX3000P instrument (Stratagene). Amplification was performed on the Applied Biosystems 9700 thermal cycler. PowerPlex® 16 (Promega) was used to amplify samples per manufacturer's instructions using 32 cycles. Samples were analyzed on a 3130XL genetic analyzer (Applied Biosystems) and electrophoresed at 3kV for 3 or 10 seconds. All extracts protected with DNAstable ® LD in this study were stored at room temperature in a foil-lined moisture barrier bag with desiccant added. The duration of each experiment was approximately one month.

MATERIALS and METHODS (cont.)

Storage Study

A batch DNA extract (buccal) quantified at 0.26 ng/µl was aliquoted (50 µl; 13ng total DNA) in triplicate for: (1) -20°C storage; (2) dry storage protected with DNAstable ® LD (+DS-LD) at room temperature (RT); and (3) dry storage unprotected at RT. This experiment was repeated for an additional cycle of re-dehydration/ hydration and re-freezing at -20°C, and average peak heights assessed.

Simulated Stress Study

The same batch DNA extract quantified at 0.26 ng/µl was also aliquoted (50 µl) in triplicate for: (1) dry storage + DS-LD at 54°C; and (3) dry storage unprotected at 54°C.

Previously Frozen Extracts Study

Ten previously extracted DNA samples (2 hair, 2 blood, 2 buccal, 2 epithelial fractions and 2 sperm fractions) and two reagent blanks were brought to 100µl using sterile water and quantified. Each 100µl extract was split three ways for: (1) initial amplification; (2) dry storage +DS-LD at room temperature (RT); and (3) -20°C storage.

Mixture Study

Male:female mixtures were created from known buccal DNA extracts (1:1, 1:2, 1:4, 1:10, 10:1, 4:1 and 2:1), and total number of dropout alleles or alleles below the reporting threshold of 100RFU (DO) were assessed in samples with and without DS-LD.

Low Concentration DNA Study

One replicate from each test condition from the *Storage Study* was serially diluted (10^{-1} and 10^{-2}) to create two sets of low level DNA samples that quantified at ~0.02 ng/µl and ~0.002 ng/µl, respectively. Five replicates of each set of diluted DNA's were stored in the original test condition and assessed.

RESULTS

Storage Study

No significant differences in quantification (QF) and amplification results were observed for samples stored with or without DS-LD or @ -20°C. Results from the second cycle of re-dehydration/hydration showed a significant reduction in average peak heights for samples stored without DS-LD versus those stored with DS-LD or stored @ -20°C. A slight reduction in quantification results for DNA recovered was also noted for samples stored without DS-LD (Table 1).

Simulated Stress Study

DNA recovered from samples stored without DS-LD and exposed to 54°C was approximately half that (~0.05 ng/µl) of samples stored with DS-LD (~0.10 ng/µl) compared to initial DNA concentration (0.26 ng/µl). Samples stored without DS-LD were highly degraded compared to those stored with DS-LD after amplification (Figure 1).

Previously Frozen Extracts Study

No significant differences were noted for quantification and amplification results between samples stored with DS-LD or @ -20°C compared to initial results, with the exception of one hair sample. All samples quantified at > 0.20 ng/µl, except the one hair sample. A full profile was amplified from this hair extract stored with DS-LD; degraded amplified product resulted after @ -20°C storage (see QF and amplification results in Figure 2 for the notable hair sample).

Mixture Study

Fewer DO alleles were observed for mixture samples stored with DS-LD than were noted initially or for samples stored without DS-LD. The majority of DO alleles occurred in the 1:10 and 10:1 mixture proportions, but DO alleles were observed in other mixture proportions as well. DO alleles were assessed for 3sec and 10sec injection times (Table 2A and 2B, respectively).

RESULTS (cont.)

Low Concentration DNA Study

All replicates with DS-LD produced complete profiles for the 10^{-1} dilution series (~0.02 ng/µl). Many DO alleles were noted in samples stored without DS-LD and at -20°C for the 10^{-1} dilution series (48 and 9 DO alleles, respectively). DO alleles were noted for all test conditions for the 10^{-2} dilution series (~0.002 ng/µl), however samples with DS-LD produced more alleles than other test conditions. No alleles were observed in any of the five replicates for samples stored without DS-LD for the 10^{-2} dilution series; few alleles were observed after storage at -20°C (one replicate is shown for each test condition and dilution series in Figure 3).

