DNA-TECHNOLOGY

DNA-TECHNOLOGY

FEMOFLOR®II
FEMOFLOR®AlphaScreen
FEMOFLOR®DeltaScreen

New line of FEMOFLOR® tests: clinical significance

REFERENCE

List of Abbreviations

AV	aerobic vaginitis
BVAB1, 2, 3	BV-associated bacteria
BV	bacterial vaginosis
CIN	cervical intraepithelial neoplasia
CMV	cytomegalovirus
CS	cervicotype
CST	community state type
HPV	human papillomavirus
HSV	herpes simplex virus
PID	pelvic inflammatory disease
STI	sexually transmitted infections
VVC	vulvovaginal candidiasis

FEMOFLOR®:
A new generation of unique tests for women

FEMOFLOR® is a line of PCR tests for the comprehensive assessment of the urogenital tract microbiota in women, developed by Russian scientists and clinicians in 2008. The principle implemented in the first test, FEMOFLOR®16, remains relevant and continues to be widely used in clinical practice. However, scientific discoveries and technical capabilities have expanded our understanding of the clinical significance of the microbiota. This led to the creation of the new FEMOFLOR® line.

The FEMOFLOR® test kits employ a comprehensive approach to assessing a woman's microbiome – we evaluate the balance of the vaginal microbiota by comparing the proportions of different microorganism groups within the total amount of bacteria. This allows to obtain a comprehensive microbiome profile and reliably identifies the dominant microbiota groups associated with the development of symptoms. This innovative approach, created and introduced into clinical practice by DNA-Technology, has significant advantages compared to other diagnostic methods: bacterial typing, virus detection, and diagnostics of mixed or anaerobic dysbiosis. The same principle is also used in FEMOFLOR®II.

NEW TESTS WITH A LONG HISTORY

FEMOFLOR®	**FEMOFLOR®II**
FEMOFLOR®16 FEMOFLOR®Screen	FEMOFLOR®II FEMOFLOR®AlphaScreen FEMOFLOR®DeltaScreen
2009	**2025**

More than 15.000.000 assays
More than 16 years on the market
45 countries

The new FEMOFLOR line includes the **FEMOFLOR®II, FEMOFLOR®AlphaScreen and FEMOFLOR®DeltaScreen tests.**

- **FEMOFLOR®II** is a universal test with maximum capabilities.
- **FEMOFLOR®AlphaScreen** is a shortened version for diagnosing bacterial vaginosis.
- **FEMOFLOR® DeltaScreen** is a complex test for diagnostics of infectious and inflammatory urogenital diseases.

All tests include control parameters – **total bacterial load** and **human genomic DNA**. These parameters are used to assess the biomaterial collection quality. Total bacterial load is also used to calculate the relative quantity of microorganisms in the sample.

The most comprehensive test, **FEMOFLOR®II**, includes an analysis of the microbiota's state: a detailed normal microbiota assessment (determining the amount of lactobacilli and bifidobacteria, lactobacilli typing), an expanded list of opportunists and detection of pathogens (major STIs, herpesviruses, and HPV).

An important feature of the test is the lactobacilli typing, which allows to assess how effectively the normal microbiota is performing its protective function. The expanded list of opportunists includes bacteria associated with bacterial vaginosis, aerobic vaginitis, mixed vaginitis, vulvovaginal candidiasis, and others. This enables precise identification of the disease's etiological agents and the correct selection of therapy.

The test also includes the detection of major STI pathogens – *Chlamydia trachomatis, Mycoplasma genitalium, Neisseria gonorrhoeae*, and *Trichomonas vaginalis*. These pathogens can often be present asymptomatically or with mild, non-specific symptoms. Their inclusion in FEMOFLOR®II – a microbiome profiling kit – ensures that they are ruled out as etiological agents. This is crucial for correctly determining the cause of dysbiotic disorders and selecting the appropriate therapy to restore the microbiome.

In terms of its purpose, FEMOFLOR®II can be considered the new version of the FEMOFLOR®16 test.

