



Bode BioSafe™ Swab: Preventing Crime Scene “Culture” from Degrading DNA Evidence

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Introduction

A combination of new technologies introduced into the market and the increasing impact of the use of DNA to solve crimes has led to both increased reliance on DNA and more collections. One often overlooked area is the impact that biological materials, collected along with the samples at the crime scene, can have on the resulting DNA profile. Bacteria, fungi, and enzymes, such as DNases, can have a dramatic impact on the stability of a DNA sample possibly degrading it before it reaches an analyst’s bench for extraction and amplification. This presentation will describe the studies performed on mock crime scene samples to examine the specific effects of microbes and enzymes on the collected samples, the resulting DNA profiles, and introduce a point of collection preservative system; The Bode BioSafe Swab.

Biological materials were deposited on various mock crime scene surfaces. After deposition, the samples were placed outside in the environment for a few days to simulate and stimulate normal bacteria, fungal, and enzymatic activity that can occur at a crime scene. The samples were collected using a standard cotton swab and placed in a swab box or using The Bode BioSafe Swab. Real time and accelerated aging experiments were performed on the samples by placing them in various temperature and humidity conditions.

Significant degradation of biological evidence can occur in a very short period when the sample is not optimally collected and stored. The Bode BioSafe swab decreased sample degradation and increased the number of complete profiles obtained. The full results presented from this study will include MPS data on the different bacterial species collected alongside the genetic evidence, the quantification and degradation index values, and DNA profiling success rates.

Materials

The Bode BioSafe Swab



Standard Cotton Swab and Swab Box



Mock Crime Scene Surfaces



PowerPlex is a registered trademark of Promega Corporation.
QIASymphony is a registered trademark of QIAGEN Group.
GeneMapper and Quantifier are registered trademarks of Thermo Fisher Scientific

Methods

Sample Collection

- Biological fluids or touch samples were deposited on a variety of mock crime scene surfaces.
- After drying and environmental storage, the samples were collected with either a cotton swab or the Bode BioSafe Swab using the wet/dry method.
- The BioSafe Swabs and cotton swabs in swab boxes were stored in various temperature (22°C and 65°C) and humidity (ambient and elevated >60%) conditions.

Zone of Inhibition Analysis

- Cotton filter paper was treated with BioSafe Reagent, Tetracycline, or left untreated.
- 4.7mm disks were removed from the filter paper.
- The disks were placed on a nutrient agar plate that had been inoculated with a representative environmental sample obtained from a worn shoe.
- The nutrient agar plate was incubated at 37°C for 16 hours.
- Each plate was visually inspected for bacterial growth and inhibition.

Traditional DNA Analysis

- Swab heads were sampled and placed in microcentrifuge tubes.
- Extraction was performed using the QIAGEN QIASymphony®.
- Quantification was performed using the ThermoFisher Quantifier™ Trio Kit.
- If needed samples were concentrated prior to amplification with Promega’s PowerPlex® Fusion.
- Capillary Electrophoresis performed using a 3500XL Genetic Analyzer.
- Samples analyzed using GeneMapper® ID-X

Phylogenetic Analysis

- Swab heads were sampled and placed in microcentrifuge tubes.
- Extraction was performed using the QIAGEN QIASymphony.
- Quantification was performed using the ThermoFisher Quantifier Trio Kit.

