

Nucleic acid isolation

A high-throughput workflow for profiling vaginal microbiota

Summary

Utilizing an Applied Biosystems™ MagMAX™ high-throughput sample extraction kit and high-throughput PCR on Applied Biosystems™ TaqMan™ OpenArray™ panels allows for fast turnaround time, and simple and efficient processing. This high-throughput workflow is characterized by:

- Sample to answer in <8 hours
- Semi-automatable, single workflow incorporating DNA isolation from various vaginal microbes, including gram-positive and gram-negative bacteria, yeasts, viruses, and protozoa
- Compatibility with multiple storage and collection solutions such as ThinPrep™, SurePath™, ESwab™, and MicroTest™ M4RT tests, and more

Introduction

Research has expanded our understanding of how microbiota affect reproductive health (e.g., fertility, susceptibility to sexually transmitted infections, pregnancy) and embryonic health. Much of the groundwork for vaginal microbe identification was established using traditional techniques like complex culture preparation, specialized microscopy, and sequencing. Analyzing vaginal microbes by traditional techniques often takes days or weeks, and these techniques have sensitivity limitations that newer molecular biology methods do not [1]. To better understand the relationships between vaginal microbiota and

female reproductive health, a robust workflow for microbial nucleic acid extraction and analysis that is easier, faster, and less expensive than traditional microbiology workflows is needed.

Over the last decade, bacterial 16S ribosomal RNA (rRNA) sequencing has been adopted by researchers who study the microbiome of the female reproductive system [2]. This type of analysis is generally limited to highly specialized laboratories that can afford the equipment, laboratory specialists, and other high costs associated with 16S rRNA gene sequencing.

Profiling commensal and pathogenic vaginal microbiota, including gram-positive and gram-negative bacteria, yeasts, viruses, and protozoa, is feasible with one simple end-to-end workflow. It includes nucleic acid extraction, purification, and interrogation of 34 microbial targets (Table 1). Prokaryotic 16S rRNA and human RNase P gene (*RPPH1*) targets serve as controls. The vaginal microbiota profiling workflow is compatible with several sample collection and storage systems, including the HOLOGIC™ ThinPrep™ Pap Test, the BD SurePath™ Liquid-Based Pap Test, the Copan Diagnostics ESwab™ Specimen Collection and Transport System, and Thermo Scientific™ MicroTest™ M4RT kits. Most laboratories can process 96 samples in less than 8 hours with this workflow. Depending on the laboratory setup and availability of staff and equipment, more samples could potentially be processed in one day. The full workflow from sample to results is shown in Figure 1.



Figure 1. Full workflow for vaginal microbiota profiling. Nucleic acid can be extracted from samples in a 96-well plate and purified in less than 2 hours. Profiling results can be obtained with the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System in less than 6 hours [3].

Table 1. Pathogens and other species that can be detected using the TaqMan OpenArray Vaginal Microbiota Comprehensive Plate.

Targets and controls	Species		
Bacteria	<i>Atopobium vaginae</i>	<i>Ureaplasma urealyticum</i>	<i>Mycoplasma hominis</i>
	<i>Bacteroides fragilis</i>	<i>Lactobacillus gasseri</i>	<i>Prevotella bivia</i>
	BVAB2	<i>Lactobacillus iners</i>	Uncultured <i>Megasphaera</i> 1
	<i>Enterococcus faecalis</i>	<i>Lactobacillus jensenii</i>	Uncultured <i>Megasphaera</i> 2
	<i>Escherichia coli</i> O18	<i>Mobiluncus curtisii</i>	<i>Haemophilus ducreyi</i>
	<i>Gardnerella vaginalis</i>	<i>Mobiluncus mulieris</i>	<i>Chlamydia trachomatis</i> *
	<i>Lactobacillus crispatus</i>	<i>Streptococcus agalactiae</i> (group B)	<i>Neisseria gonorrhoeae</i> *
	<i>Staphylococcus aureus</i>	<i>Mycoplasma genitalium</i>	<i>Treponema pallidum</i> *
Yeasts (fungi)	<i>Candida albicans</i>	<i>Candida lusitanae</i>	<i>Candida dubliniensis</i>
	<i>Candida parapsilosis</i>	<i>Candida glabrata</i>	<i>Candida tropicalis</i>
	<i>Candida krusei</i>		
Viruses	HSV1	HSV2*	
Protozoa	<i>Trichomonas vaginalis</i>		
Controls	Prokaryotic 16S rRNA gene	Xeno**	
	Human RNase P gene (<i>RPPH1</i>)	<i>Bacillus atrophaeus</i> **	

* Only available on the TaqMan OpenArray Vaginal Microbiota Comprehensive Plate.

