Evaluation of the Biomatrica™ SampleMatrix® Room-Temperature DNA Storage System

12 Month and 45 Month Data

Los Angeles County Sheriff's Department Scientific Services Bureau

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Introduction

Traditionally, the long–term storage of purified DNA involves freezing samples, typically at –20°C or less until needed for future testing. However, numerous, inherent problems are encountered when employing this methodology for long-term storage including equipment failure and evaporative loss. For forensic samples, preservation is of paramount importance as retesting and future technologies are always considerations. Additionally, tremendous increases in sample throughput and a growing demand for ample storage capacity have served as the impetus for evaluating novel methods for the storage of purified DNA extracts.

The BioMatrica™ SampleMatrix® technology, inspired by the natural phenomenon of anhydrobiosis, is a technology that employs a synthetic polymer to protect DNA. In the same manner as anhydrobiosis protects living organisms, it also allows for the long-term, stable storage of DNA. Use of the SampleGard/SampleMatrix® system involves the application of liquid samples to storage plates that contain the SampleMatrix® polymer. Samples are subsequently air-dried and become encapsulated, protecting them from potential environmental sources of degradation and other sources of product loss. This innovative approach to sample storage essentially eliminates the need for freezer storage.

Anhydrobiosis: *A form of cryptobiosis.* The desiccated, ametabolic state that some microscopic organisms can enter in response to adverse environmental conditions. This state allows organisms to survive for long periods of time until conditions become favorable enough to support life.

Purpose

This evaluation serves to determine the efficacy of the Biomatrica SampleGard™ and SampleMatrix® system versus conventional, –20°C storage of purified nucleic acids. Previous work captured data from approximately one year of room temperature and frozen storage of typical forensic samples. Data presented here summarizes the findings of approximately 45 months of storage under the same conditions.

Materials & Methods – Sample Preparation

DNA was extracted using the Qiagen EZ1 DNA Tissue Kit and DNA Forensic Card with the BioRobot® EZ1 from dried blood stains, semen and oral swabs received from multiple, known donors from the Los Angeles County Sheriff's Department Scientific Services Bureau.

Extracts were quantified using Real-Time PCR technology with the Applied Biosystems (ABI) Quantifiler™ Human DNA Quantification Kit and the ABI Prism® 7500 Real-Time PCR System.

Equal amounts of DNA from each body fluid source were placed on the SampleGard/SampleMatrix® system and stored at room temperature and also placed in 600 µL microcentrifuge tubes and stored at –20°C.

Materials & Methods – Stability at Room Temperature and –20°C

Samples were loaded onto the SampleGard/SampleMatrix® system in triplicate for room temperature storage. Quantification was performed at 12 month and 45 month end-point times. Identical samples were stored by conventional means at -20 °C for the same time periods.

At the 45-month end-point time, samples were amplified using the Applied ABI Polymerase Chain Reaction Short Tandem Repeat (STR) AmpFℓSTR® Identifiler Kit and were loaded onto a ABI Prism 310 Genetic Analyzer. Generated data was analyzed using Genemapper ID-X software (ABI).

Results — Time Interval Quantification

Samples stored on the SampleGard/SampleMatrix® system were rehydrated using sterile molecular biology grade water at a volume equivalent to the amount of sample originally placed onto the system (~15 µL). Once completely hydrated, samples were then subjected to the same Real-Time PCR quantification methods employed prior to storage. Samples stored under –20 °C conditions were allowed to thaw and were quantified in the same manner. All samples were quantified in duplicate.

Figures 1 through 3 show the quantitative results of extracts from the various biological fluids stored at both room temperature and –20 °C for 12 months and 45 months. Figure 4 summarize all the data for comparison.

Figure 1: *Average Total DNA Yields - Semen Extracts Stored on SampleMatrix: 12 and 45 Months*

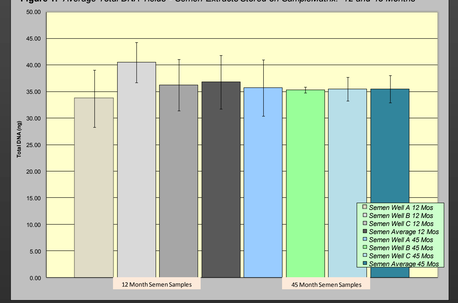


Figure 2 - *Average Total DNA Yields - Bloodstain Extracts Stored on SampleMatrix: 12 and 45 Months*