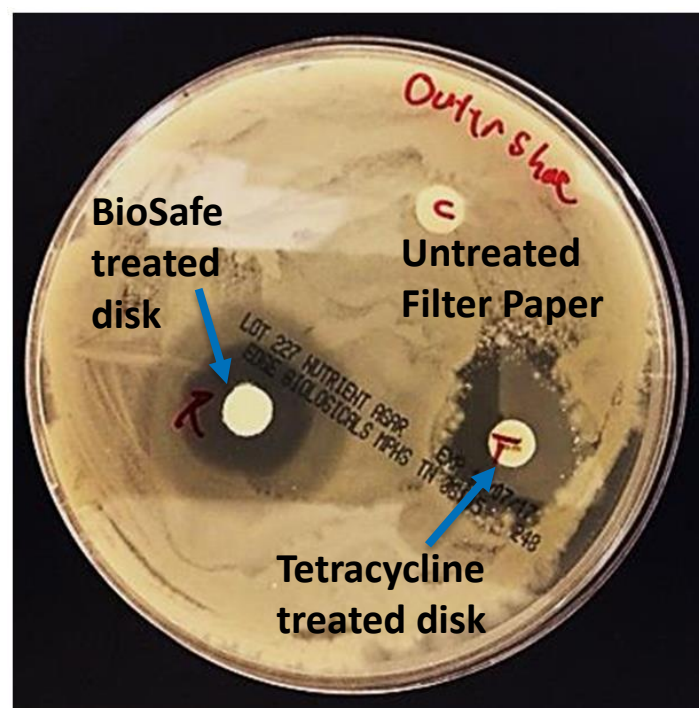
Target	Analysis Region
Bacteria	V3 and V4 regions of 16s RNA
Fungi	Internal Transcribed Spacer (5.8S) and Large Subunit Ribosomal Genes

- Sequencing performed using the Illumina MiSeq.

Target	Taxonomic Database Analysis
Bacteria	Illumina Greengenes Database
Fungi	MiSeq Reporter and QIIME 2

Microbiology Results

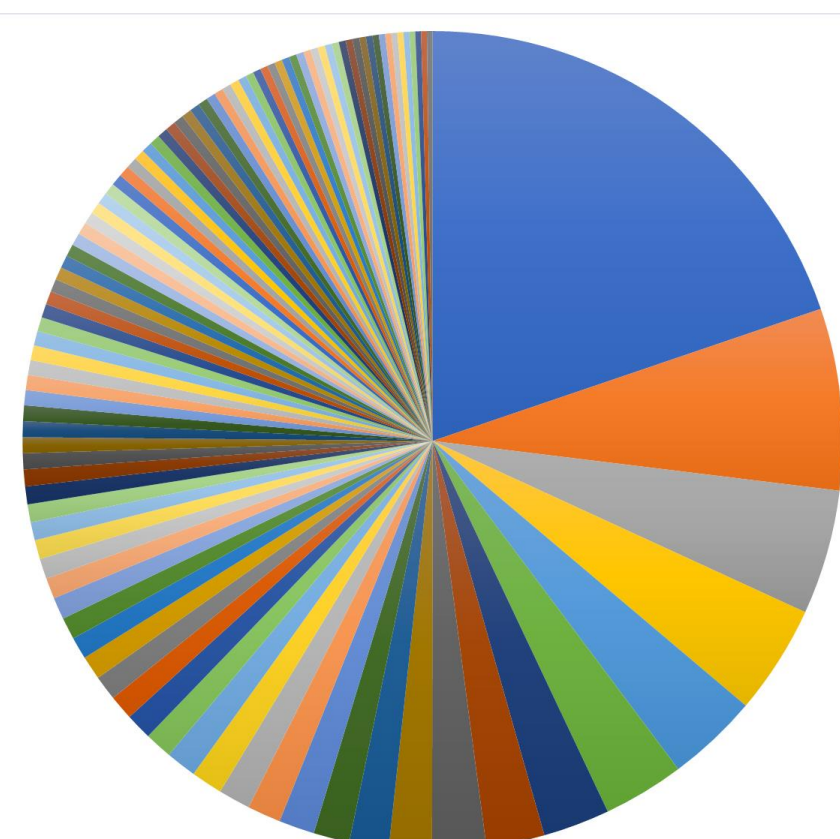
Zone of Inhibition



A clear zone on inhibition was observed surrounding both the BioSafe treated disk and the Tetracycline (a known antibiotic) treated disk. The untreated filter paper did not inhibit bacterial growth or activity.