** Only available on the TaqMan OpenArray Vaginal Microbiota Comprehensive Plate v2.

Materials and methods

Vaginal swabs were collected from 241 individual donors by collection agency partners using a general ESwab™ vaginal swab collection protocol, and stored in 1 mL of collection medium. Each 1 mL sample was transferred to a single well in a Thermo Scientific™ KingFisher™ plate for 96 deep-well format. Samples in each plate were processed using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra Kit and a specific vaginal microbiota profiling protocol (MAN0015935). Extractions were performed on the Thermo Scientific™ KingFisher™ Flex Purification System using the A25597_Vaginal script. DNA extracted from each sample was then quantified via real-time PCR using the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. The Applied Biosystems™ TaqMan™ Vaginal Microbiota Amplification Control served as a positive control. We also evaluated whether the workflow would enable detection of all commensal and pathogenic microbiota included on the Applied Biosystems™ TaqMan™ OpenArray™ Vaginal Microbiota Comprehensive Plate, without requiring special treatment or handling.

Results and discussion

Pathogens and other species that were detected in the samples analyzed using the vaginal profiling workflow are summarized in Table 2. Common vaginal microbiota were detected in every sample from the cohort. Among the species detected, 71.5% were gram-positive or gram-negative bacteria. *Trichomonas vaginalis* was detected in only one of the samples. The remaining 28.4% of the organisms detected were a mixture of gram-variable bacteria, human gDNA from host cells, viruses, and yeasts. Across the 241 samples analyzed, 97% of the targets in the Applied Biosystems™ TaqMan™ OpenArray™ Vaginal Microbiota panel were detected at least once (Figure 2). Robust detection of bacteria, fungi (yeasts), viruses, and protozoa was indicated by C_t values that fell within the expected range for the QuantStudio 12K Flex Real-Time PCR System (Figure 3). *Treponema pallidum* was the only species in the panel that was not detected. *T. pallidum* is the spirochete that causes syphilis. Failure to detect *T. pallidum* could have simply been due to its absence, since the donors in this cohort were characterized as asymptomatic.