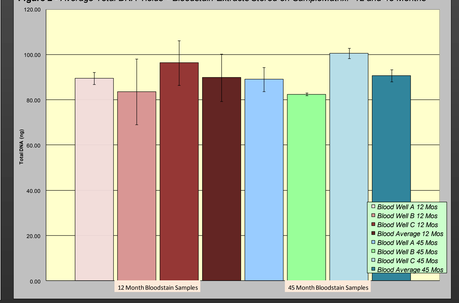


Figure 3 - *Average Total DNA Yields - Buccal Extracts Stored on SampleMatrix: 12 and 45 Months*

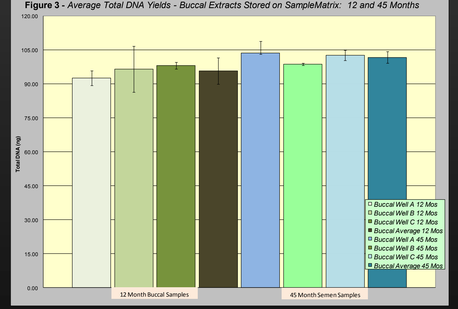
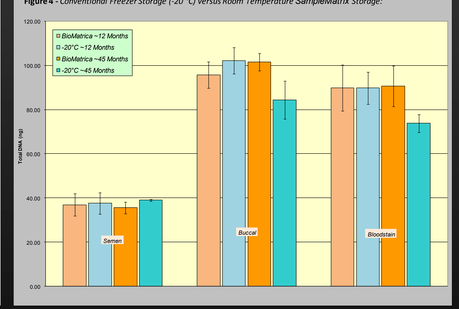


Figure 4 - *Conventional Freezer Storage (-20 °C) versus Room Temperature SampleMatrix Storage:*



Results — STR Amplification and Typing

The STR data generated from samples stored on the SampleGard/SampleMatrix® system were analyzed to determine if full STR profiles could be generated at the 45 month end-point time. Figures 5, 6, and 7 show the analyzed STR data from those samples.

Figure 5: *STR data for Semen Extracts (Room Temperature, 45 Months)*

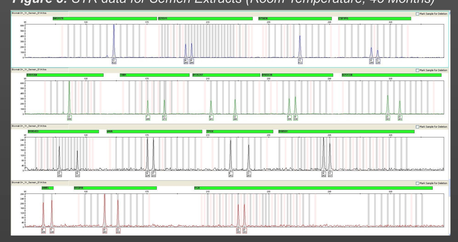


Figure 6*: STR data for Buccal Extracts (Room Temperature, 45 Months)*

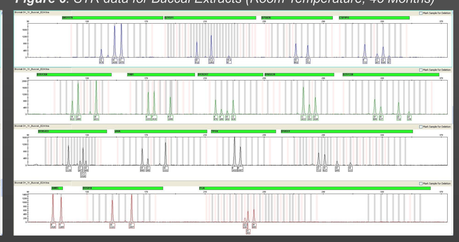
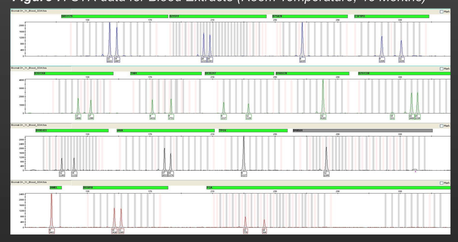


Figure 7*: STR data for Blood Extracts (Room Temperature, 45 Months)*



Discussion

Data obtained at the 45-month end-point time indicates that samples stored at room temperature using the SampleGard/SampleMatrix® system are stable and perform as well as samples stored under frozen storage. Additionally, in comparing data between 12-month and 45-month end-point times there does not appear to be any significant loss of DNA. Initially, STR amplification and typing of these samples was not part of the evaluation plan. However, in preparation for a formal validation of this product, it was important to determine if samples stored at room temperature using this system would be amenable to STR amplification and typing. Samples stored at –20°C were also subjected to STR amplification and typing and also yielded similar results (not displayed). It should be noted that samples prepared for this evaluation were not from a single source and thus concordance testing was not performed. Future testing will entail similar testing at the 5-year end-point time.

A more comprehensive, formal validation of this product is anticipated in the near future and will incorporate some of the data gathered thus far.