THE TOP 4

Considerations for Successful and Actionable Vaginal Microbiome Profiling

Bio-Me
Microbiome Profiling

Bio-Me AS

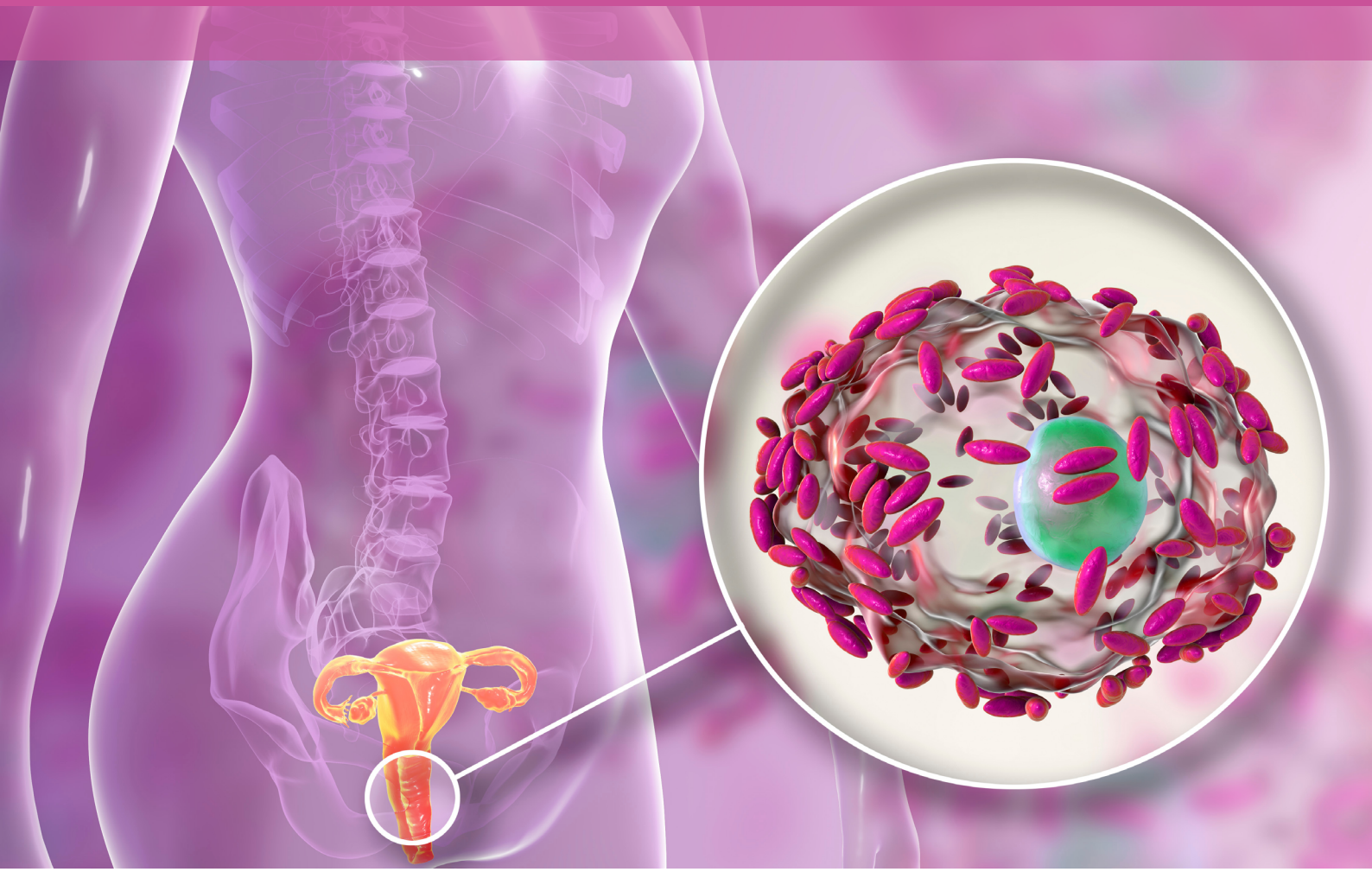
Oslo, Norway

Introduction

The vagina is colonized by millions of microbes (e.g., bacteria, fungi and viruses) that are integral to the vaginal microbiome[1,2].