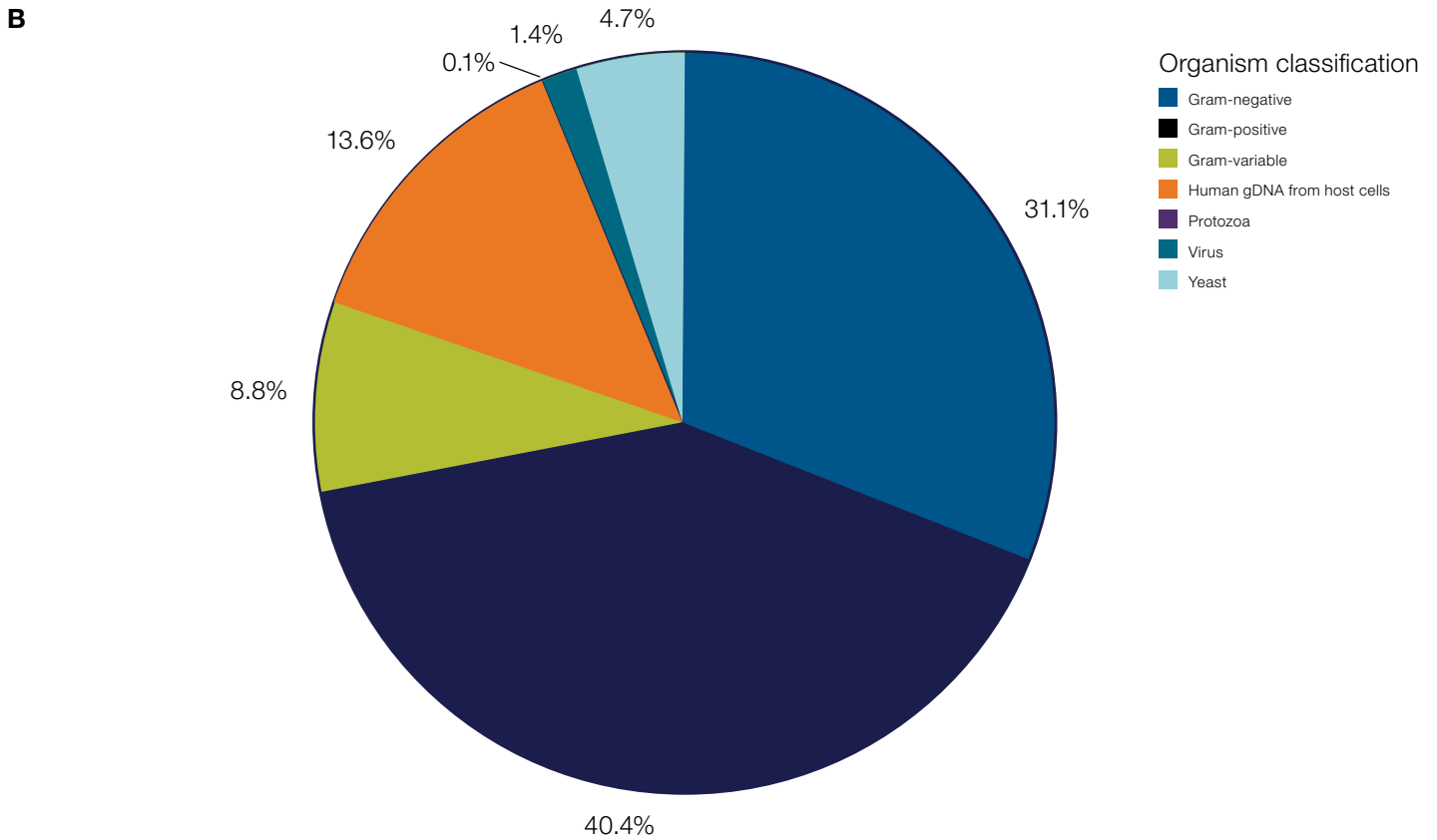
