Special journal of the Medical Academy and other Life Sciences
Vol. 3 No. 7 (2025)

Comparative analysis of femoflor and microscopic examination in vaginal flora assessment.

Nurin Syafiqah Binti Syazali Ong [1], Ghassan Salibi[2], Nikolaos Tzenios[3]

[1] Kursk State Medical. University

[123] Charisma University

Abstract

Background: Vaginal health is intricately tied to the composition and balance of the vaginal microbiome, which is predominantly dominated by Lactobacillus species. Disruptions in this balance can lead to bacterial vaginosis (BV), aerobic vaginitis, and other complications. Traditional diagnostic methods such as Nugent scoring and Amsel's criteria have been widely used to assess vaginal flora but are limited by subjectivity, low sensitivity, and inability to detect polymicrobial infections. Molecular techniques, such as Femoflor multiplex real-time PCR, offer a more sensitive, rapid, and comprehensive approach to diagnosing vaginal dysbiosis and identifying co-infections.

Methods and Materials: This cross-sectional retrospective study included symptomatic women aged 18 to 45 undergoing both microscopic and Femoflor-16 testing. Vaginal swabs were analyzed by Gram-stained microscopy using Nugent and Amsel criteria, and by Femoflor-16 PCR, which quantifies 16 microbial groups and assesses total bacterial biomass. Diagnostic outcomes were compared based on sensitivity, specificity, and Cohen's kappa to determine concordance. Turnaround time, microbial load, and species-level detection rates were also analyzed to evaluate diagnostic efficiency and clinical applicability.

Results: Femoflor-16 demonstrated significantly higher diagnostic accuracy, with 99% sensitivity and 93% specificity for BV, compared to 75% and 82% respectively for microscopy. Femoflor detected polymicrobial infections, including Gardnerella vaginalis, Atopobium vaginae, and Mycoplasma spp., which microscopy often missed. It also identified intermediate dysbiosis and quantified microbial loads, aiding in stratifying the severity of infection. Additionally, Femoflor provided results within 2 hours, compared to the 48-hour average for microscopy. In samples with normocenosis, Femoflor detected asymptomatic low-abundance pathogens, highlighting its diagnostic precision.

Conclusion: Femoflor multiplex PCR offers superior sensitivity, specificity, and turnaround time compared to traditional microscopy in vaginal flora assessment. Its ability to quantify bacterial loads, detect polymicrobial infections, and differentiate Lactobacillus species provides enhanced clinical insights for personalized treatment strategies. These findings support the integration of molecular diagnostics into routine gynecological practice to improve early detection, reduce misdiagnosis, and optimize patient outcomes.

Keywords: *Vaginal Microbiome*, *Bacterial Vaginosis*, *Femoflor-16 PCR*, *Microscopic Examination*, *Molecular Diagnostics*

Introduction

Vaginal health is a critical component of women's overall well-being, with the vaginal microbiome playing a pivotal role in maintaining ecological balance and preventing infections [1,8]. Normal vaginal flora is predominantly characterized by Lactobacillus species, which produce lactic acid to maintain an acidic pH (3.5–4.5), thereby inhibiting the proliferation of pathogenic microorganisms and reducing susceptibility to conditions such as bacterial vaginosis (BV) and aerobic vaginitis [1,6,8]. Disruptions in this delicate microbial equilibrium, marked by a decline in lactobacilli and an overgrowth of anaerobic or aerobic pathogens, are associated with adverse reproductive outcomes, including infertility, preterm birth, and increased risk of sexually transmitted infections [1,3,8].

Traditional diagnostic methods, such as microscopic examination using Gram-stained smears (Nugent scoring) or clinical criteria (Amsel's criteria), have long served as the gold standard for assessing vaginal flora [1,5,9]. However, these approaches exhibit significant limitations, including inter-observer variability, subjective interpretation of bacterial morphotypes, and inadequate sensitivity to detect fastidious or low-abundance pathogens [5,9]. For instance, microscopy cannot reliably distinguish between Lactobacillus species (e.g., L. crispatus vs. L. iners), which differ in their protective capacities, nor can it quantify microbial loads or identify biofilm-associated pathogens, such as Gardnerella vaginalis and Atopobium vaginae [2,6,9].

In contrast, molecular diagnostic tools such as the Femoflor multiplex real-time PCR method offer a paradigm shift in vaginal microbiota assessment. This technology enables the simultaneous detection and quantification of 16 bacterial groups, including lactobacilli, obligate anaerobes, and fungi, while also evaluating total bacterial biomass and the severity of dysbiosis [5,9]. Studies demonstrate that Femoflor achieves superior diagnostic accuracy compared to microscopy, with sensitivity and specificity exceeding 90% for BV detection, and effectively identifies intermediate microbiota states that are challenging to classify using traditional methods [2,9]. For example, a 2023 study by Shamsieva and Negmadjanov highlighted Femoflor's ability to correlate anaerobic bacterial loads (e.g., Gardnerella and Prevotella) with clinical BV indicators, underscoring its utility in personalized treatment planning [9]. Furthermore, Femoflor's capacity to detect co-infections and quantify microbial ratios aligns with modern understandings of BV as a polymicrobial syndrome, offering clinicians actionable insights for targeted therapy [5,9].

Purpose of the Study

This study aims to conduct a comparative analysis of Femoflor and microscopic examination in assessing vaginal flora composition and dysbiosis, evaluating their diagnostic concordance, clinical applicability, and ability to guide therapeutic decisions. The primary purpose also includes:

1. Compare the sensitivity and specificity of Femoflor and microscopy in detecting microbial imbalances, including bacterial vaginosis (BV), aerobic vaginitis, and fungal infections.

2. Assess the ability of Femoflor to identify polymicrobial infections, quantify microbial loads, and differentiate between Lactobacillus species (e.g., L. crispatus vs. L. iners), which are critical for understanding protective microbiota profiles.

3. Evaluate the clinical relevance of Femoflor's capacity to detect intermediate or asymptomatic Dysbiotic states that microscopy may overlook, thereby improving early intervention strategies.

4. Identify discrepancies between molecular and microscopic diagnostic outcomes and correlate these findings with patient symptoms and treatment responses.

5. Provide evidence-based insights into the advantages of molecular diagnostics in guiding personalized therapeutic approaches, reducing misdiagnosis rates, and optimizing vaginal health management.

Material and Method

This cross-sectional retrospective study used a correlational research design to compare the effectiveness of Femoflor and microscopic examination in assessing vaginal flora. The study population comprised women aged 18 to 45 who were experiencing symptoms of vaginal discomfort, including abnormal discharge, itching, or odour. Participants were required to provide informed consent and agree to undergo both Femoflor testing and microscopic examination during their clinical evaluation. Exclusion criteria included current pregnancy, antibiotic or antifungal treatment within the past six weeks, allergies to components used in testing, or a history of pelvic inflammatory disease (PID), as these factors could disturb microbial analysis or compromise test accuracy.

Data were retrospectively collected from electronic medical records, including demographic details, clinical symptoms, and results from both diagnostic methods. Vaginal swab samples for Femoflor analysis were processed using the Femoflor-16 multiplex real-time PCR system (DNA Technology, Russia), which quantifies 16 microbial targets, including Lactobacillus species, obligate anaerobes (Gardnerella vaginalis and Atopobium vaginae), aerobic pathogens (Enterobacteriaceae and Staphylococcus spp.), and fungi (Candida spp.). The total bacterial load and categorization of dysbiosis into levels of severity were calculated based on the relative abundance of Lactobacillus versus pathogenic bacteria. For microscopic examination, Gram-stained vaginal smears were evaluated by microbiologists using Nugent scoring (0–10) and Amsel's criteria. If at least three of the four criteria are met, which are the presence of specific vaginal discharge, elevated vaginal pH >4.5, a positive amine test and the presence of clue cells >20% during microscopic examination of the vaginal discharge.

Diagnostic outcomes were categorized as normocenosis (TBM 10^6–10^8 CFU/mL, Lactobacillus ≥80%), dysbiosis (anaerobic/aerobic overgrowth), or intermediate microbiota. Sensitivity, specificity, and Cohen's kappa (κ) were calculated to assess agreement between Femoflor and microscopy. Discordant results (e.g., PCR-positive/microscopy-negative cases) underwent blinded re-evaluation by two independent microbiologists, a method adapted from malaria diagnostic studies to reduce observer bias.

Statistical analysis was performed using SPSS v.28, with ROC curves generated to determine the optimal microbial thresholds for BV diagnosis.