The study of the human vaginal microbiome is broadening our understanding of vaginal and reproductive health and facilitating development of novel diagnostics and therapeutics aimed at optimizing vaginal health and treating specific conditions ranging from bacterial vaginosis (BV) to infertility[2].

Most vaginal microbiome studies currently utilize next-generation sequencing (NGS) approaches, specifically amplicon sequencing and shotgun metagenomics[3]. In this report we will go through four key factors to consider when selecting a method for vaginal microbiome profiling.



1.

Resolution

Amplicon-based NGS, targeting the bacterial 16S rRNA gene or the fungal ITS1 region, has been traditionally used for vaginal microbiome profiling. While this method is cost-effective, it is not suitable for resolving taxa reliably to the species level and is, in fact, usually limited to genus-level resolution. A recent breakthrough in NGS platforms has been the development of long-read technologies, such as that offered by Oxford Nanopore Technologies. While full-length 16S rRNA gene amplicon sequencing enabled by these platforms has been shown to give better resolution at the species level than short-read technologies[4], long-read platforms tend to have lower per base accuracy, higher total costs, and lower throughput[5,6]. In contrast, shotgun metagenomic sequencing targets not only the 16S rRNA gene/fungal ITS1 region, but a microbiome's collective genomic information, which allows for taxonomic identification at the species and strain level[3,7].

Higher resolution is relevant when species within a genus, or strains within a species, differ in their genomic composition in a manner that may lead to distinct functional capabilities. For example, meaningful species- and strain-level diversity has been discovered for *Gardnerella* and *Gardnerella vaginalis* respectively[8,9], and this heterogeneity is the subject of much research because *G. vaginalis* has been repeatedly linked to the pathogenesis of BV[10,11]. Likewise, strain variation within the highly beneficial vaginal species *Lactobacillus crispatus*, may have implications for strain selection for Live Biotherapeutic Products (LBPs) or for donor screening for vaginal microbiome transplant (VMT) procedures[8,9,12].

1. Resolution (cont.)

Unfortunately, the increased resolution provided by shotgun metagenomics comes at a cost. Vaginal samples are rich in host DNA (discussed further below), which impacts test sensitivity and reproducibility and necessitates costly ultradeep sequencing. In addition, result interpretation depends on complex data analysis[6], typically requiring access to expert bioinformaticians, expensive software and proprietary databases. These issues particularly affect the use of shotgun metagenomics for women's health at-home-testing as they increase turnaround time and limit accuracy and profitability. For companies engaged in microbiome R&D, these challenges are equally relevant as the number of samples analyzed increases – for example, when profiling a high number of clinical trial participants, monitoring vaginal probiotic/LBP engraftment or assessing the effects of a vaginal product over time.

Bio-Me has developed Precision Microbiome Profiling (PMP™), a platform for rapid, comprehensive and accurate profiling of the vaginal microbiome that is designed to address the issues that impact sequencing based-methods. PMP™ uses a highly parallelized quantitative PCR (qPCR)-based approach which accurately and at a low cost, detects microbes in vaginal samples with species- and strain-level resolution.

2. Host DNA

Vaginal samples can be challenging to analyze as they typically contain high levels of host DNA, which can represent more than 95% of the total extracted DNA[3,13,14]. Amplicon sequencing uses preamplification of the target regions (16S rRNA gene or ITS1) prior to sequencing and can thus be used in samples highly contaminated with host DNA. However, as mentioned above, such methods are unsuitable for reliably achieving (sub)species-level resolution.

