Evaluating the Effectiveness of Microbial DNA Extraction Kits for Intimate Samples

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INTRODUCTION

The primary type of forensic evidence used in sexual assault cases is sperm, however sperm may not always be left behind [1-3]. Another issue with sperm evidence is its low level of persistence and trouble with differential extraction [3, 4]. Due to these issues, microbial evidence may be an option to use in conjunction with sperm.

The human microbiome is comprised of the bacteria that live on a person's body [5]. It is known that different parts of the body have different types and amounts of bacteria [6] as well as inter- and intra-variability [7]. Microbial evidence may be a good complement to sperm as it is more robust and can be detected after a longer period of time [5]. Bacterial species can be distinguished by 16S rRNA sequencing, which contains species-specific variable regions [8].

In this study, the biases and differences of five microbial DNA extraction kits were examined with vaginal and epithelial microbiome standards using next generation sequencing (NGS). The extraction kit with the highest efficacy was used on vaginal and penile swabs. NGS was performed to establish the baseline microbiome profile of pre-coitus male and female intimate areas.

MATERIALS AND METHODS

Sample Collection For Phase I, cotton swabs were spiked with 33.33 µL of ATCC® vaginal microbiome whole cell mix (N=25) and ATCC® epithelial microbiome whole cell mix (N=25) (Manassas, VA). For Phase II, volunteers (N=10 male and 10 female) swabbed their penis or vagina on 2 days with 2 swabs per day. Samples were collected under SHSU IRB-2020-166.

DNA Extraction For Phase I, five extraction kits were used: Invitrogen® Purelink™ Microbiome DNA Purification Kit (San Francisco, CA), PDQeX prepGEM Bacteria Kit (Charlottesville, VA), Qiagen® QIAamp® DNA Mini Kit (Germany), Qiagen® DNeasy® PowerSoil Pro Kit, and Qiagen® DNeasy® UltraClean® Microbial Kit. For Phase II, the best performing kit during Phase I was selected.

DNA Quantification Extracted DNA was quantified with the Invitrogen® Qubit® fluorometer with the dsDNA HS Assay kit.

Library Preparation and Sequencing Library preparation was performed using the Earth Microbiome Project and Illumina® 16S Metagenomic Sequencing Library Preparation protocol with 341F and 805R primer sets targeting the V4 region of the 16S gene. ATCC® vaginal and epithelial microbiome genomic standards were also added as a control. NGS was performed on the Illumina® MiSeqFGx™ using V3 reagent chemistry, 600 cycles, and 300 bp paired end sequencing.

Data Analysis Data was analyzed using Illumina® 16S BaseSpace software and the Greengenes database.

RESULTS AND DISCUSSION

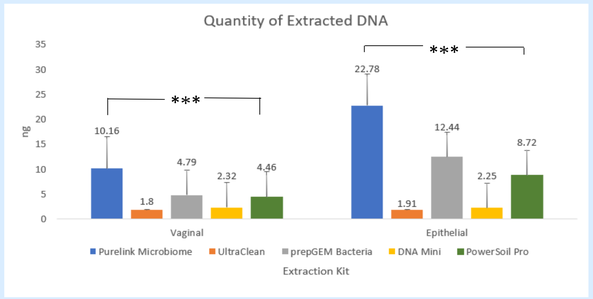


Figure 1. Quantification of eluted DNA from the five tested extraction kits. Data shown as average ± SEM. (N=50)
*** = p<0.001 for single-factor ANOVA.

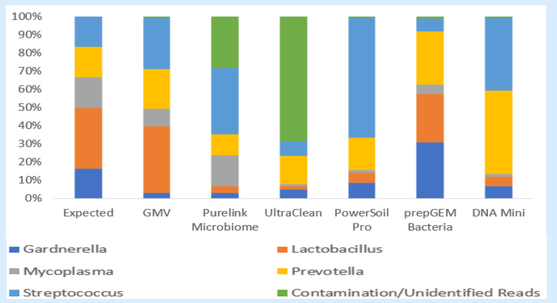


Figure 2. Relative abundance of bacteria at the genus level from ATCC vaginal standards.
Expected refers to relative abundance of bacteria comprised in the ATCC standard.
GMV denotes the genomic mix ATCC vaginal standard used as a sequencing control.

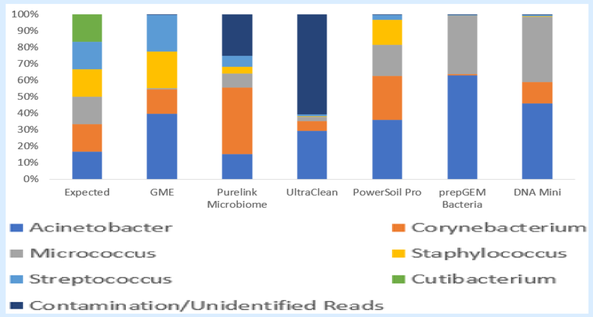


Figure 3. Relative abundance of bacteria at the genus level from ATCC epithelial standards.
Expected refers to relative abundance of bacteria comprised in the ATCC standard.
GME denotes the genomic mix ATCC vaginal standard used as a sequencing control.

