B131 "Touch Microbiome" vs. "Touch DNA": Exploring Potentials and Limitations Toward Forensic Personal Identification

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Learning Overview: After attending this presentation, attendees will understand the potential and the limitations that microbiome studies have in providing information on the donor who touched an item ("touch microbiome") in comparison with the standard DNA fingerprinting methods ("touch DNA").

Impact on the Forensic Science Community: This presentation will impact the forensic science community in terms of competence by showcasing the importance of the study and analysis of the "touch microbiome" as an alternative/integrative way to obtain intelligence information useful for solving forensic caseworks, overcoming the limitations involving "touch DNA" evidence.

The rising usage of Next Generation Sequencing (NGS) in forensic contexts allowed for the analysis of microorganisms to become an auxiliary tool to conduct personal identification analyses.[1] Among the microbiota easy to find at the crime scene, the skin "microbial footprint" is highly individual, stable over time, and easily released into the surrounding environment. Microbiota analyses have been successfully admitted as evidence in court cases, but the lack of accurate forensic reference databases currently limits their use.[2,3] On the other side, the increased sensitivity in the simultaneous amplification of different Short Tandem Repeats (STR) has enabled forensic geneticists to recover DNA profiles from highly degraded samples and low DNA content evidence, including those generated when a person touches a surface or an object ("touch DNA"). Although the forensic community has been talking about "touch DNA" for over 20 years, there are still some dark sides, such as the origin of this DNA, its transfer dynamics, its ability to withstand different environmental conditions, and the risks associated with its secondary transfer.[4,5]

This study wanted to compare the sensitivity and specificity of these two different methods ("touch microbiome" versus "touch DNA") for forensic identification purposes and highlight their strength and weaknesses. Eleven volunteers of both sexes and of different ages filled in a questionnaire on their lifestyles and on any previous or current pathologies and treatments they had. Each individual's palm and fingers were sampled using three sterile swabs ("skin sample"), then volunteers deposited their fingerprints on a glass slide that was then swabbed ("fingerprint sample"). Microbiome DNA and human DNA were extracted respectively using QIAamp® PowerFecal Pro DNA and ChargeSwitch® Forensic DNA from each sample. DNA was quantified with NanoDrop™, then the V4 region of 16S rRNA gene was analyzed using the Illumina® MiSeq® platform whereas human STRs were amplified with the AmpFℓSTR® NGM SElect™. NGS data were analyzed using R scripts normally used in metabarcoding ecology studies.

This study obtained good quality microbiome profiles from 20 out of 22 samples analyzed and found a significant difference between the composition of the "skin" and the "fingerprint" samples. Timing between washing and samplings showed an effect on the relative abundance of *Actinobacteria spp.* in both sample types, and pairwise comparisons showed a relative increase of *Proteobacteria spp.* and decrease of *Firmicutes spp.* and *Actinobacteria spp.* in fingerprint samples versus their skin counterpart. STR profiling of "touch DNA" samples showed discrepancies between skin and fingerprint samples (e.g., missing peaks for some markers in all samples but one).

While additional analyses of the results are currently in progress, this study can anticipate that microbiome profiles provided information that could potentially be used to identify an individual where "touch DNA" also failed.

Reference(s):

1. Oliveira M., Amorim A. Microbial forensics: new breakthroughs and future prospects. *Appl Microbiol Biotechnol*. 102 (24):10377–10391, 2018.
2. Spagnolo E.V., Stassi C., Mondello C., Zerbo S., Milone L., Argo A. Forensic microbiology applications: a systematic review. *Legal Med*. 36: 73–80, 2019.
3. Neckovic A., van Oorschot R.A.H., Szkuta B., Durdle A. Challenges in Human Skin Microbial Profiling for Forensic Science: A Review. *Genes*. 11, 1015, 2020.
4. Lacerenza D., Aneli S., Omedei M., Gino S., Pasino S., Berchialla P., Robino C. A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers. *Forensic Sci. Int. Genet*. (22):44–53, 2016.
5. Raymond J.J., van Oorschot R.A., Gunn P.R., Walsh S.J., Roux C. Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes. *Forensic Sci. Int. Genet*. 4 (1):26–33, 2009.

Microbiome, Touch DNA, Next Generation Sequencing

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