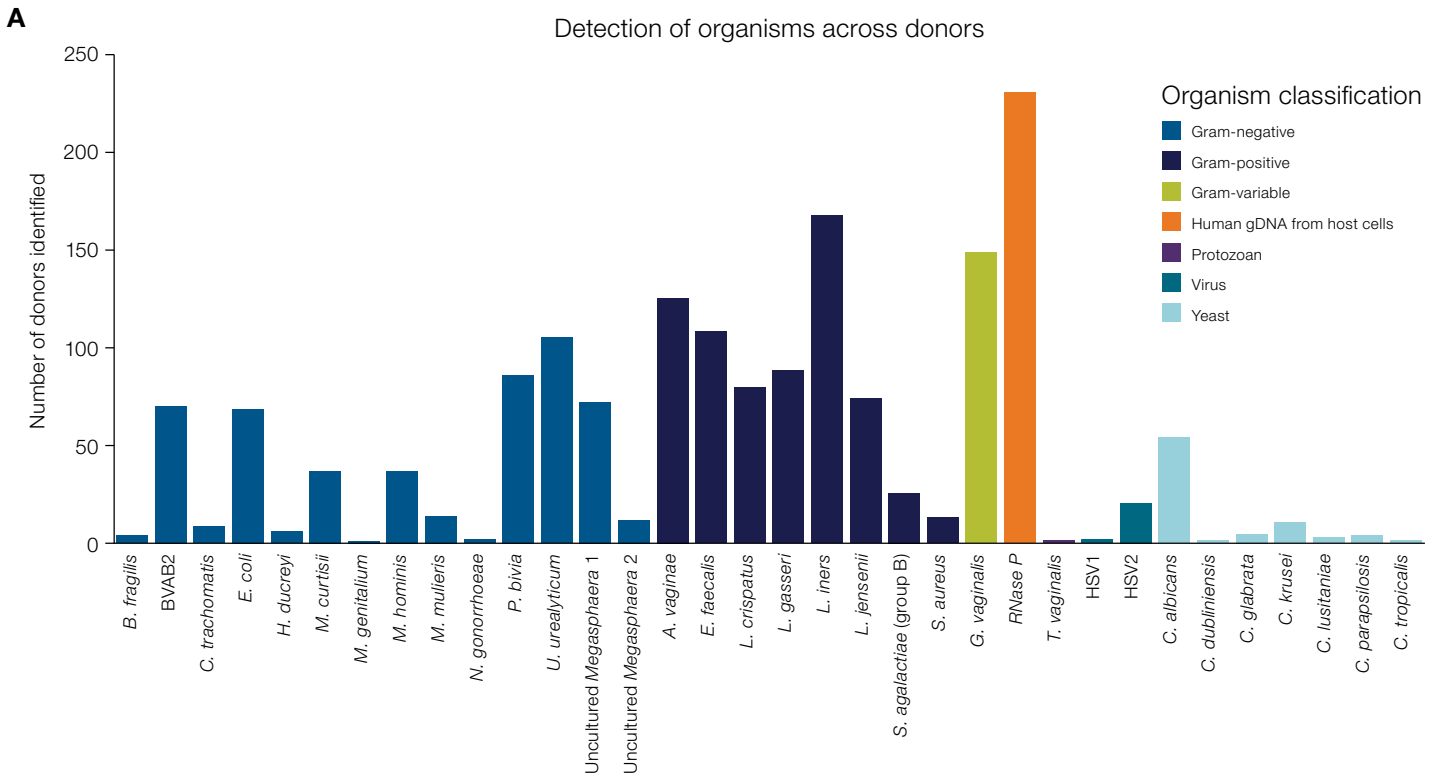