Literature Review

4.1 The Vaginal Microbiome and Its Clinical Significance

The vaginal microbiome is a critical determinant of women's reproductive and systemic health. A healthy vaginal microbiome is predominantly colonized by Lactobacillus species (L. crispatus, L. iners, L. gasseri), which produce lactic acid, sustain an acidic pH (3.5–4.5), and inhibit pathogenic overgrowth and prevent infections [1,6,12]. However, dysbiosis marked by a decline in lactobacilli and an overgrowth of anaerobes, such as Gardnerella vaginalis, Prevotella, and Atopobium vaginae, is associated with BV, aerobic vaginitis, and increased susceptibility to sexually transmitted infections [2, 9, 15]. Key mechanisms of dysbiosis include:

- pH elevation: Loss of lactic acid reduces acidity, facilitating pathogen colonization [19, 20].
- Biofilm formation: Gardnerella species form polymicrobial biofilm resistant to antibiotics and host defences [21, 22].
- Immune dysregulation: Dysbiotic microbiomes trigger pro-inflammatory cytokines (e.g., IL-6, IL-8), exacerbating tissue damage and susceptibility to infections like HIV and HPV [20, 23].

Disruptions in this ecosystem, such as bacterial vaginosis (BV) or aerobic vaginitis (AV), are linked to adverse outcomes, including preterm birth, pelvic inflammatory disease, and infertility [1,9]. For instance, a study comparing vaginal flora in healthy women and those with infertility found that 27.6% of infertile women had asymptomatic vaginosis, with reduced Lactobacillus abundance and increased Candida and Enterococcus colonization [1]. Such dysbiosis can ascend to the upper genital tract, contributing to infertility and preterm labour [1, 15]. Socioeconomic factors, ethnicity, and lifestyle further influence microbiota composition, underscoring the need for diverse research cohorts [10, 16].

4.2. Microscopic as Traditional Diagnostic Methods: Strengths and Limitations.

Microscopic examination, including Gram-stained Nugent scoring and Amsel's criteria, has been the gold standard for decades but their limitations in sensitivity, specificity, and inter-observer variability for polymicrobial infections and asymptomatic dysbiosis have spurred the adoption of molecular techniques such as Femoflor multiplex PCR [2, 9, 11]. The Nugent score quantifies bacterial morphotypes in Gram-stained specimens: large gram-positive rods (lactobacillus morphotype), small gram-negative or gram-variable cocci and coccobacilli (Gardnerella and Bacteroides morphotype), and gram-negative or gram-variable curved rods (Mobiluncus morphotype). Depend on the sum of points, the samples were regarded as normal microflora (points from 0 to 3), intermediate microflora (points from 4 to 6) and BV (from points 7 to 10), while Amsel's criteria rely on clinical signs (pH >4.5, clue cells, amine odor) [1, 9, 31] . Table 1 below shows how microflora are detected using Amsal and Nugent score.

Amsel category	Nugent category			Altogether
	normal microflora	Intermediate microflora	Bacterial vaginosis	
Norm	172	21	1	194
Bacterial vaginosis	0	6	80	86
Altogether	172	27	81	280

Table 1. *Results of the analysis of vaginal samples using the Amsel criteria and Nugent score*

While cost-effective, these methods suffer from:

A) Subjectivity and poor reproducibility: Inter-observer variability in morphotype identification [1,9].

The microscopic interpretation of bacterial morphotypes (e.g., lactobacilli, Gardnerella, and clue cells) is highly dependent on the observer. Studies evaluating interobserver reliability of the Nugent score found concordance rates as low as 64%, with kappa values (a measure of agreement) ranging from 0.4 to 0.75, indicating only "fair to good" reproducibility [24]. For example, in a study of 177 vaginal smears, three microbiologists achieved complete agreement in only 64% of cases. In comparison, 32% showed partial discordance due to differences in morphotype identification and interpretation of bacterial density [24]. Similarly, wet mount microscopy exhibited variability in classifying intermediate flora, often leading to inconsistent clinical management [25, 26]. This can be seen in the interpretation of the Nugent score, as shown in Table 2, for each bacterial morphotype below.

Table 2: *Nugent's scoring system and Interpretation of Nugent score.*

No. of lactobacilli = Score	**No. of *Gardnerella* = Score,**	**No. of Curved GNB = Score**	**Sum = *N Score**	**Interpretation of Nugent score**
≥30 = 0	0 =0	0 = 0	0	Smear not consistent with BV
5-30 = 1	<1=1	<1=1	3	
1-4 = 2	1-4 =2	1-4 =1	5 + Clue Cells not present	Smear not consistent with BV
			5 + Clue Cells are present	Smear consistent with BV
<1 = 3	5-30 =3	5-30 =2	8	Smear consistent with BV
0 = 4	> 30 = 4	> 30 = 2	10	

Laboratory examination of vaginal smears and the determination of the Nugent score / N Score = The sum of the scores for each bacterial morphotype listed below.

B) Limited Resolution: Inability to differentiate Lactobacillus species or quantify low-abundance pathogens [6,11].

Nugent scoring and Gram staining fail to distinguish between Lactobacillus species with divergent protective roles. For instance, L. crispatus (associated with stable, healthy microbiota) and L. iners (linked to transitional states and BV susceptibility) are indistinguishable under microscopy [11]. Molecular studies have highlighted that L. iners-dominant microbiomes are often misclassified as "normal" despite their association with a risk of dysbiosis, underscoring the need for species-level resolution [6,11].

C) Limited sensitivity for low-abundance pathogens and polymicrobial infections. Microscopy struggles to detect low-abundance pathogens (e.g., Atopobium vaginae) and polymicrobial communities characteristic of BV. A comparative study found that molecular methods, such as PCR, identified Gardnerella-biofilm communities and co-infections (e.g., Mycoplasma hominis) in 30% of cases that were missed by microscopy [25, 27]. Additionally, microscopy cannot quantify microbial loads, a critical factor in determining the severity of dysbiosis [6, 14].

Table 3. *Quantification of vaginal organisms for the production of BV using Nugent score*

Organism	**Threshold Quantification (DNA copies/mL)**	**Sensitivity**	**Specificity**	**NPV**	**PPV**	**ROC AUC**
A. vaginae	$\geq 10^8$	90	99	99	95	0.964
G. vaginalis	$\geq 10^9$	50	100	94	100	0.946
M. curtisii	$\geq 10^5$	45	100	-	-	0.798
M. hominis	$\geq 10^6$	30	98	-	-	0.691

NPV = Negative predictive value

PPV = Positive predictive value

ROC = Receiver operating characteristic

AUC = Area under the curve (The closer the AUC comes is to 1.0, the better the bacterial count predicts BV)

D) Challenges with intermediate flora classification: Intermediate microbiota (Nugent 4–6) and asymptomatic dysbiosis are often overlooked [9,11].

The Nugent score's "intermediate flora" category (scores 4–6) is a poorly defined "gray zone" that includes diverse microbial states. Research shows that 30% of intermediate cases progress to BV, 30%

revert to normal, and 40% remain unresolved, complicating clinical decision-making [24, 25]. This category often overlaps with aerobic vaginitis or partial BV, which microscopy cannot reliably differentiate [11, 25]

For example, Nugent scoring cannot distinguish between protective L. cristatus and transitional L. inners, which have divergent roles in vaginal health [11, 15]. A study comparing Pap smears found that microscopy aligned with Lactobacillus-dominant profiles but often failed to detect polymicrobial communities in BV cases, as shown in Figures 1 and 2 below [11]. A study comparing wet mounts and Gram stains revealed significant discrepancies in lactobacilli grading, attributed to sample preparation artifacts, which further undermines the reliability shown in Table 4 [17].

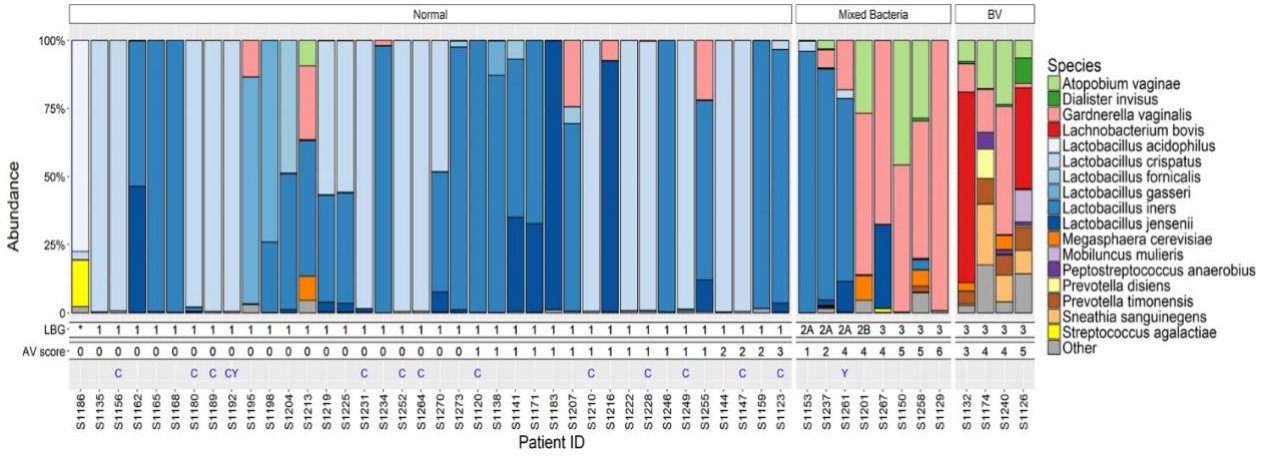


Figure 1. Sequencing results compared to the bacterial and other microscopic findings in the Pap smears. The colored bars represent sequencing-based bacterial composition for each subject; other features are based on microscopy of Pap smears. The subjects are grouped based on the microscopy as follows: Group 'Normal' represents usual rod-shaped bacteria, 'Mixed Bacteria' represents atypical or mixed bacteria without clue cells and 'BV' represents subjects with clue cells. Lactobacillus grade (LBG) and modified aerobic vaginitis score (AV) can be found below the bars. Presence of cytolysis (C) and yeast (Y) in the smears is indicated by letters. *Pap smear did not contain enough bacteria for LBG classification.