Shotgun metagenomics, on the other hand, while providing high resolution, is deeply impacted by the amount of human DNA in vaginal samples. Although reads from host contamination can be removed post-sequencing during data processing, most of the generated reads have been expended on sequencing the human genome and do not offer any information on the vaginal microbes. It is also possible to deplete host DNA from a sample prior to sequencing and thereby improve the ratio of informative bacterial DNA in a vaginal sample. However, host depletion further increases costs and studies investigating these approaches have shown that they introduce taxonomic biases[5] which are particularly detrimental when studying the microbiome. Regardless of the approach used to deal with high host DNA content, shotgun metagenomics results in increased costs to achieve a sequencing depth for vaginal microbes that is sufficient to yield sensitive and reproducible results[6].

In contrast to shotgun metagenomics, the qPCR-based approach central to Bio-Me PMP™ is not negatively impacted by host DNA, because it is a targeted method.

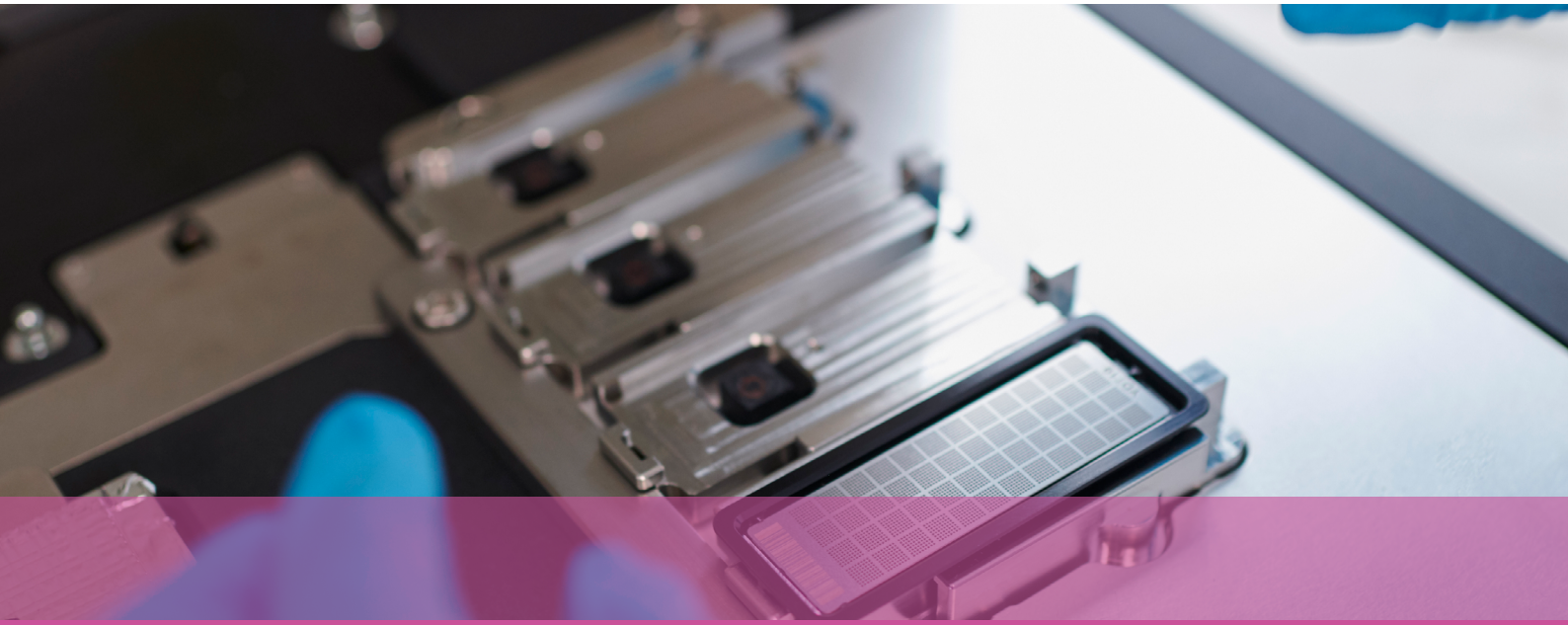
3. Speed

Sequencing methodologies have a relatively high turnaround time (often measured in weeks), which is unacceptable in the vaginal health field because vaginal microbiomes can shift rapidly and repeatedly, e.g., with menses[15–17].