Figure 2. Detection of organisms and percentages of organisms detected out of 241 donors profiled. (A) Number of donors who tested positive for organisms and species included in the TaqMan OpenArray Vaginal Microbiota panel. **(B)** Distribution of targets detected via qPCR.

Table 2. Species detected in vaginal swabs from 241 donors using the vaginal microbiota profiling workflow.

Pathogen	Species	Number of donors identified per target	Percentage of donors identified per target
Bacteria	<i>Atopobium vaginae</i>	125	51.87%
	<i>Bacteroides fragilis</i>	4	1.66%
	BVAB2	70	29.05%
	<i>Chlamydia trachomatis</i> *	9	3.73%
	<i>Enterococcus faecalis</i>	108	44.81%
	<i>Escherichia coli</i> O18	69	28.63%
	<i>Gardnerella vaginalis</i>	148	61.41%
	<i>Haemophilus ducreyi</i>	6	2.49%
	<i>Lactobacillus crispatus</i>	79	32.78%
	<i>Lactobacillus gasseri</i>	88	36.51%
	<i>Lactobacillus iners</i>	167	69.29%
	<i>Lactobacillus jensenii</i>	74	30.71%
	<i>Mobiluncus curtisii</i>	37	15.35%
	<i>Mobiluncus mulieris</i>	14	5.81%
	<i>Mycoplasma genitalium</i>	1	0.41%
	<i>Mycoplasma hominis</i>	37	15.35%
	<i>Neisseria gonorrhoeae</i> *	2	0.83%
	<i>Prevotella bivia</i>	86	35.68%
	<i>Staphylococcus aureus</i>	13	5.39%
	<i>Streptococcus agalactiae</i> (group B)	26	10.79%
<i>Treponema pallidum</i> *	–	–	
Uncultured <i>Megasphaera</i> 1	72	29.88%	
Uncultured <i>Megasphaera</i> 2	12	4.98%	
<i>Ureaplasma urealyticum</i>	105	43.57%	
Fungi	<i>Candida albicans</i>	54	22.41%
	<i>Candida dubliniensis</i>	1	0.41%
	<i>Candida glabrata</i>	5	2.07%
	<i>Candida krusei</i>	11	4.56%
	<i>Candida lusitanae</i>	3	1.24%
	<i>Candida parapsilosis</i>	4	1.66%
	<i>Candida tropicalis</i>	1	0.41%
Viruses	HSV1	2	0.83%
	HSV2*	21	8.71%
Protozoa	<i>Trichomonas vaginalis</i>	1	0.41%
Controls	<i>Bacillus atropheus</i> **	–	–
	Human RNase P gene (<i>RPPH1</i>)	230	95.44%
	Prokaryotic 16S rRNA gene	–	–
	Xeno**	–	–