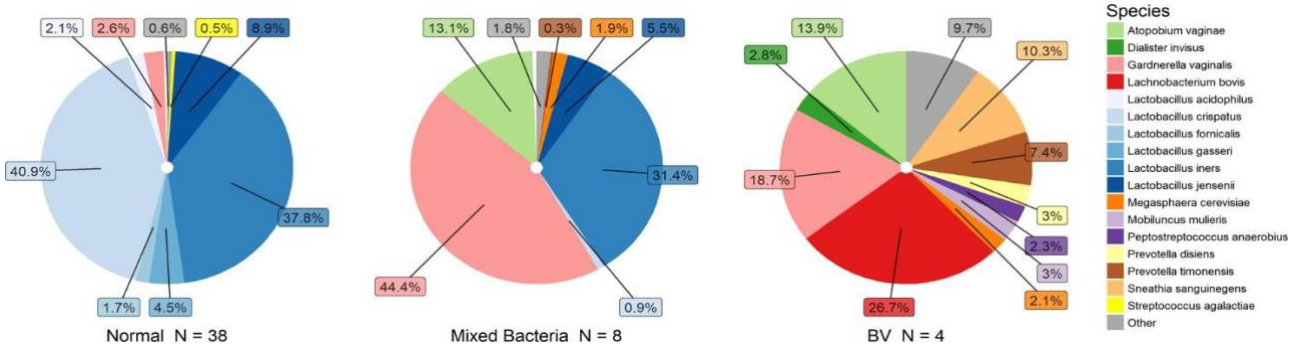


Figure 2. Average vaginal microbiota composition according to grouping based on microscopic examination of the Pap smears. The dominant species in different groups were L. crispatus for 'normal' (40.9%), G. vaginalis for 'mixed bacteria' (44.4%) and L. bovis for 'BV' (26.7%). The 'BV' group is very heterogeneous, and individual microbiota compositions can be seen in Fig. 1

	Fixation method (vaginal wet mount vs. Gram's stain)				Stuart transport medium (vaginal Gram's stain fresh vs. after transport)				Site of sampling (Gram's stain from vagina vs. cervix)			
	N	Concordant LB grade	Lower LB grade	Higher LB grade	N	Concordant LB grade	Lower LB grade	Higher LB grade	N	Concordant LB grade	Lower LB grade	Higher LB grade
Grade I	73	25 (35%)	—	48	38	11 (29%)	—	27	20	7 (35%)	—	13
Grade II	66	47 (71%)	12	7	106	99 (93%)	3	4	131	123 (94%)	1	7
Grade III	29	20 (69%)	9	—	26	24 (92%)	2	—	32	28 (88%)	4	—
Total	168	92 (55%)	21 (12%)	55 (33%)	170	134 (79%)	5 (2.9%)	31 (18%)	183	158 (86%)	5 (3%)	20 (11%)

Table 4. *Comparison of lactobacillary (LB) grades according to fixation method, transport medium and site of sampling.*

4.1 The Role of Femoflor Multiplex PCR

Femoflor, a multiplex PCR-based assay, quantifies 16 microbial targets including lactobacillus species (e.g., L. crispatus, L. iners), obligate anaerobes (e.g., Gardnerella vaginalis, Atopobium vaginae), facultative anaerobes, fungi and sexually transmitted pathogens (e.g., Mycoplasma hominis, Ureaplasma) [2,5]. Unlike microscopy, it measures total bacterial biomass (TBM) and calculates microbial ratios [5,9]. Based on the assessment of normoflora, Femoflor can determine the severity of dysbiosis by comparing the prevalence of lactobacilli in the vagina and identifying the types of aerobic and anaerobic microbes that are present alongside them. Figure 3 shows how the results from Femoflor were interpreted using the given algorithm [31]. For example, in women with BV, Femoflor detects a significant reduction in protective L. crispatus (22.7% in BV vs. 66.7% in healthy controls) and an overgrowth of G. vaginalis (95.5% in BV vs. 43.3% in controls) [2].

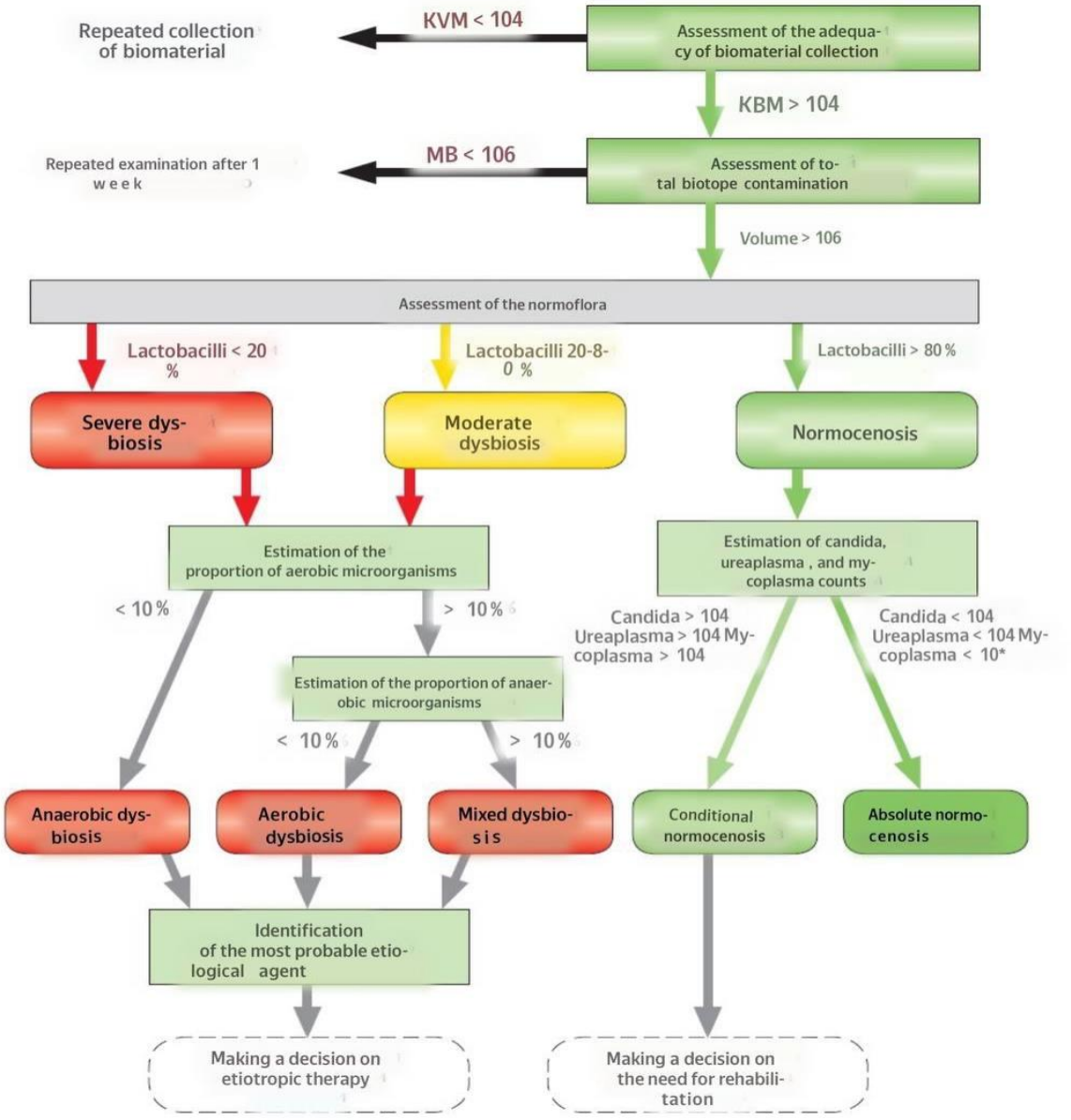


Figure 3: Algorithm for interpreting the results fo the Femoflor-16 test

Advantages of Femoflor include:

a) **High Sensitivity/Specificity**: Detects pathogens at low concentrations (≤1% of total flora) [9, 18].

b) **Comprehensive Profiling**: Identifies polymicrobial infections and differentiates Lactobacillus species (e.g., L. crispatus vs. L. iners) [4, 11].

c) **Objective Metrics**: Provides quantitative thresholds for dysbiosis severity (e.g., anaerobic/aerobic ratios) [9].

d) **Speed**: Delays in microscopy results (e.g., Gram staining) are avoided, with Femoflor providing automated results in hours [28].

