



### A42 DNAgard<sup>®</sup>Tissue as a Room Temperature Microbial Preservation System for Forensic Soil Samples

*Tiffany A. Vickman, BS\**, *Pamela J. Staton, PhD*, and *Dishari Mukherjee, MSFS*, Marshall University Forensic Science Center, 1401 Forensic Science Drive, Huntington, WV 25701; and *G. Michele Yezzo, BS*, PO Box 244, West Jefferson, OH 43162

After attending this presentation, attendees will become aware of the different storage techniques used for soil evidence; DNAgard<sup>®</sup>Tissue (Biomatrix) as a biological soil sample preservation system; and microbial community profiling (MCP) as a forensic tool.

This presentation will impact the forensic science community by evaluating a novel use for DNAgard<sup>®</sup>Tissue as an in-lab or on-scene forensic sample storage/preservation medium.

DNAgard<sup>®</sup> consists of a specialized liquid solution that allows for the immediate stabilization of the DNA from cells and tissues. According to Biomatrix, this method allows for samples to be stored at room temperature for up to six months. In addition, this presentation will compare commonly used and novel storage techniques, e.g., length in storage per storage condition, as well as its impact on DNA quality and quantity. This will provide a basis for future research in microbial community profiling as a forensic tool and, in particular, how microbial DNA profiles may be used for evaluation of soil as evidence. The soil environment is dominated mainly by prokaryotes whose diversity can vary greatly. Bacteria are the most dominant form of these microbial communities found in soil whereby bacterial DNA profiling may provide an opportunity for linking soil samples with one another. MCP can easily be performed in forensic laboratories as many, if not all, crime laboratories have the required capillary electrophoresis genetic analyzers and PCR equipment to perform this analysis. Collection and storage of soil samples is a critical aspect in preparation for microbial DNA analysis. Traditional storage techniques used by forensic laboratories include either storing soil samples at -20°C or at room temperature. Traditional soil analysis often involves drying and sieving of samples prior to evaluation. While this may be essential for chemical and microscopic analysis of soil components and profiles, such procedures may have lethal and destructive effects on microbial communities.

The goal of this study was to determine the best method for storing soil samples for microbial DNA analysis. Seven storage conditions were compared: (1) room temperature; (2) -20°C freezer; (3) DNAgard<sup>®</sup>Tissue at room temperature; (4) -20°C freezer then air-dried; (5) -20°C freezer then oven-dried; (6) room temperature then air-dried; and, (7) room temperature then oven-dried. Each week, for a total of 5 weeks, soil samples were removed from storage and tested in triplicate. Microbial DNA was extracted using PowerLyzer™ PowerSoil<sup>®</sup> DNA Isolation Kit (MO BIO Laboratories, Inc.), a commercial kit chosen for its reported ability to extract good quality DNA from difficult environmental samples. This method is also reported to remove all humic substances and other PCR inhibitors. Following extraction, the microbial DNA was analyzed using a Nanodrop 2000c Spectrophotometer (Thermo Scientific) where the A260/280 ratio and sample concentration were determined. Preliminary testing revealed that storing soil samples in DNAgard<sup>®</sup>Tissue for up to five weeks resulted in extracted DNA of higher quality and yielded more consistent results when compared to other storage conditions. This study provides fundamental information to proceed to the next phase of testing which is microbial community profiling performed on an Applied Biosystems (AB) GeneAmp<sup>®</sup> PCR System 9700 with amplified products separated on the AB 3130xl Genetic Analyzer. The data generated will be analyzed using AB GeneMapper<sup>®</sup> ID Software v3.2.1.

**Soil, Microbial, DNAgard**