CONCLUSIONS

DNAstable ® LD was beneficial for the recovery of DNA quantity and quality for all types of samples with variable initial DNA quantities. Most notable was the protective effect of DNAstable ® LD on samples exposed to high temperatures and for low template DNA samples (< 0.05 ng/µl). Based on the results from this validation, the ASCDL intends to use DNAstable ® LD to store retained DNA extracts at room temperature.

REFERENCES

Biomatrica, 2012. DNAstable ®/DNAstable ® LD Handbook: Protocols for Sample Protection, Sample Recovery and Downstream Applications.

Frippiat et al., 2010. Evaluation of novel forensic DNA storage methodologies. Forensic Science International - Genetics. November 5(5):386-92.

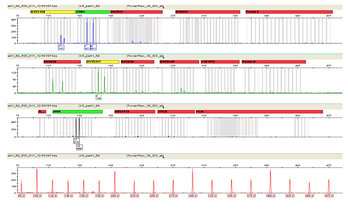
Lee et al., 2011. Assessing a novel room temperature DNA storage medium for forensic biological samples. Forensic Science International - Genetics. January 6(1):31-40.

Table 1. Average quantification (QF) values and average peak heights (PH) after a second cycle of hydration/drying or re-freezing for the -20°C test condition.

	Test Condition		
	-20°C	+DS-LD	dry @ RT
AVE QF value (ng/µl)	0.229	0.231	0.184
AVE PH (RFU)	892	886	417

FIGURE 1. Simulated stress conditions @ 54°C.

Unprotected, dried and stored @ RT; QF = 0.05 ng/µl



+ DS-LD, dried and stored @ RT; QF = 0.10 ng/µl

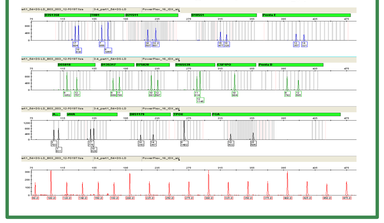


Table 2. Number of drop-out alleles or alleles below the 100RFU analytical threshold ("DO") for created male: female mixtures injected for 3 seconds (A) and 10 seconds (B).

(A)

Mixture Proportion	DO alleles		
	Initial	+DS-LD	dry @ RT
1:1	0	0	0
1:2	0	0	3
1:4	5	8	5
1:10	18	14	17
10:1	8	4	18
4:1	0	0	0
2:1	0	0	0
TOTAL	31	26	43

(B)

Mixture Proportion	DO alleles		
	Initial	+DS-LD	dry @ RT
1:1	0	0	0
1:2	0	0	1
1:4	0	0	1
1:10	4	3	4
10:1	2	1	3
4:1	0	0	0
2:1	0	0	0
TOTAL	6	4	9

FIGURE 2. Previously frozen DNA extract from a hair sample.

Initial amp QF = 0.016 ng/µl | re-frozen @ -20°C QF = 0.006 ng/µl | +DS-LD QF = 0.011 ng/µl

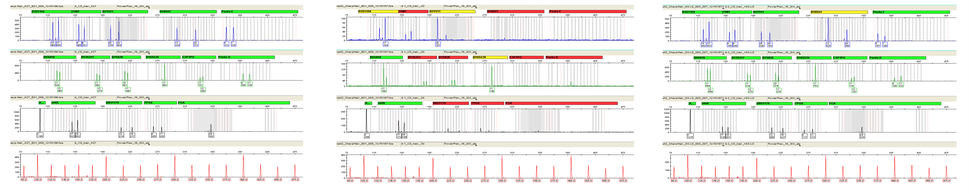
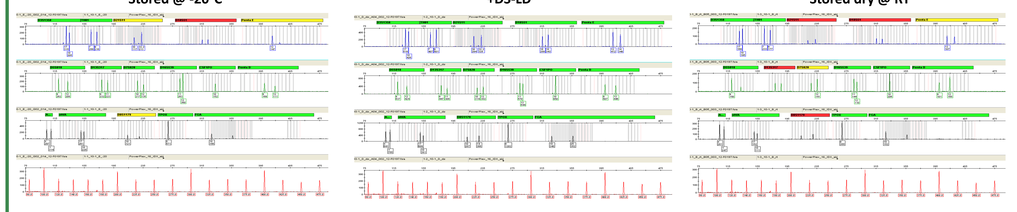


FIGURE 3. Low concentration DNA samples.

10^{-1} dilutions QF ≈ 0.02 ng/µl (3sec)

Stored @ -20°C | +DS-LD | Stored dry @ RT



10^{-2} dilutions QF ≈ 0 - 0.002 ng/µl (10 sec)

Stored @ -20°C | +DS-LD | Stored dry @ RT