FEMOFLOR®AlphaScreen and **FEMOFLOR®DeltaScreen** are shortened versions of the FEMOFLOR®II. Both panels include a brief analysis of the normal microbiota (*Lactobacillus* non-*iners* and *Lactobacillus iners*), as well as opportunistic mycoplasmas (*Mycoplasma hominis, Ureaplasma urealyticum*, and *Ureaplasma parvum*) and *Candida* spp., including *Candida albicans*.

FEMOFLOR®AlphaScreen provides a detailed assessment of anaerobic opportunists associated with bacterial vaginosis (*Gardnerella vaginalis, Fannyhessea vaginae, Mobiluncus* spp., *Anaerococcus* spp./*Peptostreptococcus* spp., *Bacteroides* spp./ *Porphyromonas* spp./ *Prevotella* spp., *Sneathia* spp./ *Leptotrichia* spp./ *Fusobacterium* spp., *Megasphaera* spp./*Veillonella* spp./ *Dialister* spp., BVAB1 /BVAB2/ BVAB3). It is specifically designed for diagnosing bacterial vaginosis and monitoring the effectiveness of its treatment.

FEMOFLOR®DeltaScreen is an upgraded version of the original FEMOFLOR®Screen. It includes an evaluation of main microbiota state parameters and detection of pathogens.

How to choose the test?

Clinical situation	FEMOFLOR®				
	FF®16	FF®II	Screen	Delta Screen	Alpha Screen
Infectious diseases of reproductive system					
Inflammatory symptoms	●	●	●	●	
Symptoms of BV	●	●			●
Asymptomatic cases					
Preparation for pelvic surgery	●	●	●	●	
Prophylactic examinations	●	●	●	●	
Treatment monitoring					
Treatment of infectious diseases	●	●	●	●	
BV treatment	●	●			●
Obstetrics					
Pregnancy planning, ART	●	●	●	●	
Prophylactic examinations during pregnancy	●	●	●	●	
Infertility, miscarriage	●	●	●	●	

Comparing of FEMOFLOR® tests

	FEMOFLOR®				
	FF®16	FF®II	Screen	Delta Screen	Alpha Screen
Number of tubes	16	16	8	8	8
Indicators					
Human genomic DNA	●	●	●	●	●
Total bacterial load	●	●	●	●	●
Normal microbiota					
Lactobacillus spp.	●	●	●	●	●
L. crispatus		●			
L. jensenii/ L. mulieris		●			
L. gasseri/ L. paragasseri		●			
L. iners		●		●	●
L. non-iners				●	●
Bifidobacterium spp.		●			
Aerobes					
Staphylococcus spp.	●	●		●	
Streptococcus spp.	●	●		●	
Streptococcus agalactiae		●			
Enterobacteriaceae	●	●		●	
Enterococcus spp.		●		●	
Haemophilus spp.		●		●	
Anaerobes					
Gardnerella vaginalis	●	●	●	●	●
Fannyhessea vaginae (Atopobium vaginae)	●	●		●	●
Mobiluncus spp.	●	●		●	●
Anaerococcus spp.		●			●
Peptostreptococcus spp.	●	●			●

	FEMOFLOR®				
	FF®16	FF®II	Screen	Delta Screen	Alpha Screen
Bacteroides spp./*Porphyromonas* spp./*Prevotella* spp.	•	●			●
Sneathia spp./*Leptotrichia* spp./ *Fusobacterium* spp.	•	●			●
Megasphaera spp./ *Veillonella* spp./*Dialister* spp.	•	●			●
BVAB1 / BVAB2 / BVAB3		●			●
Mycoplasmas					
Ureaplasma urealyticum	spp.	●	spp.	●	●
Ureaplasma parvum	•	●	•	●	●
Mycoplasma hominis	•	●	•	●	●
Yeast fungi					
Candida spp.	•	●	•	●	●
Candida albicans		●		●	●
Sexually transmitted infections (STI)					
Chlamydia trachomatis		●	•	●	
Mycoplasma genitalium	•	●	•	●	
Neisseria gonorrhoeae		●	•	●	
Trichomonas vaginalis		●	•	●	
Herpesviruses					
HSV1		●	•	●	
HSV2		●	•	●	
CMV		●	•	●	
Human papillomaviruses					
HPV 16		●			
HPV 18		●			
HPV 45		●			
HPV 31/ 33/ 35/ 39/ 51/ 52/ 56/ 58/ 59/ 66/ 68		●			