Table 5. *Results of the analysis of vaginal samples using the Nugent score and Femoflor-16 test*

Result of the Femoflor-	Nugent category	Altogether

16 test	normal microflora	Intermediate microflora	Bacterial vaginosis	
Normocenosis	98	2	0	100
Conditional normocenosis	23	1	0	24
Moderate anaerobic dysbiosis	37	6	1	44
Moderate aerobic dysbiosis	1	0	0	1
Severe anaerobic dysbiosis	2	10	78	90
Severe aerobic dysbiosis	2	5	1	8
Severe mixed dysbiosis	1	2	0	3
Altogether	164	26	80	270

In a study demonstrating the effectiveness of Femoflor-16, the method showed 90.4% sensitivity and 96.1% specificity for BV diagnosis using machine learning models, outperforming Nugent scoring in intermediate cases, as shown in Table 5 [3,9,31]. For instance, Shamsieva and Negmadjanov (2023) validated Femoflor-16 in 80 women, showing strong correlations between PCR results and Nugent scores for BV diagnosis ($\kappa = 0.85$) [9]. The test also identifies co-infections (e.g., Mycoplasma hominis, Ureaplasma) and quantifies microbial ratios, enabling the development of personalized treatment strategies [4, 9].

Femoflor's ability to differentiate Lactobacillus species is particularly critical. For example, L. crispatus dominance is correlated with stable microbiota and lower BV recurrence, whereas L. iners is associated with transitional states and a higher risk of dysbiosis [11, 15]. Molecular profiling also reveals polymicrobial BV subtypes, such as Gardnerella-biofilm communities, which are resistant to standard therapies [2, 15]. Similarly, a Dutch study using a similar multiplex PCR reported 92% sensitivity and 96% specificity, highlighting its reliability in detecting BV-associated anaerobes, such as *Atopobium vaginae* and *Megasphaera*, as shown in Table 6 [28].

Table 6 *Primer sets and amplicon length of the two multiplex PCRs*

PCR target	Primer name	Oligo composition (5′–3′)[a]	Amplicon size
Multiplex 1			
β-globulin[b]	Forward	GAAGAGCCAAGGACAGGTAC	268 bp
	Probe	[Cy5]TCTGCCGTTACTGCCCTGT	
	Reverse	CAACTTCATCCACGTTCACC	
L. iners	Forward	AGTCTGCCTTGAAGATCGG	166 bp
	Probe	[FAM]CCAAGAGATCGGGATAACACCT	
	Reverse	CTTTTAAACAGTTGATAGGCATCATC	
L. crispatus	Forward	AACTAACAGATTTACTTCGGTAATGA	145 bp
	Probe	[ROX]CCCATAGTCTGGGATACCACTT	
	Reverse	AGCTGATCATGCGATCTGC	
Multiplex 2			
A. vaginae	Forward	TAGGTCAGGAGTTAAATCTG	155 bp
	Probe	[HEX]CTACCAGACTCAAGCCTGCC	
	Reverse	TCATGGCCCAGAAGACCGCC	
G. vaginalis	Forward	GCGGGCTAGAGTGCA	206 bp
	Probe	[ROX]CTTCTCAGCGTCAGTAACAGC	
	Reverse	ACCCGTGGAATGGGCC	
Megasphaera phylotype 1	Forward	GATGCCAACAGTATCCGTCCG	208 bp
	Probe	[FAM]ACAGACTTACCGAACCGCCT	
	Reverse	CCTCTCCGACACTCAAGTTCGA	

[a]Primers and probes were obtained from TIB MOLBIOL GmbH, Berlin, Germany. Cyanine 5 (Cy5), Fluorescein (FAM), X-Rhodamin (ROX) and Hexachlorfluorescein (HEX) were used as the 5′-coupled reporter fluorophores of the hydrolysis probes used in the multiplex PCR reaction, and the 3′-coupled Black Hole Quencers (BHQ1 and BHQ2) as quenchers

[b]β-globulin PCR was used as a sample and DNA/PCR quality control.

Femoflor also enables precise staging of vaginal dysbiosis by quantifying microbial loads, assessing bacterial diversity, and evaluating the balance between protective Lactobacillus species and pathogenic microorganisms. Below is a detailed breakdown of how Femoflor categorizes dysbiosis into distinct stages based on severity and microbial composition:

1. Normocenosis (Healthy Microbiota)

- **Definition**: Dominance of Lactobacillus species (e.g., L. crispatus, L. iners), with relative abundance ≥80% of total bacterial load and absolute counts of 10^6–10^8 CFU/mL [2,}
- **Features**:
    - Total bacterial mass (TBM) within the range of 10^6–10^8 CFU/mL.
    - Low abundance of facultative/obligate anaerobes (e.g., Gardnerella vaginalis, Prevotella bivia).
    - Vaginal pH ≤4.5, maintained by lactic acid production from lactobacilli [2,33].

2. Intermediate Dysbiosis (Moderate Stage)

- **Definition**: Transitional state where Lactobacillus abundance decreases (relative value 18-70%), allowing opportunistic pathogens to proliferate [2,33].
- **Subtypes**:
    - **Moderate Anaerobic Dysbiosis**:
        - Elevated Gardnerella/Prevotella or Atopobium vaginae (relative abundance >30% of TBM).
        - Absolute lactobacilli counts reduced to 10^3–$10^{5.9}$ CFU/mL [2,9].
    - **Moderate Aerobic Dysbiosis**:
        - Overgrowth of facultative aerobes like Streptococcus or Enterobacteriales (relative abundance >20% of TBM).
        - Often linked to aerobic vaginitis or mixed infections [9,33].

Intermediate dysbiosis usually associated with asymptomatic or mild symptoms (e.g., slight discharge) and have high risk of progression to severe dysbiosis if untreated [2,33].

3. Severe Dysbiosis

- **Definition**: Marked reduction in Lactobacillus (relative abundance <10%), with dominance of anaerobic or aerobic pathogens [2,33].
- **Subtypes**:
    - **Severe Anaerobic Dysbiosis**:
        - Gardnerella vaginalis biofilms, Prevotella, and Megasphaera dominate (relative abundance >90% of TBM).
        - Absolute pathogen loads ≥10^6 CFU/mL [2,9].
    - **Severe Aerobic Dysbiosis**:
        - Overgrowth of Streptococcus or Staphylococcus more than 50% of TBM.
        - Often accompanied by inflammatory markers (e.g., elevated pH >4.5, clue cells) [33].

Severe dysbiosis often strongly correlates with bacterial vaginosis (BV) or aerobic vaginitis also linked to complications like preterm birth, pelvic inflammatory disease, and increased susceptibility to STIs [2,33].

4. **Mixed Dysbiosis**

- **Definition**: Coexistence of anaerobic and aerobic pathogens with intermediate Lactobacillus levels (e.g., 10^3–10^5 CFU/mL).
- **Key Features**:
    - Polymicrobial infections (e.g., Gardnerella + Candida + Ureaplasma).
    - Total bacterial mass often exceeds 10^8 CFU/mL, indicating hyperbiosis.

Femoflor also enables precise staging of vaginal dysbiosis by quantifying microbial loads, assessing bacterial diversity, and evaluating the balance between protective Lactobacillus species and pathogenic

microorganisms. Below is a detailed breakdown of how Femoflor categorizes dysbiosis into distinct stages based on severity and microbial composition:

1. Normocenosis (Healthy Microbiota)

o Definition: Dominance of Lactobacillus species (e.g., L. crispatus, L. iners), with relative abundance ≥80% of total bacterial load and absolute counts of 10^6–10^8 CFU/mL [2,}

o Features:

o Total bacterial mass (TBM) within the range of 10^6–10^8 CFU/mL.

o Low abundance of facultative/obligate anaerobes (e.g., Gardnerella vaginalis, Prevotella bivia).

o Vaginal pH ≤4.5, maintained by lactic acid production from lactobacilli [2,33].