For results to enable appropriately timed actions, they need to be generated quickly (days). For this reason, in clinical practice, qPCR tests are advantageous compared with sequencing approaches because they are fast (as well as accurate and highly cost-effective). Accordingly, numerous qPCR tests have been developed to support diagnosis of vaginal disease (such as BV).

However, a challenge of conventional qPCR when applied to microbiome profiling is the limitation in analyzing many microbial targets simultaneously. For this reason, most vaginal qPCR tests that have been developed only analyze 2-15 targets[18–21], rather than providing a comprehensive vaginal microbiome profile.

Bio-Me PMP™ platform relies on a highly parallelized qPCR-based approach that can comprise hundreds of species or strain-specific assays per test panel, sufficient to enable a comprehensive characterization of the vaginal microbiome rapidly, with ready-to-use results available 2-5 days after sample arrival.



4. Absolute Quantification

Estimation of bacterial abundances by NGS approaches is based on the counts of reads mapped to a reference genome (or a part of it). The resulting semi-quantitative output is presented as relative abundances of all detected taxa. This poses a challenge, as the use of relative abundances can lead to misleading results[22]. In other words, microbial load of a taxon may increase, decrease or remain constant while relative abundance measurements could move the opposite way (Figure 1).

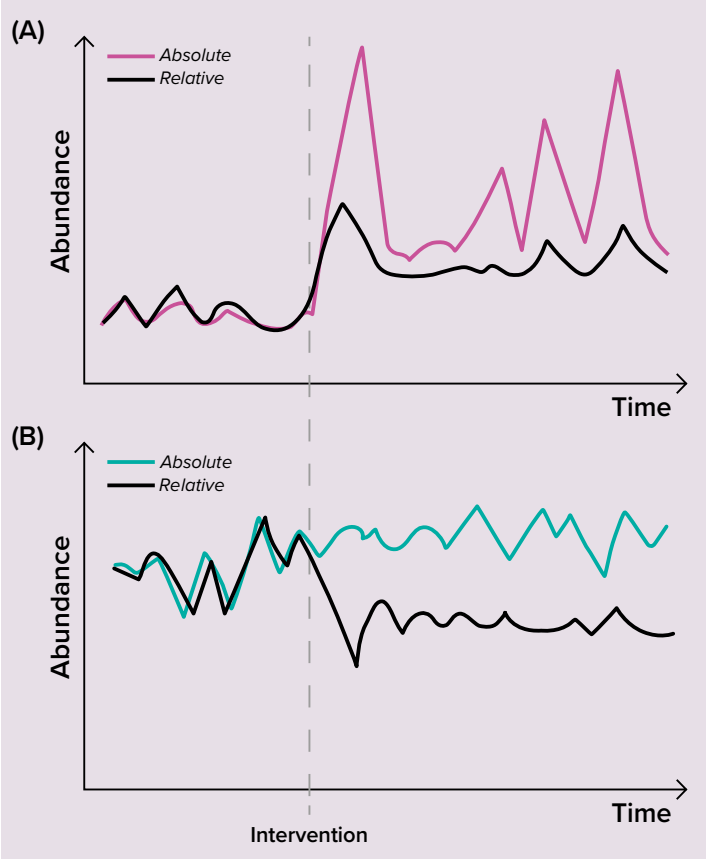


Figure 1. The use of relative abundance can produce false positives. (A) The abundance of a taxon is increased after an intervention (pink line). This increase is also reflected in its relative abundance (black line). (B) Another taxon is not influenced by the intervention (green line). However the intervention has a negative effect on its relative abundance (black line) due to the increased relative abundance for the taxon in (A). Based on Jian et al.

A related example is the fact that the total bacterial biomass (referred to as microbial load) in a patient can change independently from taxon relative abundances.