Vaginal microbiota balance is the foundation of female reproductive health

The vaginal microbiome is a dynamic ecosystem that fluctuates throughout life depending on menstrual cycle, pregnancy, hormonal changes, diet, lifestyle and exposure to various medications, including antibiotics (1). Changes in the composition of the vaginal microbiota may occur at different stages of a woman's life, including childhood, puberty, pregnancy and menopause (2,3).

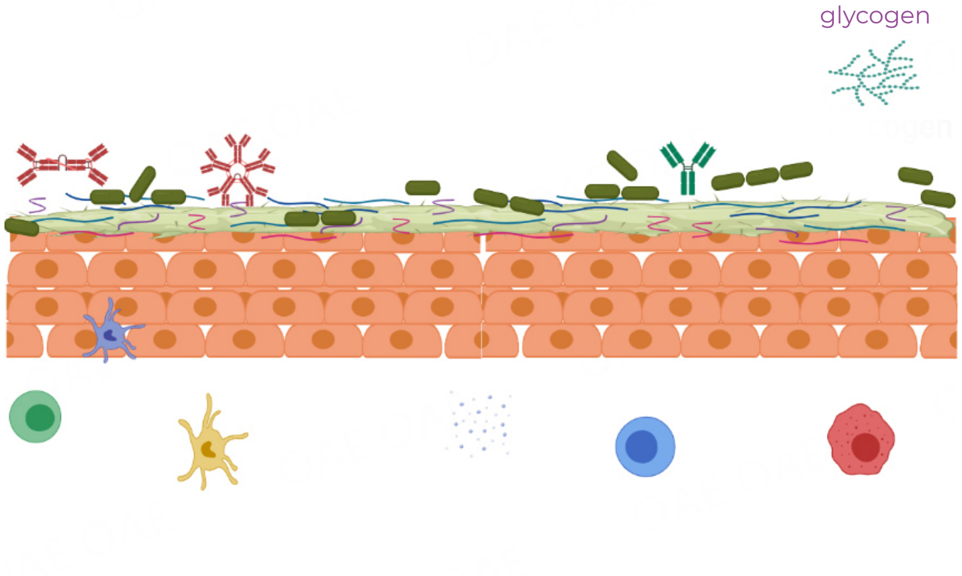
A balanced vaginal microbiota plays an important role in maintaining women's reproductive health. It is characterized by the prevalence of normal microbiota representatives and low quantities of opportunistic microorganisms. Normal microbiota induces the expression of defensins — a specific type of vaginal antimicrobial peptides that prevent pathogen-specific proteins from binding to epithelial cells of the genital tract mucosa (10). Dysbiosis is observed when the relative quantity of normal microbiota representatives falls below 80% of the total bacterial load and the percentage of opportunistic microorganisms rises. Dysbiosis may manifest as bacterial vaginosis, aerobic vaginitis, vulvovaginal candidiasis, and other conditions, depending on the particular groups of microorganisms that caused the imbalance (4-9).

Disturbances of the cervico-vaginal microbiota are associated with increased susceptibility to sexually transmitted infections, development of cervical intraepithelial neoplasia (CIN), and pregnancy complications. Correcting vaginal microbiota dysbiosis reduces the risk of pathology and reproductive health problems.

