Spectrophotometric Comparison of Storage and Preservation Methods on Trace Soil

Tiffany Vickman, B.S.[a], Dishari Mukherjee, MSFS[a], G. Michele Yezzo, B.S.[b]., Pamela Staton, Ph.D[a]
[a]Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701
[b]Forensic Consultant

ABSTRACT

Soil is frequently encountered as evidence in criminal investigations. The major fraction of DNA extracted from soil is microbial in origin. PCR-based Microbial Community Profiling (MCP) is a technique that seeks to link persons and objects to specific places or suspects and victims to one another. Optimization of sample storage procedures is an essential first step for investigating MCP reliability for forensic analysis. This study assessed the impact of three storage and preservation methods on the quality and quantity of trace soil DNA utilizing spectrophotometry. Results demonstrated that soil stored in DNAgard® Tissues & Cells (Biomatrica) and that stored at -20 °C yielded high quality DNA in sufficient quantity for MCP analysis. A blinded MCP pilot study was performed using Automated Ribosomal Intergenic Spacer Analysis (ARISA) MCP where intra-sample clustering of replicates and no clustering of inter-sample replicates occurred when these storage methods were employed.

INTRODUCTION

Soil samples were stored using two commonly used forensic soil storage techniques, -20 °C and room temperature; as compared to sample storage in DNAgard® Tissues & Cells at room temperature. The focus of the study was to determine storage method impact on DNA quality and quantity. Proper storage and preservation of forensic soil samples is a critical step in preparation for downstream DNA analysis.

MATERIALS AND METHODS

Three sites were chosen to represent distinct locations in Huntington, West Virginia. Two soil samples were collected from different plots at the Marshall University Crime Scene House. The remaining sample was collected approximately one mile away at the Marshall University Forensic Science Center.

Figure 1. Soil sample being placed into the MoBio PowerLyzer™ 24 Bench Top Bead-Based Homogenizer

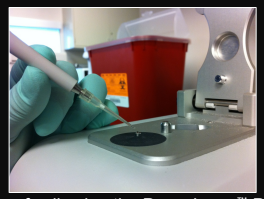

Figure 2. Sample placement on the NanoDrop™ 2000c

Total genomic DNA was extracted from 250 mg of soil using the PowerLyzer™ PowerSoil® DNA Isolation Kit and the PowerLyzer™ 24 Bench Top Bead-Based Homogenizer (MO BIO Laboratories, Inc.) and then analyzed using a NanoDrop™ 2000c Spectrophotometer (Thermo Scientific) for quality and quantity of each sample. DNAgard® Tissues & Cells, a room temperature DNA stabilization solution, was employed as a novel approach to determine its utility for trace soil microbial community preservation. ARISA was performed on samples stored in both DNAgard® Tissues & Cells and at -20 °C. For this process, DNA extracts were amplified using bacterial primers on a GeneAmp® PCR System 9700 (Applied Biosystems, AB) and the amplified products were separated on an AB 3130xl Genetic Analyzer. Statistical analysis was performed using data from AB GeneMapper® ID Software v3.2.1 with PISCES Conservation Ltd. Community Analysis Package 4.0.

RESULTS

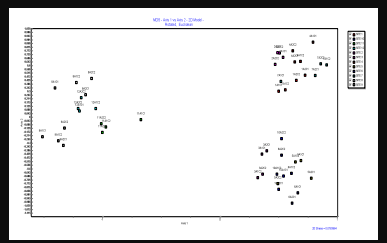

Figure 3. Euclidean model showing clustering of soil samples from three sites based upon ARISA MCP testing

Sample Storage	Mean Quantity	Mean A260/280	Std. Dev. A260/280
Stored in DNAgard®	6.144 ng/µl	1.860	0.137
Room Temp	21.978 ng/µl	2.057	0.177
-20 °C	23.356 ng/µl	1.827	0.102

Table 1. DNA quantity and A260/280 ratios for soil stored for five weeks using each method

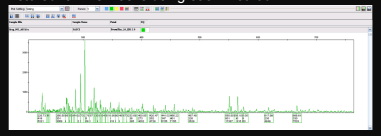

Figure 4 Example of a Bacterial Community Profile of a soil sample generated using ARISA

DISCUSSION

Over a period of five weeks, soil stored at -20 °C and soil stored in DNAgard® Tissues & Cells had A260/280 quality ratios of 1.827 and 1.860 respectively, which were well within the ideal range of 1.8-2.0. Although soils stored in DNAgard® yielded significantly lower DNA quantity, A260/280 ratios were still in the ideal range for quality with adequate DNA available for ARISA MCP analysis. This low quantity could be due to the DNAgard® solution diluting the soil sample during the extraction process. Further research should be performed using the optional DNAgard® dry down procedure in order to determine if the quantity of DNA extracted will increase. This procedure involves evaporation of the DNAgard® Tissues & Cells solution prior to extraction and can preserve the samples for up to one year at room temperature. ARISA MCP was performed on samples where these two preservation methods were utilized. Euclidian clustering shows that the samples collected from each of the three sites could be distinguished from one another.

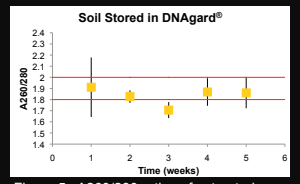

Figure 5. A260/280 ratios of extracted DNA from soil stored in DNAgard® Tissues & Cells over a 5 week period

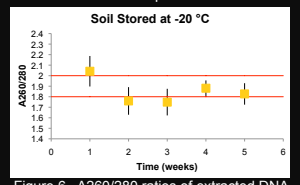

Figure 6. A260/280 ratios of extracted DNA from soil stored at -20 °C over a 5 week period

CONCLUSIONS & FUTURE STUDIES

Based on the findings of this study, soil samples will be stored at -20 °C until more research is performed on DNAgard® Tissues & Cells. In order to improve on the quantity of DNA extracted from soil stored in DNAgard®, the optional dry-down procedure should be evaluated to determine if higher DNA yields will result. A sensitivity study is planned to determine the least amount of soil sample required for ARISA MCP based on these storage methods.

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