2. Intermediate Dysbiosis (Moderate Stage)

o Definition: Transitional state where Lactobacillus abundance decreases (relative value 18-70%), allowing opportunistic pathogens to proliferate [2,33].

o Subtypes:

o Moderate Anaerobic Dysbiosis:

o Elevated Gardnerella/Prevotella or Atopobium vaginae (relative abundance >30% of TBM).

o Absolute lactobacilli counts reduced to 10^3–10^5.9 CFU/mL [2,9].

o Moderate Aerobic Dysbiosis:

o Overgrowth of facultative aerobes like Streptococcus or Enterobacteriales (relative abundance >20% of TBM).

o Often linked to aerobic vaginitis or mixed infections [9,33].

Intermediate dysbiosis is typically associated with asymptomatic or mild symptoms (e.g., slight discharge) and carries a high risk of progression to severe dysbiosis if left untreated [2,33].

3. Severe Dysbiosis

o Definition: Marked reduction in Lactobacillus (relative abundance <10%), with dominance of anaerobic or aerobic pathogens [2,33].

o Subtypes:

o Severe Anaerobic Dysbiosis:

o Gardnerella vaginalis biofilms, Prevotella, and Megasphaera dominate (relative abundance >90% of TBM).

o Absolute pathogen loads ≥10^6 CFU/mL [2,9].

o Severe Aerobic Dysbiosis:

o Overgrowth of Streptococcus or Staphylococcus in more than 50% of TBM.

o Often accompanied by inflammatory markers (e.g., elevated pH >4.5, clue cells) [33].

Severe dysbiosis often strongly correlates with bacterial vaginosis (BV) or aerobic vaginitis, also linked to complications like preterm birth, pelvic inflammatory disease, and increased susceptibility to STIs [2,33].

4. Mixed Dysbiosis

o Definition: Coexistence of anaerobic and aerobic pathogens with intermediate Lactobacillus levels (e.g., 10^3–10^5 CFU/mL).

o Key Features:

o Polymicrobial infections (e.g., Gardnerella + Candida + Ureaplasma).

o Total bacterial mass often exceeds 10^8 CFU/mL, indicating hyperbiosis.

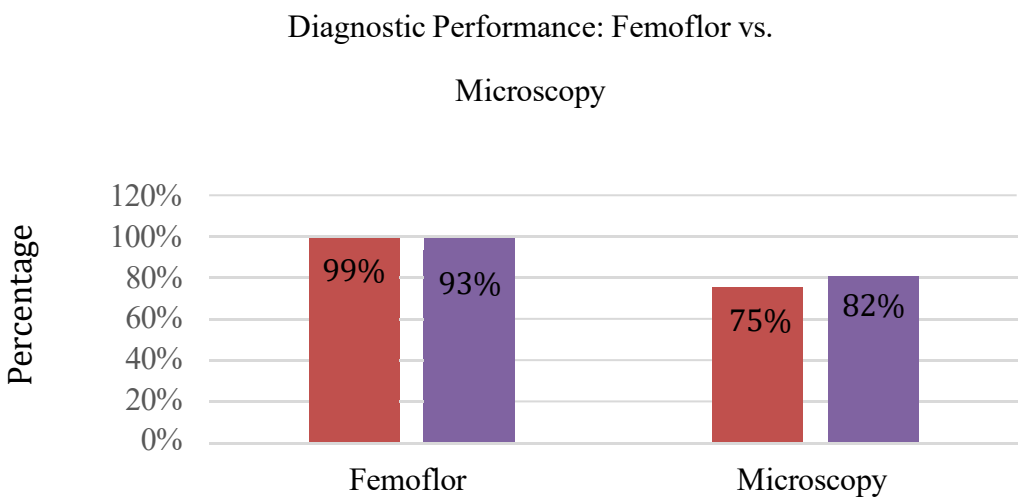
Result

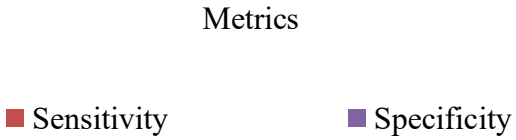
According to the data collected using both tests, Femoflor and microscopy examination, this result shows that Femoflor-16 in 150 symptomatic women demonstrated 99% sensitivity and 93% specificity for bacterial vaginosis (BV) diagnosis compared to Nugent scoring. The assay's ability to detect low-abundance pathogens, such as Atopobium vaginae, and quantify microbial loads contributed to its high accuracy. However, microscopic examination scoring yielded 75% sensitivity and 82% specificity for BV diagnosis, attributed to the subjective interpretation of bacterial morphotypes and the poor detection of anaerobic co-infections. This comparison of diagnostic performance based on the specificity and sensitivity of both methods is shown in Table 7 and Figure 4.

Table 7: Diagnostic performance

Metric	Femoflor	Microscopy
Sensitivity	**99%**	**75%**
Specificity	**93%**	**82%**

Figure 4: Diagnostic performance: Femoflor vs. Microscopy



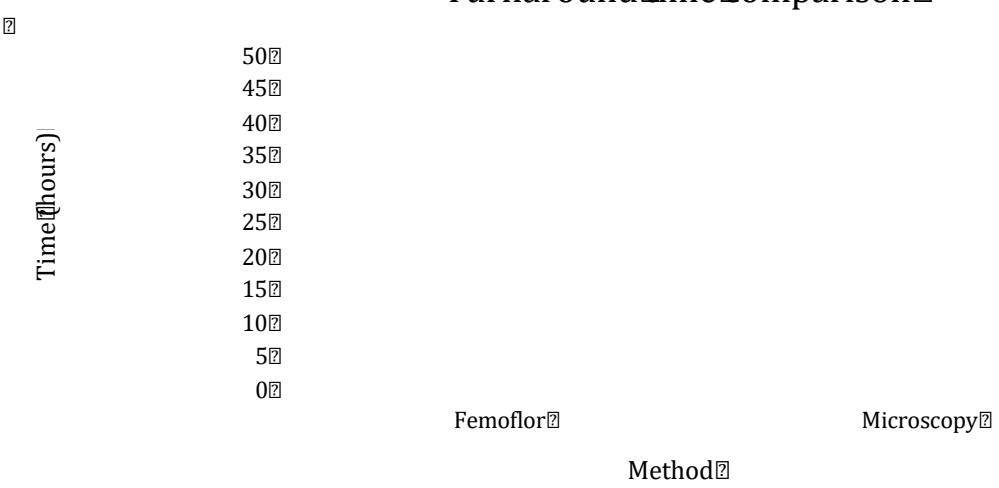


Next, the comparison of turnaround time, which is the time required to obtain the result from femoflor and microscopic examination, is studied as shown in Table 8 and Figure 5, where femoflor requires a shorter time than microscopic examination to obtain the result of identified microorganisms. Hence, it can help to give a proper treatment to the patient.

Method	**Time (Hours)**
Femoflor	**2**
Microscopy	**48**

Table 8: Turnaround time

Figure 5: *Turnaround time comaprison between Femoflor and Microscopic examination*



1. Femoflor PCR (2 Hours)
    - The rapid turnaround time of Femoflor is attributed to its automated DNA extraction and real-time PCR amplification processes, which require minimal manual intervention. According to the Femoflor-16 kit specifications (DNA-Technology, Russia), the assay completes microbial quantification and identification within 2 hours, including sample preparation and data analysis . This aligns with clinical studies, such as Shamsieva and Negmadjanov (2023), which reported same-day results for Femoflor in outpatient settings, enabling timely therapeutic decisions .

2. Microscopic Examination (48 Hours)
    - Traditional microscopy involves labor-intensive steps such as Gram staining, slide preparation, and manual scoring, often leading to delays. A study comparing molecular and microscopic methods noted that microscopy results typically took 24–

48 hours in clinical laboratories due to batching practices and inter-observer validation requirements. For instance, Nugent scoring requires multiple evaluations by trained microbiologists to ensure consistency, contributing to prolonged reporting times.

Femoflor detected Lactobacilli in 85% of samples, outperforming microscopy by 15 percentage points (70%). This discrepancy arises because microscopy cannot differentiate Lactobacillus species (e.g., L. crispatus vs. L. iners), which vary in protective roles, nor quantify their abundance. Molecular methods, such as Femoflor, utilize species-specific primers to identify and quantify lactobacilli, even at low concentrations. Similarly, Gardnerella vaginalis/Prevotella bivia/Porphyromonas were detected in 50% of Femoflor samples, compared to 30% by microscopy. These anaerobic bacteria form biofilms resistant to microscopy's visual detection, whereas PCR amplifies their DNA regardless of growth conditions. Femoflor's ability to detect Mycoplasma (18%) and Ureaplasma (10%), which microscopy missed entirely, reflects PCR's superiority in identifying fastidious organisms requiring specialized culture media. Microscopy lacks sensitivity to detect these cell-wall-deficient bacteria, whereas Femoflor targets their genetic material directly. Atopobium vaginae, a BV-associated anaerobe, was identified in 15% of Femoflor samples vs. 3% by microscopy, as molecular methods bypass the need for labour-intensive staining protocols.