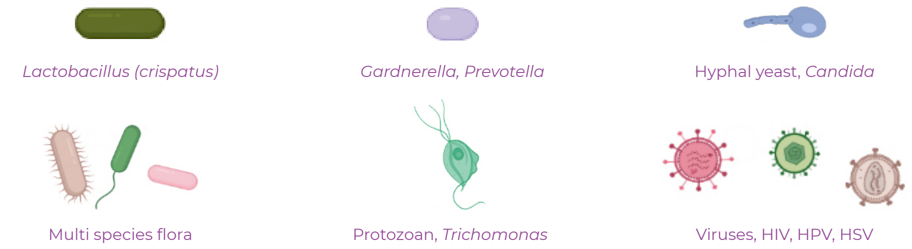
Eubiosis

Stable microbiome
L. crispatus dominant

[Lactic acid]$^{\text{high}}$ ↑
pH 4.5
intact mucins



Commensals / Pathogens



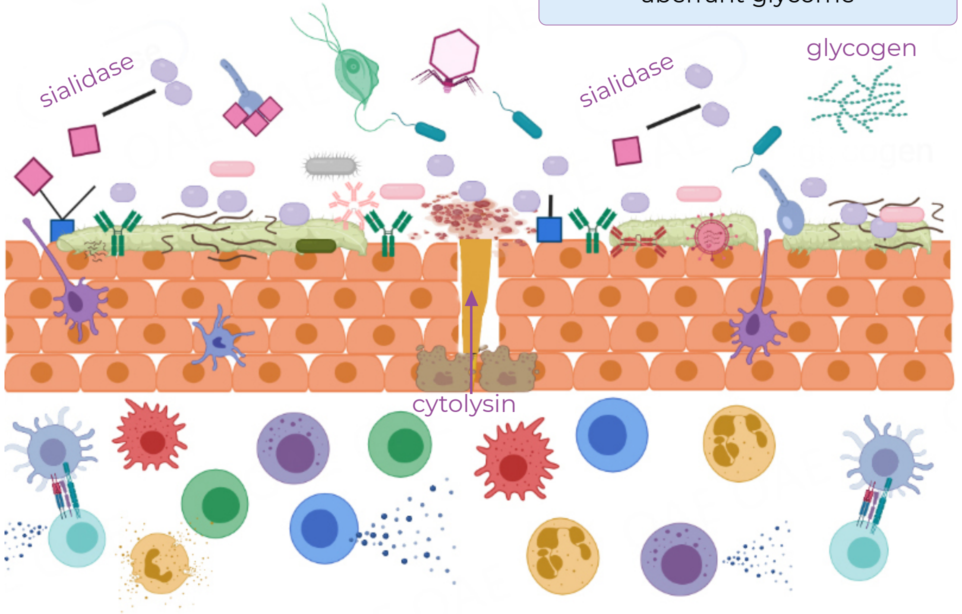
Eubiosis. *Lactobacillus crispatus* is considered the most preferential species in the vaginal microbiome, as it provides the lowest pH in the vaginal biotope through its production of lactic acid.

Adapted from Sanozky-Dawes et all. (210)