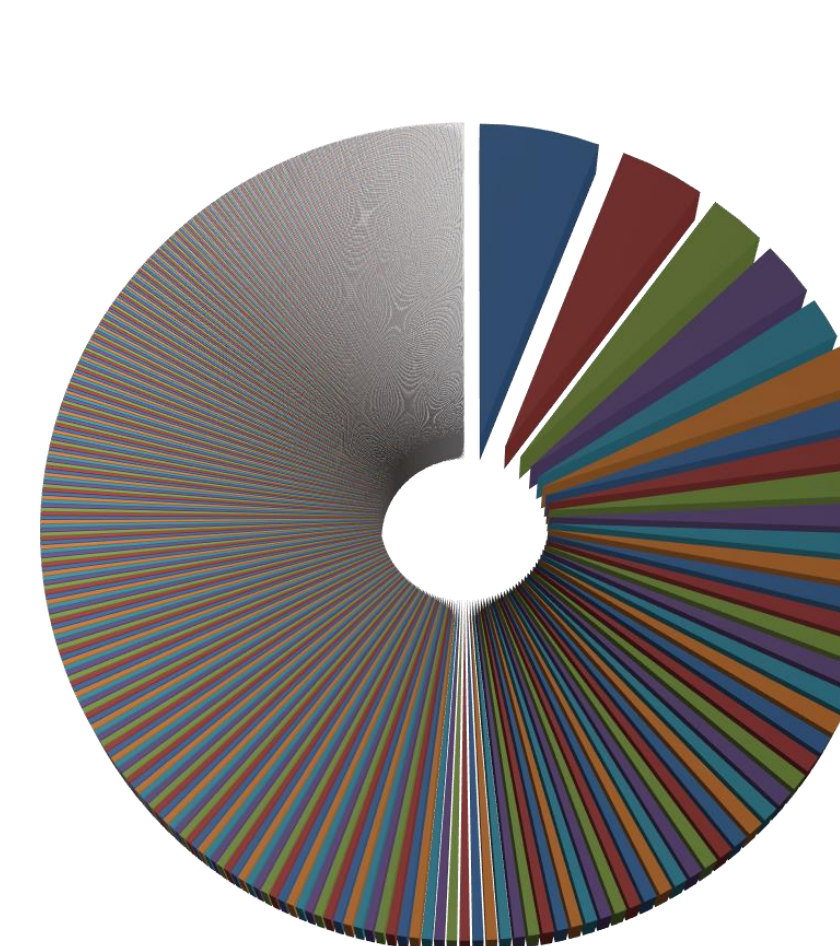
Phylogenetic Analysis

Blood Sample from a Wood Plank



- ✓ 61 Orders
- ✓ 243 Species Identified
- ✓ Top Species
- Janthinobacterium agaricidamnorum

