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### **B94 The Individualization of Pubic Hair Bacterial Communities and the Effects of Storage Time and Temperature**

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After attending this presentation, attendees will better understand a novel forensic technique using the pubic hair microbiome that has the potential to link individuals who have come into contact with each other. Additionally, attendees will understand the impact of storage time and storage temperature on the pubic hair microbiome.

This presentation will impact the forensic science community by introducing a novel forensic method with the potential to associate two individuals in a sexual assault. Attendees will also be informed regarding how to best store these types of samples for future analysis.

A potential application of microbial genetics in forensic science is the detection of transfer of the pubic hair microbiome between individuals during sexual intercourse using high-throughput sequencing. In addition to the primary need to show whether the pubic hair microbiome is individualizing, another aspect that must be addressed before using the microbiome in criminal casework is the impact of storage on the microbiome of samples recovered for forensic testing.

To test the effects of short-term storage, pubic hair samples were collected from volunteers and stored at room temperature (~20°C), refrigerated (4°C), and frozen (-20°C) for one week, two weeks, four weeks, and six weeks in addition to a baseline sample, followed by amplification and sequencing of the V3/V4 region of the 16S rRNA gene. Individual microbial profiles ( $R^2 = 0.69$ ) and gender ( $R^2 = 0.17$ ) were the greatest sources of variation between samples. Because of this variation, individual and gender could be predicted using Random Forests supervised classification in this sample set with an overall error rate of  $2.7\% \pm 5.8\%$  and  $1.7\% \pm 5.2\%$ , respectively. There was no statistically significant difference attributable to time of sampling or temperature of storage within individuals. Further work on larger sample sets will quantify the temporal consistency of individual profiles and define whether transfer between sexual partners can be detected. For short-term storage ( $\leq$  six weeks), the microbiome recovered was not significantly affected by the storage time or temperature, suggesting that investigators and crime laboratories can use existing evidence storage methods.

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#### **Bacterial Forensics, Human Microbiome, Storage**