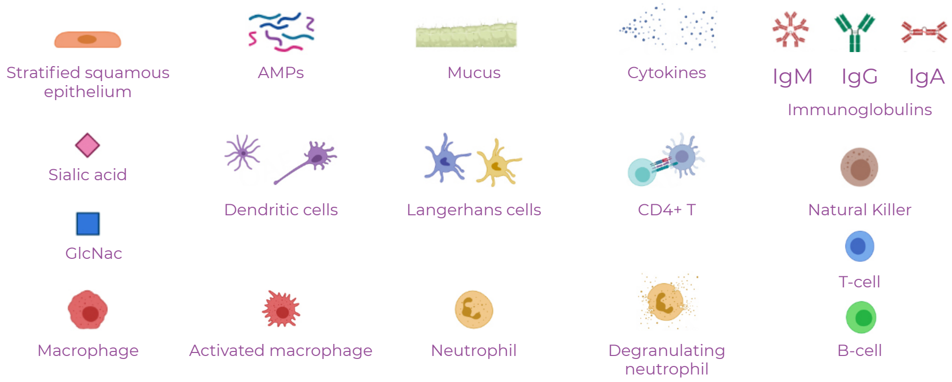
Dysbiosis

[Lactic acid]$^{\text{low}}$ ↓
pH > 5.0
mucin breakdown

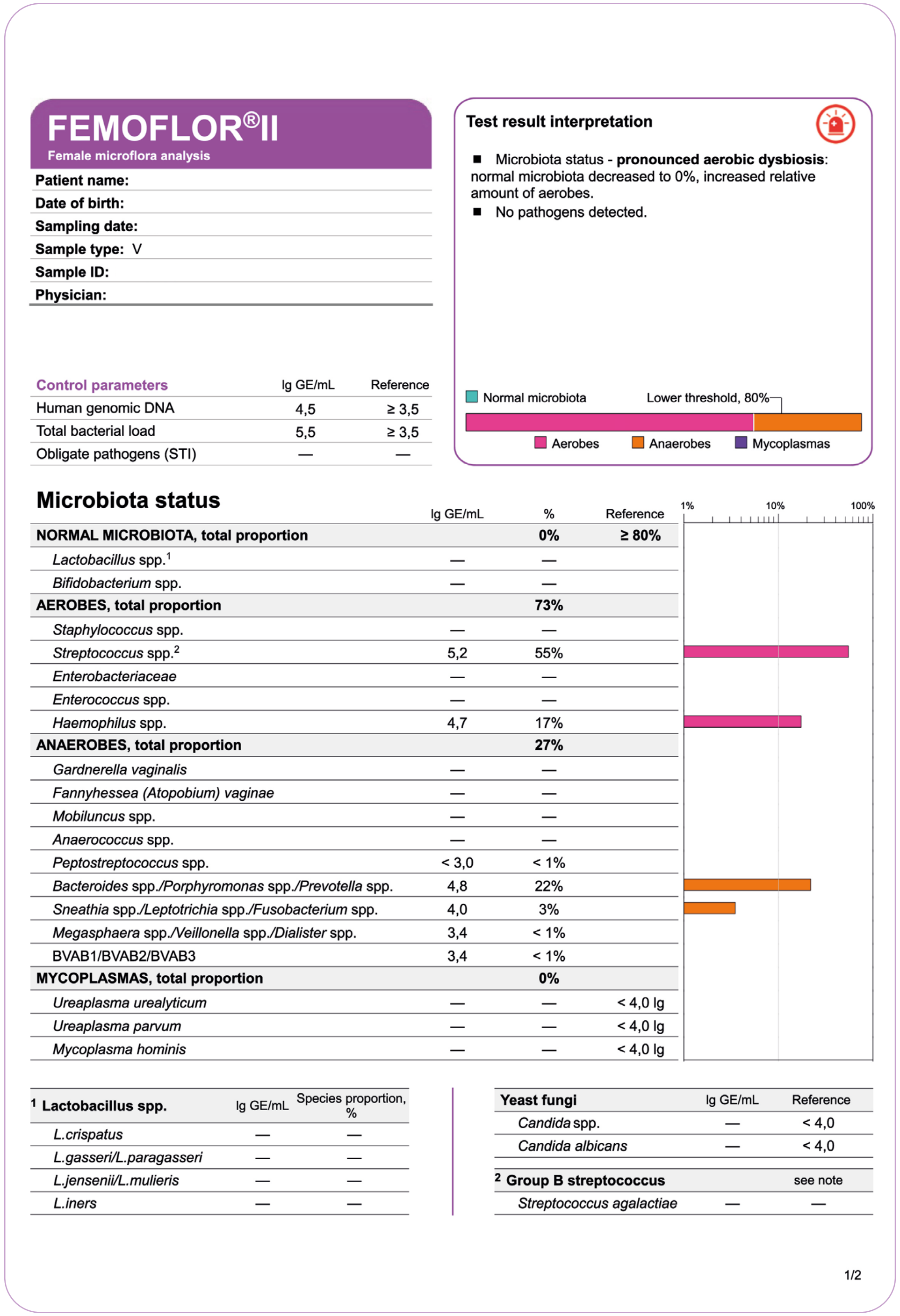
Polymicrobial biofilm
aberrant glycome



Host Factors and Cells



Dysbiosis. The decline of *Lactobacillus* spp. causes a rise in pH, enabling the establishment of microorganisms like *Prevotella* and *Gardnerella* that form biofilms. These bacteria produce virulence factors like hydrolytic enzymes (e.g., sialidases) and cytolysins, which degrade mucins and epithelial cells and disrupt immune defense. This, in turn, encourages further colonization by other pathogens and opportunistic microorganisms.