Touch Sample from a Knife

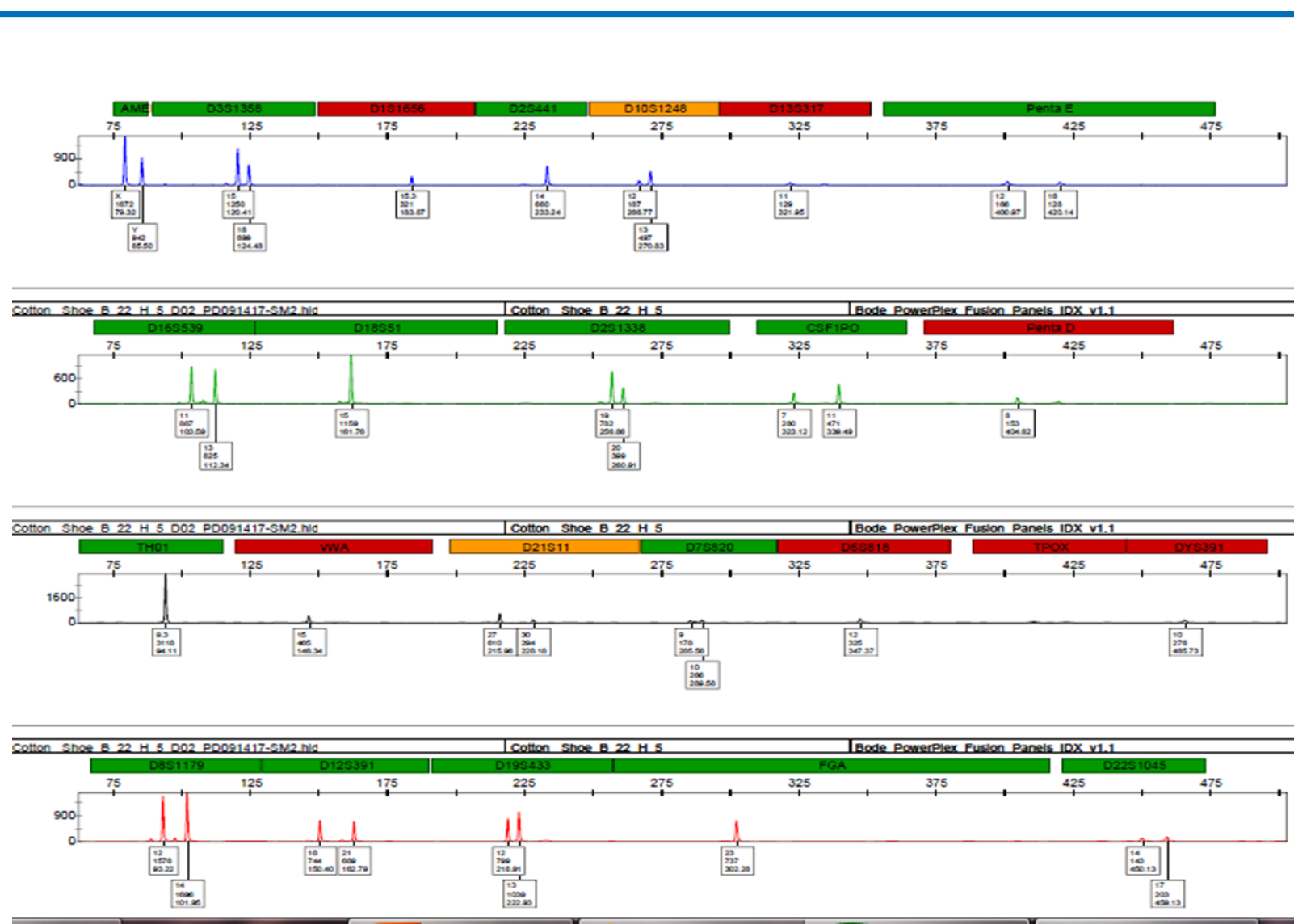


- ✓ 54 Orders
- ✓ 478 Species Identified
- ✓ Top 5
- Pseudomonas azotogormans
- P. lundensis
- Tepidomonas ignava
- Acidovorax temperans
- Tepidomonas taiwanensis

