

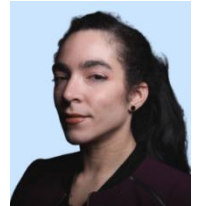


CAIRIBU UROBIOME RESEARCH INTEREST GROUP (U-RIG)

CAIRIBU U-RIG KNOWLEDGE EXCHANGES

EXECUTIVE SUMMARY 2/20/2026 U-RIG KNOWLEDGE EXCHANGE

U-RIG KNOWLEDGE EXCHANGE FEAT: KEISHA HARRISON, PHD



Goal of CAIRIBU Urobiome RIG Knowledge Exchanges: Facilitate knowledge exchange and spur collaborations in the urobiome and adjacent fields

Summary of Dr. Harrison's presentation

Considerations of methods and techniques for studying the urobiome

Introduction and Overview of the Bladder Microbial Niche

- [Dr. Keisha Harrison](#) introduced her presentation on theoretical frameworks that guide experimental design in researching the urobiome.
- The urinary microbiome is biologically significant and is associated with multiple urologic conditions, yet it has not been characterized to the same depth or consistency as high biomass systems.
- **The bladder is a low-microbial biomass niche.** High-microbial niches, such as the gut microbiome (colon), which has $\sim 10^{11}$ bacteria/mL. In contrast, bacterial concentration in the bladder ranges from 10^2 - 10^4 bacteria/mL. Less dense microbial ecosystems influence what researchers can detect.

Challenges of Assessing Microbiota in Low-Biomass Environments

- Small differences and how much material is collected can drastically shift what is observed. Catheterized urine typically contains 10^2 - 10^5 CFU/mL (when detectable). The location of collection and contamination sensitivity (from skin, vaginal carryover, the environment, or the kitome) must be carefully considered.
- **Background DNA from reagents, the lab environment, or host cells become a larger proportion of what is ultimately sequenced.**
- Culture-based techniques are limited by growth conditions and may not recover all taxa present, making 16S rRNA gene sequencing, a culture-independent, low-cost, and scalable technique, a transformative approach.
- As a low-biomass sample, urine's total endogenous microbial DNA may be small, while exogenous DNA from kit contamination or host cells might be abundant.

Contamination and Sequencing Variability

- Ideally, a no-template control would not contain bacterial DNA. Though in reality, microbial DNA is often detected in these controls because of contamination from reagents, extraction kits, and the general lab environment. **This contamination is stable and contained and can be a reference for filtering background signal across samples.**
- If contamination is controlled and proportional, both low and high biomass samples should yield representative community structures.
- Well-to-well spillover and DNA transfer can distort the observed community, whereby the resulting composite contains true biological signal, lab and reagent contaminants, and exogenous DNA from neighboring high biomass samples.
- Low-biomass systems require rigorous contamination control, careful plate design, and experiment-specific computational decontamination strategies.
- Sequencing is just one step in a larger workflow, and variability can enter at every stage, from collection and storage to DNA isolation and library prep.
- Collection method and preservation/storage decisions impact downstream analysis, and incomplete lysis can affect the detectability of taxa.

DNA Isolation and Lysis Methods

- DNA extraction determines what is detectable, but microbial cell envelopes vary dramatically – **no single lysis method (mechanical, enzymatic, chemical) is universally unbiased**. Combined with the urobiome's low-biomass ecosystem, these factors make sequencing more difficult.
- The cell wall of gram-positive bacteria is relatively resistant to chemical disruption alone. Once the outer membrane of gram-negative bacteria is disrupted, the thin peptidoglycan cell wall is easier to disrupt.
- Chemical lysis incorporates detergents or chytropic agents that solubilize membranes and denature proteins.
- Enzymatic lysis requires matching enzymes to cell wall chemistry to target specific bonds in the cell wall, while mechanical methods use physical force to disrupt cells and can improve recovery of organisms with robust or heavily cross-linked cell walls.

Empirical Studies on Lysis Methods

- Teng et al. (2018) used a mock oral community to compare different lysis methods and their impact on community structure. Key findings were that:
 1. No method perfectly recapitulated expected composition
 2. Bead and enzyme combinations (M5, M6) showed highest concordance
 3. Enzymatic-only and kit-only methods introduced systematic distortions
 4. Gram-positive taxa were most sensitive to extraction strategy
- Dr. Karstens and colleagues studied the impact of DNA isolation methods on observed urinary microbiome profiles.
- The study tested five commercial extraction kits on 11 voided urine samples. Results demonstrated kit-dependent variability in DNA yield and sequencing depth. Despite differences in DNA yield and concentration, kit choice did not automatically translate to differences in sequencing depth.

Kit Choice and Bias in Urobiome Studies

- Different kits can bias the apparent abundance of specific taxa.
- Karstens et al. (2021) observed that most kits did not strongly shift the community towards gram-negative organisms, but specific genera were affected.
- The Promega kit showed a trend towards lower recovery of gram-positive associated genera, while the PowerSoil kit recovered more gram-negative organisms.
- Urine's low biomass and background DNA can impact community abundances, emphasizing the need for reproducible and robust results.

Decontamination Strategies

- Visual inspection and statistical decontamination methods can help identify and remove contaminants from data.
- Given the need for contamination controls in urobiome research, Dr. Harrison recommended setting up study designs with controls to better assess and delineate contamination.

Dr. Harrison is a post-doctoral researcher at Oregon Health and Science University in the Department of Medical Informatics, Division of Bioinformatics and Computational Biomedicine. She is studying under Dr. Lisa Karstens. Dr. Harrison is a computational biologist. She obtained a PhD at Oregon State University in Microbiology and Biological Data Science where her research focused on microbiome analysis within fermentation food systems. Dr. Harrison is an Institutional Research and Academic Career Development Award postdoctoral fellow at Oregon Health and Science University. In Dr. Karsten's lab, Dr. Harrison is investigating the role of urogenital microbiota in overactive bladder and urinary tract infection.