FEMOFLOR®II

Female microflora analysis

Patient name:
Date of birth:
Sampling date:
Sample type: V
Sample ID:
Physician:

Control parameters	lg GE/mL	Reference
Human genomic DNA	4,5	≥ 3,5
Total bacterial load	5,5	≥ 3,5
Obligate pathogens (STI)	—	—

Test result interpretation

- Microbiota status - **pronounced aerobic dysbiosis**: normal microbiota decreased to 0%, increased relative amount of aerobes.
- No pathogens detected.

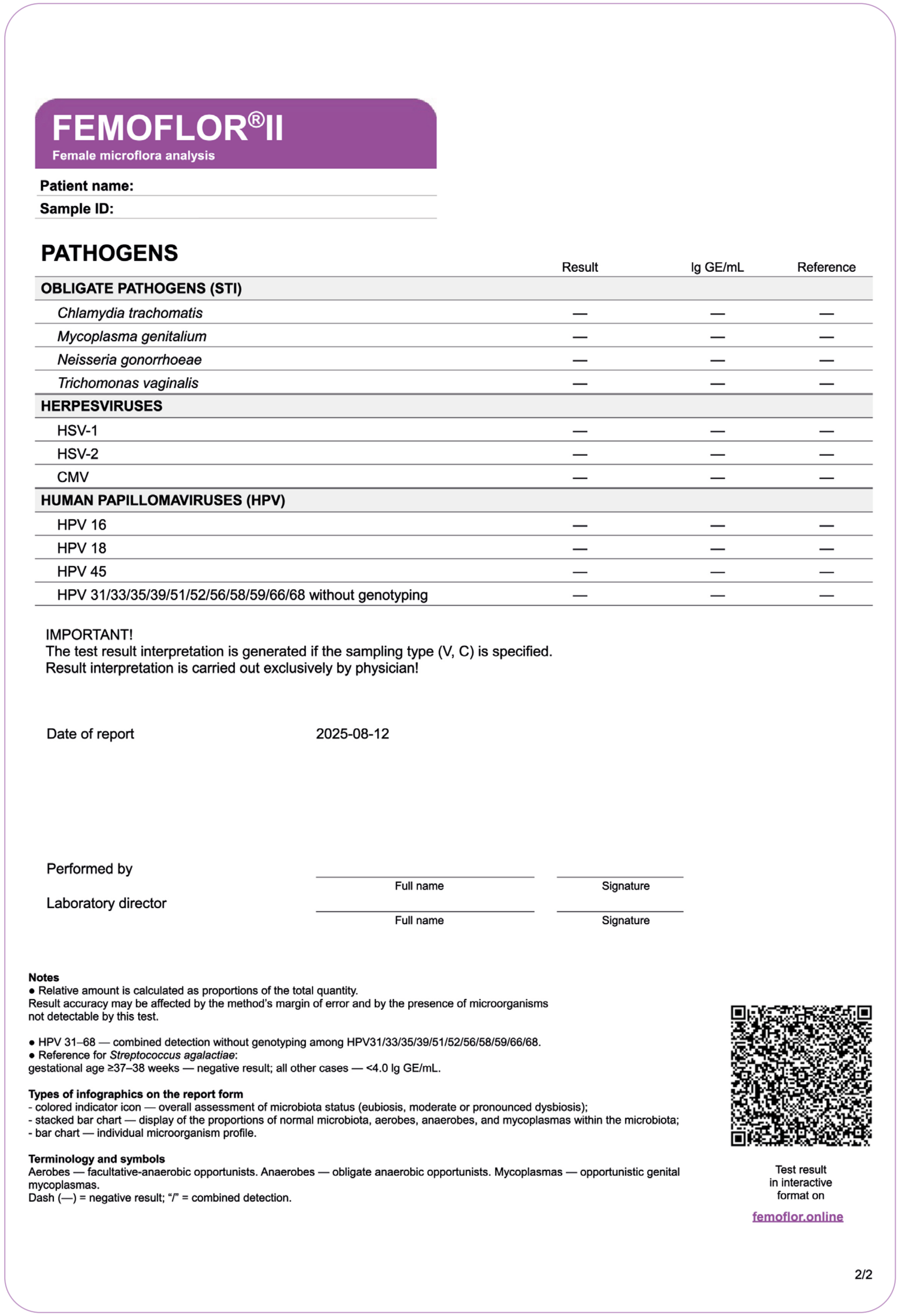
Microbiota status

	lg GE/mL	%	Reference
NORMAL MICROBIOTA, total proportion		**0%**	**≥ 80%**
Lactobacillus spp.[1]	—	—	
Bifidobacterium spp.	—	—	
AEROBES, total proportion		**73%**	
Staphylococcus spp.	—	—	
Streptococcus spp.[2]	5,2	55%	
Enterobacteriaceae	—	—	
Enterococcus spp.	—	—	
Haemophilus spp.	4,7	17%	
ANAEROBES, total proportion		**27%**	
Gardnerella vaginalis	—	—	
Fannyhessea (Atopobium) vaginae	—	—	
Mobiluncus spp.	—	—	
Anaerococcus spp.	—	—	
Peptostreptococcus spp.	< 3,0	< 1%	
Bacteroides spp./*Porphyromonas* spp./*Prevotella* spp.	4,8	22%	
Sneathia spp./*Leptotrichia* spp./*Fusobacterium* spp.	4,0	3%	
Megasphaera spp./*Veillonella* spp./*Dialister* spp.	3,4	< 1%	
BVAB1/BVAB2/BVAB3	3,4	< 1%	
MYCOPLASMAS, total proportion		**0%**	
Ureaplasma urealyticum	—	—	< 4,0 lg