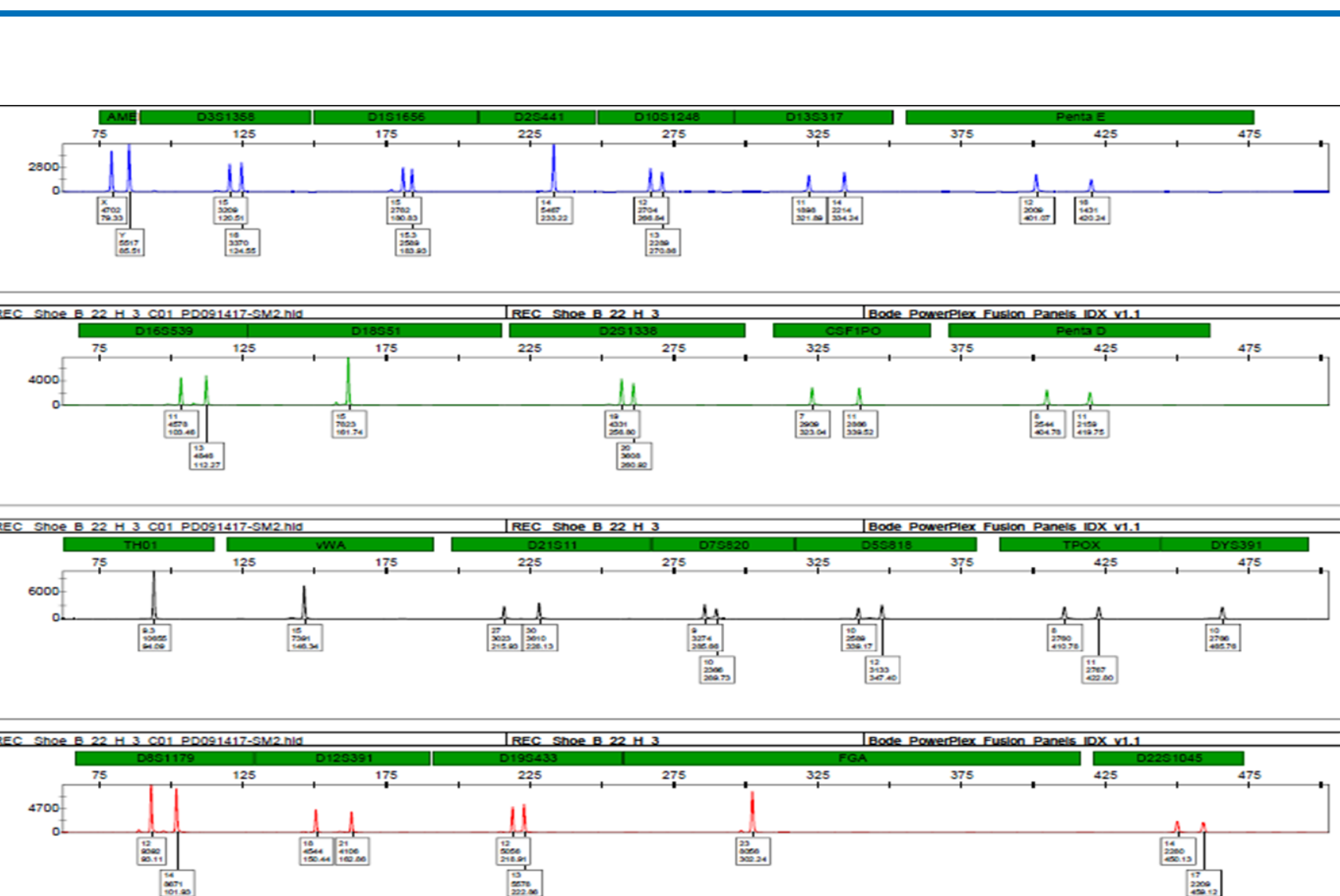
DNA Analysis Results

Samples Collected and Stored at Room Temperature and Elevated Humidity for 2 months