* Only available on the TaqMan OpenArray Vaginal Microbiota Comprehensive Plate.

** Only available on the TaqMan OpenArray Vaginal Microbiota Comprehensive Plate v2.

Gram-negative targets were detected in 105 of the 241 samples. Among the gram-positive and gram-variable species, lactobacilli were the most common: they were detected in 167 of the samples, and *L. iners* was detected most frequently. Other lactobacilli found in 88 of the samples included *L. jensenii*, *L. gasseri*, and *L. crispatus*. This was not surprising, as lactobacilli are naturally occurring bacteria that are part of the healthy vaginal microbiome population.

Additionally, gram-positive bacteria, including *Staphylococcus aureus*, *Streptococcus agalactiae* (group B), *Enterococcus faecalis*, and *Atopobium vaginae*, were detected in 125 of the samples profiled. The gram-variable species *Gardnerella vaginalis* was detected in 148 of the 241 samples. *G. vaginalis* is often associated with bacterial vaginosis, but it can also be found in healthy women.

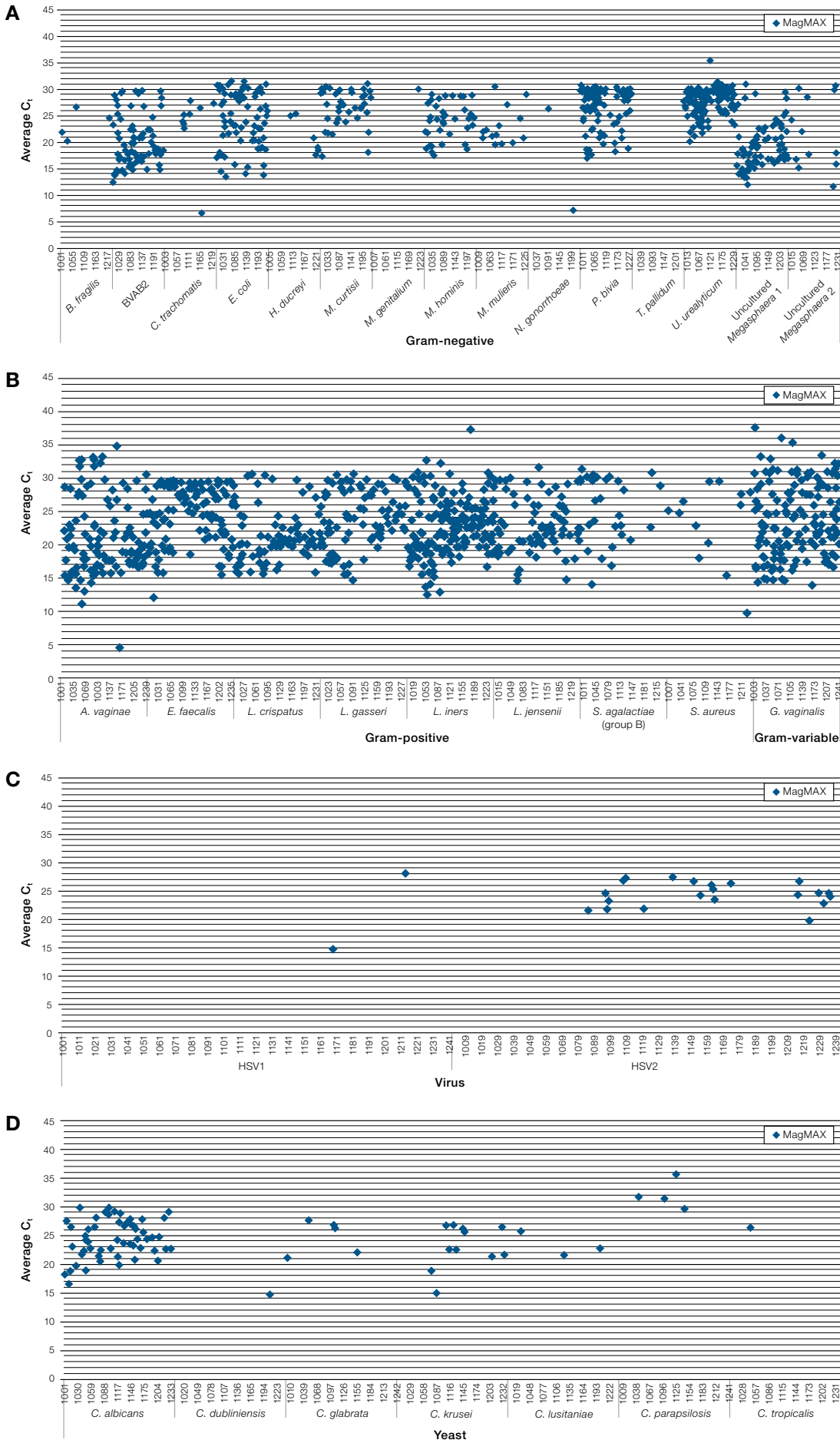


Figure 3. qPCR results for the detection of nucleic acid from vaginal microbiota in vaginal swab samples. C_t values for (A) gram-negative bacteria, (B) gram-positive bacteria, (C) viruses, and (D) yeasts.

Candida albicans was detected in each of the 54 samples that tested positive for yeasts. This and other *Candida* species occur naturally on human skin and mucous membranes, in the gut, and in the vaginal mucosa. They can cause a condition called candidiasis, commonly known as yeast infections. Protozoa and viruses were far less common. The protozoan *T. vaginalis* was detected in only one sample. HSV1 was detected in two samples, while HSV2 was detected in 21. Each of these microbes is associated with a sexually transmitted infection (STI).

Conclusions

Gram-positive and gram-negative bacteria, yeasts, viruses, and protozoa in vaginal swab samples can be efficiently detected using our vaginal microbiota profiling workflow. Up to 96 samples can be profiled in less than 8 hours using the

proposed workflow by extracting samples with the MagMAX DNA Multi-Sample Ultra Kit on the KingFisher Flex system and performing high-throughput PCR with the TaqMan OpenArray Vaginal Microbiota workflow on a TaqMan OpenArray plate and QuantStudio 12K Flex system. This method enables simple and efficient sample processing with a rapid turnaround.

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References

1. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3248621/>.
2. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7492712/>.
3. <https://www.thermofisher.com/document-connect/document-connect/html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fbrochures%2Fopenarray-vaginal-microbiota-taqman-app-note.pdf>.

Ordering information

Product	Cat. No.
Sample preparation materials and reagents	
ESwab collection kit	R723480
MagMAX DNA Multi-Sample Ultra Kit	A25597
B-PER Bacterial Protein Extraction Reagent	78243
Lysozyme Solution (50 mg/mL)	PI-90082
KingFisher Plastics for 96 deep-well format	95040450B
KingFisher Plastics for 96 standard and PCR formats	97002540B
MicroAmp Optical Adhesive Film	4360954
MicroAmp Clear Adhesive Film	4306311
Nucleic acid purification equipment	
KingFisher Flex Purification System	5400620
Real-time PCR instruments, kits, and reagents	
QuantStudio 12K Flex OpenArray Vaginal Microbiota Starter Kit	A32087
TaqMan OpenArray Vaginal Microbiota Comprehensive Plate	A39899
TaqMan Vaginal Microbiota Amplification Control	A32040
QuantStudio 12K Flex Real-Time PCR System, OpenArray block, desktop	4472380
QuantStudio 12K Flex Accufill System	4471021

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