For Enterobacteriales, Femoflor reported a 20% detection rate vs. microscopy's 5%, likely due to PCR's capacity to detect low-abundance pathogens in polymicrobial infections. Similarly, Streptococcus spp. was detected in 30% of Femoflor samples, compared to 15% by microscopy, as PCR avoids the misclassification errors inherent in morphotype-based microscopy. Femoflor identified Megasphaera/Veillonella/Dialister (20%) and Peptostreptococcus (10%), which microscopy failed to detect. These obligate anaerobes are challenging to cultivate, and their small size and irregular morphology complicate microscopic identification.

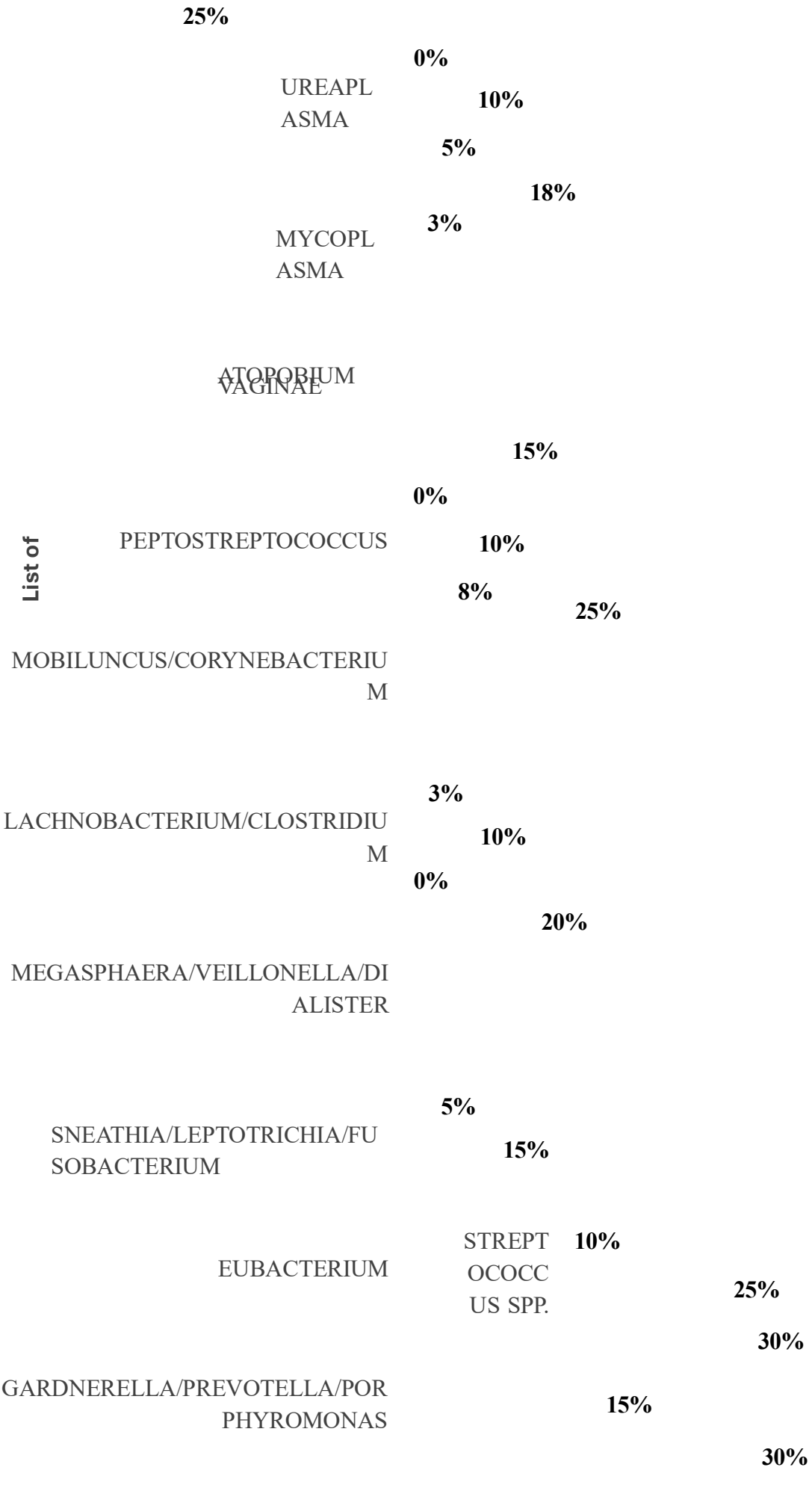
Sneathia/Leptotrichia/Fusobacterium were detected in 15% of Femoflor samples vs. 5% by microscopy, reflecting PCR's ability to resolve tightly adherent biofilm-associated species. Femoflor detected Candida in 25% of samples vs. microscopy's 18.3%, likely due to PCR's ability to identify species in mixed infections (e.g., C. albicans vs. C. krusei) without relying on culture-based isolation. Microscopy may miss low fungal loads or misclassify non-viable cells, as shown in Table 9.

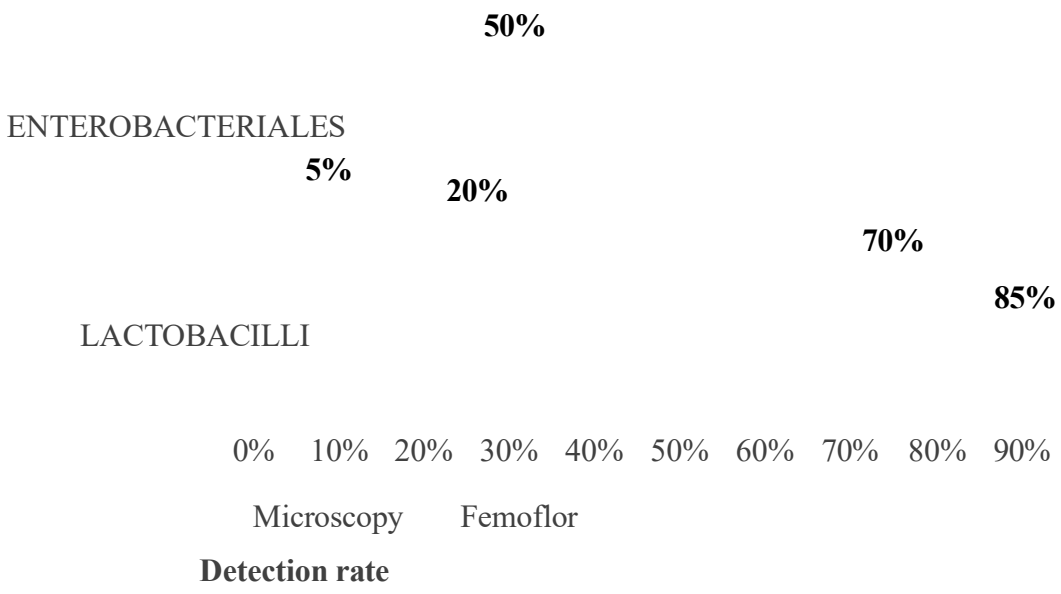
Table 9: Detection rate of vagina flora

Microorganism Detection: Femoflor vs. Microscopy

18.33%

CANDIDA


25%
0%
UREAPLASMA
10%
5%
18%
3%
MYCOPLASMA
ATOPOBIUM VAGINAE
15%
0%
PEPTOSTREPTOCOCCUS
10%
8%
25%
MOBILUNCUS/CORYNEBACTERIUM
3%
LACHNOBACTERIUM/CLOSTRIDIUM
10%
0%
20%
MEGASPHAERA/VEILLONELLA/DIALISTER
5%
SNEATHIA/LEPTOTRICHIA/FUSOBACTERIUM
15%
EUBACTERIUM
STREPTOCOCCUS SPP.
10%
25%
30%
GARDNERELLA/PREVOTELLA/PORPHYROMONAS
15%
30%
List of


50%
ENTEROBACTERIALES
5%
20%
70%
85%
LACTOBACILLI
0% 10% 20% 30% 40% 50% 60% 70% 80% 90%
Microscopy
Femoflor
Detection rate

Femoflor is also able to detect the vaginal state of health or disease, which is defined as normocenosis (absolute and relative) or dysbiosis (moderate, severe and mixed aerobic/anaerobic). Femoflor-16 classifies vaginal microbiota into normocenosis (Lactobacillus-dominant with total bacterial biomass [TBM] 10^6–10^8 CFU/mL) and dysbiosis (anaerobic/aerobic overgrowth with TBM deviations). Subcategories (absolute/relative normocenosis; severe/moderate/mixed dysbiosis) align with criteria validated in clinical studies (see Table 10).

Table 10: The results of tested samples from women's genital discharge using the Femoflor-16 test

Femoflor-16	**Sample number (%)**
Normocenosis • Absolute • Relative	23(51,1%) 11(24,4%) 12(26,7%)
Dysbiosis • Severe • Moderate • Mixed	17(37,8%) 8(47,1%) 5(29,4%) 4(23,5%)
Total number of tested samples	45

In the 11 patients with absolute normocenosis (24.4%), the amount of lactobacilli, expressed as an absolute value, ranged from 10^5.8 to 10^7.3. Their relative value, as a % of all microorganisms found in the vagina, varied from 80-85% to 100%.