Blood Stain from Shoe: Cotton Swab Collection DI Value of 5.39

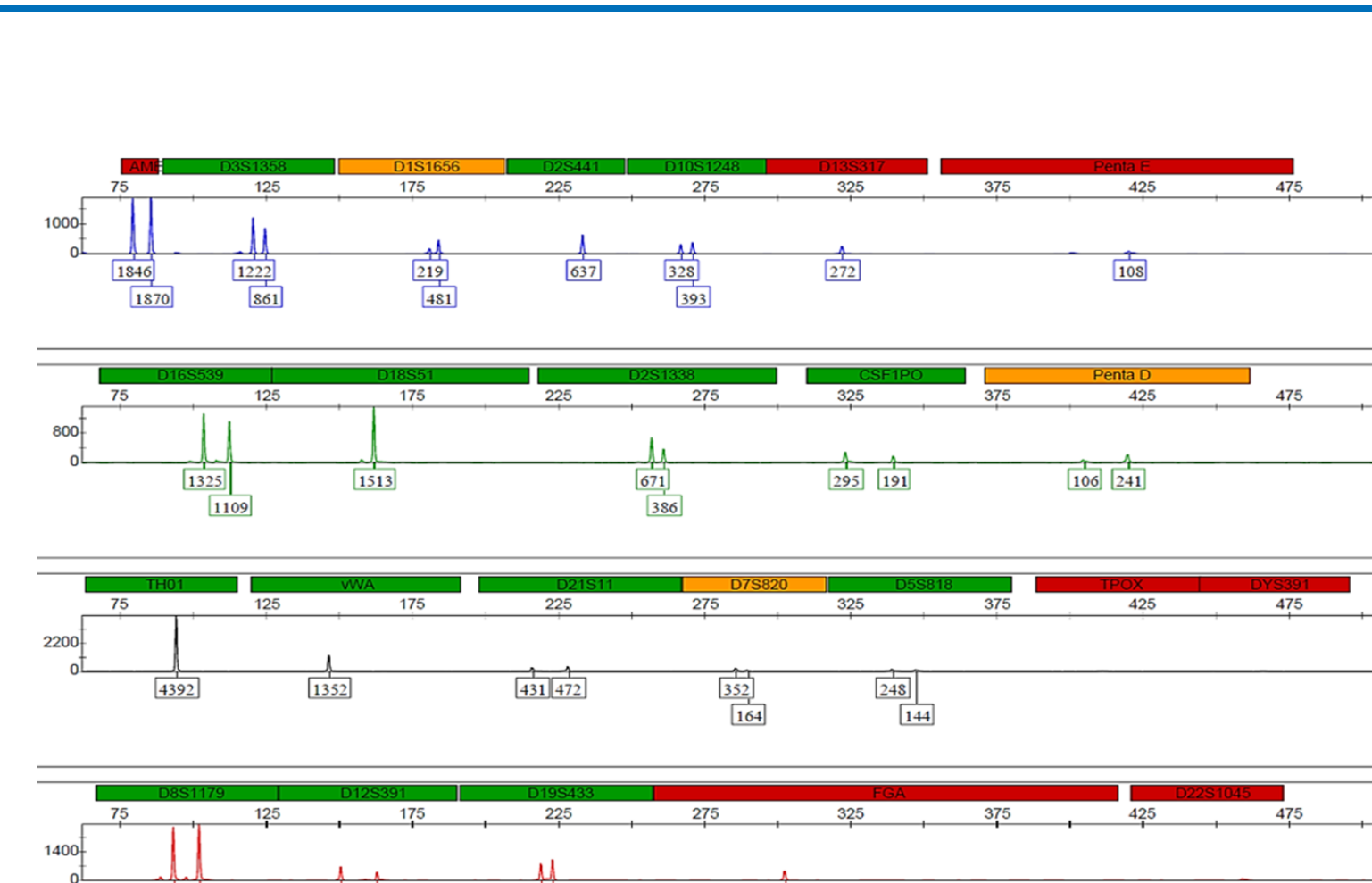


Blood Stain from Shoe: BioSafe Swab Collection DI Value of .89



Samples Collected and Stored at 65°C for 2 months-Estimated 3 Years at Room Temperature

Blood Stain from a Knife: Cotton Swab Collection DI Value of 4.86



Blood Stain from Knife: BioSafe Swab Collection DI Value of .99



DNA Profiling Success Rates

	BioSafe Swab (n=36)	Cotton Swab (n=36)
Complete Profile	97.22%	66.67%
Partial Profile	2.78%	25.00%
No Profile	0%	8.33%

Conclusions

- Hundreds of difference species of microbes were found on mock evidence samples.
- ~10% of were known to actively produce extracellular nucleases.
- The Bode BioSafe Swab inhibits the growth and activity of harmful microbes collected with evidence samples.
- Evidence collected with the BioSafe Swab yielded higher quantities of DNA and lower degradation index values.
- Thirty percent more complete profiles were obtained from evidence collected with the BioSafe Swab compared to the standard cotton swab and swab box.