By contrast, absolute quantification (number of genomes per taxon) is less vulnerable to false inferences and it is also more informative. Absolute quantification provides a crucial added level of actionable information that has importance in many health contexts, such as bacterial vaginosis[21], pregnancy[23], vaginal microbiome donor screening[12] and menopause[24].

Approaches that utilize qPCR, such as Bio-Me PMP™ technology, enable the absolute quantification of vaginal microbes, thus contributing vital information for vaginal microbiome characterization.

Bio-Me
Microbiome Profiling

Transforming Vaginal Microbiome Analysis with Precision Microbiome Profiling (PMP™)

Bio-Me has developed the **PMP™ Core Vaginal Microbiome Test** with a precise analysis of **over 50 key bacterial and fungal targets.**

The test determines the **vaginal community state type (I-V)** and quantifies microbes implicated in **bacterial vaginosis, aerobic vaginitis,** and **candida vulvovaginitis.**

In addition, the vaginal microbiome profiling results may illuminate potential risks for many important health conditions including **infertility, adverse birth outcomes and STIs** among others.

The PMP™ approach involves a highly parallellized qPCR platform that supports **comprehensive vaginal microbiome profiling** in a high throughput format.

Results are directly actionable - **no need for bioinformatics steps** for data processing.

Bio-Me **PMP™ Core Vaginal Microbiome Test** meets the needs for a vaginal microbiome profiling tool:

- **High-level resolution** - It provides the same resolution of shotgun metagenomics (down to species and subspecies levels).
- **Not impacted by host DNA** - The targeted approach used overcomes the challenges associated with a high presence of host DNA in the sample.
- **Fast** - Ready-to-use results in 2-5 days from sample arrival!
- **Absolute Quantification** - It provides the number of genomes per taxon, as well as the relative abundance for the targeted taxa.

Custom proprietary strain assays upon request!

Contact us: sales@bio-me.com

Disclaimer: This test was developed and validated by Bio-Me as a Research Use Only (RUO) vaginal microbiome profiling analysis. The results from the test are not designed to diagnose, treat, or cure any medical condition. A diagnosis needs to be confirmed via consultation with a qualified health professional, such as a registered general practitioner or gynaecologist.

Bio-Me
Microbiome Profiling

References:

1. Berg, G. et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome 8, 103 (2020).

2. Pendharkar, S., Skafte-Holm, A., Simsek, G. & Haahr, T. Lactobacilli and Their Probiotic Effects in the Vagina of Reproductive Age Women. Microorganisms 11, 636 (2023).

3. Berman, H., McLaren, M. & Callahan, B. Understanding and interpreting community sequencing measurements of the vaginal microbiome. BJOG: Int. J. Obstet. Gynaecol. 127, 139–146 (2020).

4. Matsuo, Y. et al. Full-length 16S rRNA gene amplicon analysis of human gut microbiota using MinION™ nanopore sequencing confers species-level resolution. BMC Microbiol. 21, 35 (2021).

5. Chiang, A. D. & Dekker, J. P. From the Pipeline to the Bedside: Advances and Challenges in Clinical Metagenomics. J. Infect. Dis. 221, S331–S340 (2019).

6. Petersen, L. M., Martin, I. W., Moschetti, W. E., Kershaw, C. M. & Tsongalis, G. J. Third-Generation Sequencing in the Clinical Laboratory: Exploring the Advantages and Challenges of Nanopore Sequencing. J. Clin. Microbiol. 58, (2019).

7. Feehily, C. et al. Shotgun sequencing of the vaginal microbiome reveals both a species and functional potential signature of preterm birth. npj Biofilms Microbiomes 6, 50 (2020).

8. Holm, J. B. et al. Integrating compositional and functional content to describe vaginal microbiomes in health and disease. Microbiome 11, 259 (2023).

9. Ma, B. et al. A comprehensive non-redundant gene catalog reveals extensive within-community intraspecies diversity in the human vagina. Nat. Commun. 11, 940 (2020).