Absolute normocenosis refers to a vaginal microbiome dominated by Lactobacillus species, which maintain an acidic pH (3.5–4.5) and suppress pathogenic overgrowth. However, even in this balanced state, low-abundance facultative and anaerobic bacteria may persist at non-

pathogenic levels. The data in Table 11 highlight the presence of such microorganisms in women with normocenosis, albeit at minimal relative abundances (<0.1% of total bacteria).

***Table 11**: Prevalence of in women with absolute normocenosis*

Isolates in women with absolute normocenosis	**Sample number**	**%**
Gardnerella vaginalis / Prevotellabivia / Porphyromonas	8	72,7%
Peptostreptococcus spp.	8	72,7%
Eubacterium spp.	6	54,5%
Atopobium vaginae	5	45,5%
Ureaplasma spp	3	27,3%
Staphilococcus spp.	3	27,3%
Megasphera spp/Veilonella spp/Dialister spp.	2	18,2%
Streptococcus spp.	1	9,1%
Total sample number	36	

Relative normocenosis refers to a vaginal microbiota state in which Lactobacillus species remain dominant, coexisting with low-abundance opportunistic or pathogenic microorganisms. The data from the study align with findings from recent research on vaginal microbiome dynamics and diagnostic methodologies. In 12 (26.7%) patients with relative normocenosis, the bacterial isolates detected are described in Table 12. In this group of patients, despite Lactobacillus dominance with relative abundance ≥73%, 50% of women harboured Gardnerella vaginalis, Prevotella bivia, or Candida spp. The presence of Atopobium vaginae (25%) and Ureaplasma (41.7%) organisms linked to persistent bacterial vaginosis (BV) and preterm birth suggests that

relative normocenosis may represent a transitional state. Candida spp. in 50% of cases highlights the frequent coexistence of fungal and bacterial communities, a phenomenon reported in studies of asymptomatic women. This highlights the importance of dual pathogen screening in symptomatic patients. Relative normocenosis is not a static "healthy" state but a dynamic equilibrium with clinical implications.

Table 12: *Prevalence of isolates in woman with relatives normocenosis*

Isolates in women with relative normocenosis	**Sample number**	**%**
Gardnerella vaginalis / Prevotella bivia / Porphyromonas	6	50,0%
Eubacterium spp.	6	50,0%
Candida spp.	6	50,0%
Ureaplasma spp	5	42,0%
Atopobium vaginae	3	25,0%
Streptococcus spp.	2	16,7%
Lachnobacterium spp. / Clostridium spp.	2	16,7%
Mobiluncus spp/ Corynebacterium spp.	2	16,7%
Enterobacteriales spp.	1	8,3%
Staphilococcus spp.	1	8,3%
Total sample number	34	

Dysbiosis was categorized according to severity based on the findings in 17 (37.8%) of the 40 patients tested. In 8 cases (47.1%), it is severe, in 5 cases (29.4%), moderate, and in 4 cases (23.5%), mixed. Table 13 shows the patients with severe dysbiosis and the detected

microorganisms. The absolute quantitative abundance of lactobacilli ranged from $10^{3\cdot6}$ to $10^{6\cdot1}$ CFU/mL, while their relative abundance decreased from 3–9% to 0%. Concurrently, the total microbial load demonstrated an absolute quantitative range of $>10^4$ to $10^{6\cdot8}$ CFU/mL, with a relative abundance of >40–80%.

Table 13. *Prevalence of isolates in women with severe dysbiosis.*

Isolates in women with severe dysbiosis	**Sample number**	**%**
Gardnerella vaginalis/ Prevotellabivia/Porphyromonas	8	87,5%
Atopobium vaginae	6	75,0%
Eubacterium spp.	6	75,0%
Megasphera spp/Veilonella spp/Dialister spp.	5	62,5%
Sneathia spp /Leptotrihia spp/Fusobacterium spp.	4	50,0%
Peptostreptococcus spp.	4	50,0%
Ureaplasma spp.	4	50,0%
Lachnobacterium spp./Clostridium spp.	3	37,5%
Mobiluncus spp/Corynebacterium spp.	2	25,0%
Candida spp.	2	25,0%
Enterobacteriales spp.	1	12,5%
M. hominis	1	12,5%
Total sample number	46	

The high prevalence of Gardnerella vaginalis (87.5%) and Atopobium vaginae (75%) reflects their role in biofilm formation and BV pathogenesis. These organisms are strongly correlated with severe dysbiosis due to their ability to disrupt lactobacilli dominance and elevate vaginal pH. Megasphaera/Veillonella/Dialister (62.5%) and Sneathia/Leptotrichia/Fusobacterium (50%)

are anaerobic consortia linked to BV recurrence and treatment resistance. Ureaplasma spp. (50%) and Candida spp. (25%) indicate mixed infections, complicating clinical management. Studies have noted that molecular methods, such as Femoflor-16, improve the detection of such co-infections compared to microscopy. The low detection of Enterobacteriales (12.5%) and M. hominis (12.5%) suggests these are less dominant in severe dysbiosis but may contribute to inflammation.

The microorganism and its prevalence rate in moderate dysbiosis in women were detected as shown in Table 14.

***Table 14**: Prevalence of isolates in women with moderate dysbiosis*

Isolates in women with moderate dysbiosis	**Sample number**	**%**
Gardnerella vaginalis / Prevotellabivia / Porphyromonas	4	80,0%
Atopobium vaginae	3	60,0%
Eubacterium spp.	3	60,0%
Megasphera spp / Veilonella spp /Dialister spp.	2	40,0%
Ureaplasma spp.	2	40,0%
Lachnobacterium spp./Clostridium spp.	1	20,0%
Mobiluncus spp/Corynebacterium spp.	1	20,0%
Peptostreptococcus spp.	1	20,0%
Candida spp.	1	20,0%
Total sample number	18	

Gardnerella vaginalis / Prevotella bivia / Porphyromonas are strongly associated with bacterial vaginosis (BV) and dysbiosis, with an 80% prevalence. Gardnerella forms biofilms that facilitate polymicrobial colonization, while Prevotella bivia produces sialidases that degrade vaginal mucins, increasing pH and promoting dysbiosis. A study using Femoflor-16 PCR found Gardnerella/Prevotella in 95.5% of BV cases, correlating with elevated Nugent scores. Atopobium vaginae is a fastidious anaerobe often co-detected with Gardnerella in BV. It resists standard therapies and is linked to recurrent infections. Molecular studies highlight its role in biofilm persistence. Eubacterium spp. and Megasphaera/Veillonella/Dialister, which show 60% and 40% prevalence, respectively, contribute to dysbiosis by producing amines (e.g., trimethylamine) that elevate vaginal pH. Their co-occurrence with Gardnerella exacerbates inflammation and biofilm complexity. Ureaplasma is frequently detected in dysbiotic states. While not always pathogenic, its overgrowth in low-lactobacilli environments may contribute to aerobic vaginitis or ascending infections. The reduced relative abundance of lactobacilli (15–70%) reflects compromised vaginal acidity, allowing anaerobes to thrive. Lactobacillus crispatus, a key protective species, is often replaced by transitional L. iners in moderate dysbiosis. While less common, Candida colonization in dysbiosis suggests fungal-bacterial interactions that may exacerbate symptoms.

The microorganism and its prevalence rate in mixed dysbiosis in women were detected as shown in the table 15.

***Table 15**: Prevalence of isolates in women with mixed dysbiosis*

Isolates in women with mixed dysbiosis	**Sample Number**	**%**
Gardnerella vaginalis / Prevotella bivia / Porphyromonas	3	75.0%
Streptococcus spp.	3	75.0%
Eubacterium spp.	2	50.0%
Atopobium vaginae	2	50.0%

Enterobacteriales spp.	1	25.0%
Megasphaera / Veillonella / Dialister	1	25.0%
Lachnobacterium / Clostridium	1	25.0%
Ureaplasma spp.	1	25.0%
Candida spp.	1	25.0%
Total sample number	16	

Based on Table 15, the predominance of Anaerobic and BV-associated pathogens, such as Gardnerella vaginalis and Prevotella bivia, which are key anaerobic bacteria linked to bacterial vaginosis (BV), was detected in 75% of the samples. These organisms are known to form biofilms and disrupt vaginal pH, facilitating polymicrobial infections. Streptococcus spp., detected in 75% of cases, are often associated with aerobic vaginitis (AV) and mixed dysbiosis. Their presence alongside anaerobes highlights the complexity of co-infections in dysbiotic states. Absolute Lactobacillus counts ranged from 10^3 to $10^{5.9}$, with relative abundance as low as 18%. This contrasts with healthy vaginal microbiota, where Lactobacillus typically constitutes more than 80% of the flora. Reduced lactobacilli correlate with dysbiosis severity and impaired acidification. Atopobium vaginae (50% prevalence) and Megasphaera/Veillonella (25%) are markers of persistent BV and recurrent infections. These organisms resist standard therapies and are more effectively detected via molecular methods, such as Femoflor-16. Candida spp. (25%) and Ureaplasma spp. (25%) indicate overlapping dysbiotic conditions. Such polymicrobial profiles complicate diagnosis and require multiplex PCR for accurate detection.