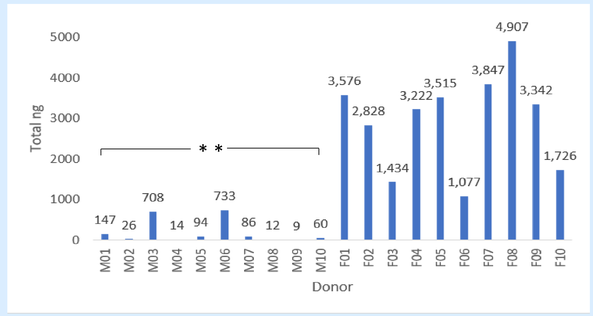


Figure 4. Quantity of DNA extracted from male and female donor swabs. (N = 80 swabs)
** = p<0.01 for single-factor ANOVA

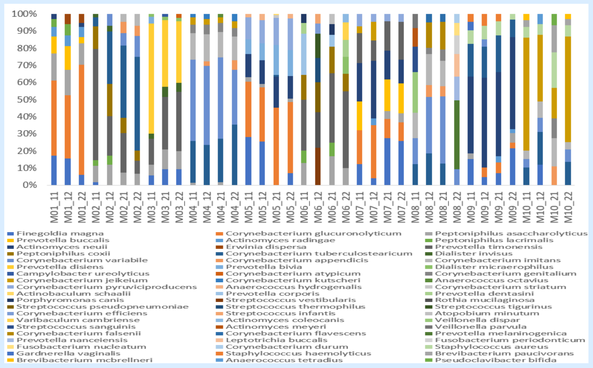


Fig 5. The relative abundance of bacteria at the species level from male donor samples. (N=40 swabs)

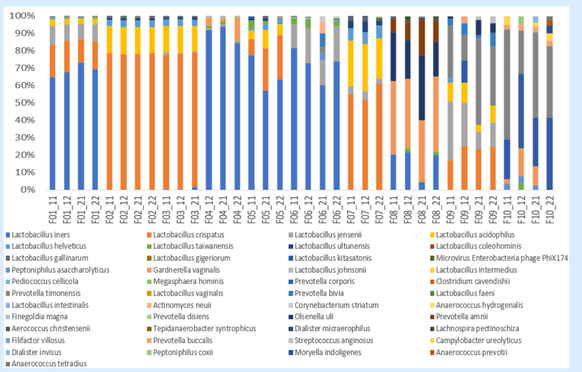
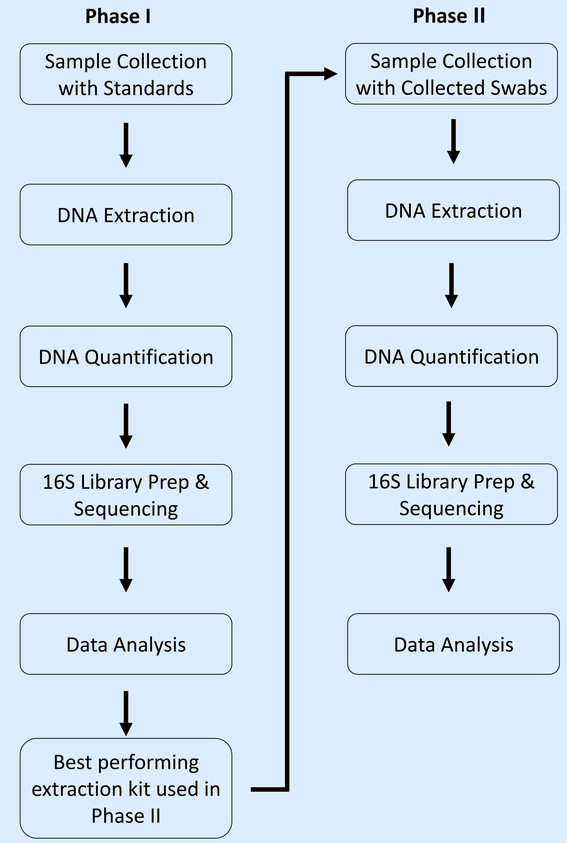


Fig 6. The relative abundance of bacteria at the species level from female donor samples. (N=40 swabs)

MATERIALS AND METHODS



CONCLUSIONS

- The Invitrogen® Purelink™ Microbiome Kit was the best performing DNA extraction kit overall when considering DNA yield and relative representation of the microbial populations present
- Female donor samples had a high relative abundance of *Lactobacillus*
- Unique genera and species were identified for all male donors and most female donors
- Male donor samples had more donor specific bacteria compared to female donor samples
- Future studies include in the investigation of transfer and persistence of genital microbiomes post-coitus and the affect showering has on the persistence of post-coital microbial transfer

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ACKNOWLEDGEMENTS

The authors would like to thank the SHSU Department of Forensic Science, SHSU Department of Biological Sciences, Trisha Brewer, Ryan Gutierrez, Damani Johnson, and the volunteers that participated in this research.