10. Hickey, R. J. & Forney, L. J. Gardnerella vaginalis does not always cause Bacterial Vaginosis. J. Infect. Dis. 210, 1682–1683 (2014).

11. Morrill, S., Gilbert, N. M. & Lewis, A. L. Gardnerella vaginalis as a Cause of Bacterial Vaginosis: Appraisal of the Evidence From in vivo Models. Front. Cell. Infect. Microbiol. 10, 168 (2020).

12. Yockey, L. J. et al. Screening and characterization of vaginal fluid donations for vaginal microbiota transplantation. Sci. Rep. 12, 17948 (2022).

13. Ahannach, S. et al. Microbial enrichment and storage for metagenomics of vaginal, skin, and saliva samples. iScience 24, 103306 (2021).

14. Baud, A. et al. Microbial diversity in the vaginal microbiota and its link to pregnancy outcomes. Sci. Rep. 13, 9061 (2023).

15. Oerlemans, E. et al. Impacts of Menstruation, Community Type, and an Oral Yeast Probiotic on the Vaginal Microbiome. mSphere 7, e00239-22 (2022).

16. Jepsen, I. E. et al. Probiotic treatment with specific lactobacilli does not improve an unfavorable vaginal microbiota prior to fertility treatment—A randomized, double-blinded, placebo-controlled trial. Front. Endocrinol. 13, 1057022 (2022).

17. Zheng, N., Guo, R., Wang, J., Zhou, W. & Ling, Z. Contribution of Lactobacillus iners to Vaginal Health and Diseases: A Systematic Review. Front. Cell. Infect. Microbiol. 11, 792787 (2021).

18. Haahr, T. et al. Vaginal Microbiota and In Vitro Fertilization Outcomes: Development of a Simple Diagnostic Tool to Predict Patients at Risk of a Poor Reproductive Outcome. J. Infect. Dis. 219, 1809–1817 (2018).

19. Hilbert, D. W. et al. Development and Validation of a Highly Accurate Quantitative Real-Time PCR Assay for Diagnosis of Bacterial Vaginosis. J. Clin. Microbiol. 54, 1017–1024 (2016).

20. Cartwright, C. P. et al. Development and Validation of a Semiquantitative, Multitarget PCR Assay for Diagnosis of Bacterial Vaginosis. J. Clin. Microbiol. 50, 2321–2329 (2012).

21. Armstrong, E. et al. Treatment Success Following Standard Antibiotic Treatment for Bacterial Vaginosis Is Not Associated With Pretreatment Genital Immune or Microbial Parameters. Open Forum Infect. Dis. 10, ofad007 (2023).

22. Jian, C., Luukkonen, P., Yki-Järvinen, H., Salonen, A. & Korpela, K. Quantitative PCR provides a simple and accessible method for quantitative microbiota profiling. PLoS ONE 15, e0227285 (2020).

23. Pacha-Herrera, D. et al. Vaginal Microbiota Evaluation and Lactobacilli Quantification by qPCR in Pregnant and Non-pregnant Women: A Pilot Study. Front. Cell. Infect. Microbiol. 10, 303 (2020).

24. Łaniewski, P. & Herbst-Kralovetz, M. M. Connecting microbiome and menopause for healthy ageing. Nat. Microbiol. 7, 354–358 (2022).

About Bio-Me

To learn more about the Bio-Me PMP™ Core Vaginal Microbiome Test, as well as our publications and ongoing projects, please contact us: **sales@bio-me.com**

The Bio-Me team holds core expertise in microbiology, molecular biology, and bioinformatics. The company has a history of collaborating with key opinion leaders in the microbiome space and leading academic institutions in Europe and the US. We serve industry and academic clients globally with our expertise in gut, vaginal, and skin microbiome profiling. In addition, we create custom assays (e.g., for proprietary strain analysis).

Bio-Me AS

Oslo Science Park
Gaustadalléen 21
N-0349 Oslo
Norway

Contact us: sales@bio-me.com
www.bio-me.com

Bio-Me
Microbiome Profiling