Conclusion

The findings of this study underscore the transformative potential of Femoflor multiplex PCR as a diagnostic tool for vaginal flora assessment, demonstrating superior accuracy and efficiency compared to conventional microscopic examination. Femoflor's ability to quantify microbial loads, differentiate Lactobacillus species (e.g., L. crispatus vs. L. iners), and detect polymicrobial

infections addresses critical limitations of traditional methods, such as Nugent scoring and Amsel's criteria, which are prone to subjectivity and poor sensitivity for low-abundance pathogens [2,5]. For instance, Femoflor's sensitivity and specificity exceeding 90% for bacterial vaginosis (BV) detection, as validated in studies of symptomatic women, highlight its diagnostic precision [2,5]. Furthermore, its capacity to stratify dysbiosis into distinct categories (e.g., moderate vs. severe, aerobic vs. anaerobic) enables clinicians to tailor therapeutic strategies based on microbial ratios and total bacterial biomass, thereby improving outcomes in recurrent or complex cases [2,5].

The clinical implications are profound: Femoflor enhances decision-making by identifying intermediate dysbiotic states often misclassified as "normal" by microscopy, such as Gardnerella - biofilm communities or co-infections with Mycoplasma and Ureaplasma, which are critical for preventing complications like preterm birth and pelvic inflammatory disease [2,5,13]. Its rapid turnaround time, which is 2 hours rather than 48 hours for microscopy, further supports timely interventions, particularly in high-risk populations [5,29]. However, challenges remain, including cost barriers in resource-limited settings and the need for standardized diagnostic thresholds across diverse populations [5,30].

In summary, Femoflor represents a paradigm shift in gynecological diagnostics, aligning with modern understandings of vaginal microbiota as a dynamic and polymicrobial ecosystem. By integrating molecular precision with clinical practicality, it paves the way for personalized medicine, reducing misdiagnosis rates and optimizing therapeutic efficacy. Future research should focus on cost-effective implementation models and longitudinal studies to validate its long-term impact on reproductive health outcomes [2, 5, 30].

References

1. Babu, G. (2017). Comparative study on the vaginal flora and incidence of asymptomatic vaginosis among healthy women and in women with infertility problems of reproductive age. *Journal of Clinical and Diagnostic Research*.
2. Savicheva, A. M., et al. (2023). Vaginal microbiota molecular profiling in women with bacterial vaginosis: A novel diagnostic tool. *International Journal of Molecular Sciences, 21*(24), 15880.
3. Joseph, R. J., et al. (2021). Finding a balance in the vaginal microbiome: How do we treat and prevent the occurrence of bacterial vaginosis? *Antibiotics, 10*(6).

4. A comparative analysis of seminal and vaginal microbiota of married couples by real-time PCR with Androflor and Femoflor reagent kits. (2017). *RSMU Press*. Retrieved May 1, 2025, from https://vestnik.rsmu.press/archive/2017/2/5/abstract?lang=en
5. Savicheva, A. M. (2023). Molecular testing for the diagnosis of bacterial vaginosis. *International Journal of Molecular Sciences, 25*(1), 449.
6. Lamont, R., et al. (2011). The vaginal microbiome: New information about genital tract flora using molecular-based techniques. *BJOG: An International Journal of Obstetrics & Gynaecology, 118*(5), 533–549.
7. Baykushev, R., & Raykova, V. (2024). Multiplex real-time PCR method as a reliable test in the routine microbiology study of vaginal microbiome in women with genital tract discharge. *Journal of IMAB, 30*(3).
8. Amabebe, E., & Anumba, D. O. C. (2018). The vaginal microenvironment: The physiologic role of lactobacilli. *Frontiers in Medicine, 5*, 181.
9. Sh, S. (2023). Study of Femoflor-16 for evaluation of vaginal microbiocenosis in women with inflammatory diseases of the genitals. *American Journal of Medicine and Medical Sciences, 3*, 276–280.
10. Health, S. (2025, May 2). Vaginal health research has lagged for years: That's starting to change. *Seed*. https://seed.com/cultured/vaginal-health-research-timeline/
11. Virtanen, S., et al. (2019). Vaginal microbiota composition correlates between Pap smear microscopy and next generation sequencing and associates to socioeconomic status. *Scientific Reports, 9*(1).
12. van der Veer, C. (2019). *Vaginal microbes in sexual health and disease* (Doctoral dissertation). Universiteit van Amsterdam.
13. Hillier, S. L., et al. (1992). Characteristics of three vaginal flora patterns assessed by Gram stain among pregnant women. *American Journal of Obstetrics and Gynecology, 166*(3), 938–944.
14. Money, D. (2005). The laboratory diagnosis of bacterial vaginosis. *Canadian Journal of Infectious Diseases and Medical Microbiology, 16*(2), 77–79.
15. Chen, X., et al. (2021). The female vaginal microbiome in health and bacterial vaginosis. *Frontiers in Cellular and Infection Microbiology, 11*, 631972.
16. Lomte, T. S. (2025, May 2). Study highlights the need for more diversity in vaginal microbiome research. *News-Medical*. https://www.news-medical.net/news/20250214/Study-highlights-the-need-for-more-diversity-in-vaginal-microbiome-research.aspx
17. Donders, G. G., et al. (1996). Assessment of vaginal lactobacillary flora in wet mount and fresh or delayed Gram's stain. *Infectious Diseases in Obstetrics and Gynecology, 4*(1), 2–6.
18. Elnifro, E. M., et al. (2000). Multiplex PCR: Optimization and application in diagnostic virology. *Clinical Microbiology Reviews, 13*(3), 559–570.
19. Li, T., et al. (2019). Evaluation of the vaginal microbiome in clinical diagnosis and management of vaginal infectious diseases. *Chinese Medical Journal, 132*(9), 1100–1103.
20. Kalia, N., Singh, J., & Kaur, M. (2020). Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: A critical review. *Annals of Clinical Microbiology and Antimicrobials, 19*(1).

21. Hugerth, L. W., et al. (2024). Defining vaginal community dynamics: Daily microbiome transitions, the role of menstruation, bacteriophages, and bacterial genes. *Microbiome, 12*(1).
22. Lambert, J. A., et al. (2013). Longitudinal analysis of vaginal microbiome dynamics in women with recurrent bacterial vaginosis: Recognition of the conversion process. *PLoS ONE, 8*(12), e82599.
23. Holm, J. B., et al. (2023). Integrating compositional and functional content to describe vaginal microbiomes in health and disease. *Microbiome, 11*(1).
24. Varghese, J., et al. (2018). Evaluation of interobserver reliability of Nugent score for diagnosis of bacterial vaginosis. *Indian Journal of Sexually Transmitted Diseases and AIDS, 39*(2), 120.
25. Durand, G., Bretelle, F., & Fenollar, F. (2016). Complications of pregnancy. In *Infectious Diseases* (pp. 498–504.e2).
26. Joesoef, M. R., et al. (1991). Reproducibility of a scoring system for Gram stain diagnosis of bacterial vaginosis. *Journal of Clinical Microbiology, 29*(8), 1730–1731.
27. Gaydos, C. A., et al. (2017). Clinical validation of a test for the diagnosis of vaginitis. *Obstetrics and Gynecology, 130*(1), 181–189.
28. Kusters, J. G., et al. (2015). A multiplex real-time PCR assay for routine diagnosis of bacterial vaginosis. *European Journal of Clinical Microbiology & Infectious Diseases, 34*(9), 1779–1785.
29. Giroux, M., et al. (2020). A randomized comparison of training programs using a pelvic model designed to enhance pelvic floor examination in patients presenting with chronic pelvic pain. *International Urogynecology Journal*.
30. Murdoch, A. I. K., et al. (2023). Determinants of clinical decision making under uncertainty in dentistry: A scoping review. *Diagnostics, 13*(6), 1076.
31. Nazarova, V. V., et al. (2017). Criteria for diagnosis of bacterial vaginosis using the test Femoflor-16. *Journal of Obstetrics and Women's Diseases, 66*(4), 57–67.
32. Sklyar, T. V., et al. (2015). Definition of women reproductive tract microflora composition using test-system "Femoflor-17". *Mikrobiolohichnyi Zhurnal (Kiev, Ukraine), 77*(5), 87–94.