Ureaplasma parvum	—	—	< 4,0 lg
Mycoplasma hominis	—	—	< 4,0 lg

[1] **Lactobacillus spp.**	lg GE/mL	Species proportion, %
L.crispatus	—	—
L.gasseri/L.paragasseri	—	—
L.jensenii/L.mulieris	—	—
L.iners	—	—

Yeast fungi	lg GE/mL	Reference
Candida spp.	—	< 4,0
Candida albicans	—	< 4,0
[2] **Group B streptococcus**		see note
Streptococcus agalactiae	—	—

1/2



FEMOFLOR®II

Female microflora analysis

Patient name:
Sample ID:

PATHOGENS

	Result	lg GE/mL	Reference
OBLIGATE PATHOGENS (STI)			
Chlamydia trachomatis	—	—	—
Mycoplasma genitalium	—	—	—
Neisseria gonorrhoeae	—	—	—
Trichomonas vaginalis	—	—	—
HERPESVIRUSES			
HSV-1	—	—	—
HSV-2	—	—	—
CMV	—	—	—
HUMAN PAPILLOMAVIRUSES (HPV)			
HPV 16	—	—	—
HPV 18	—	—	—
HPV 45	—	—	—
HPV 31/33/35/39/51/52/56/58/59/66/68 without genotyping	—	—	—

IMPORTANT!
The test result interpretation is generated if the sampling type (V, C) is specified.
Result interpretation is carried out exclusively by physician!

Date of report 2025-08-12

Performed by
Full name
Signature

Laboratory director
Full name
Signature

Notes
- Relative amount is calculated as proportions of the total quantity.
Result accuracy may be affected by the method's margin of error and by the presence of microorganisms not detectable by this test.

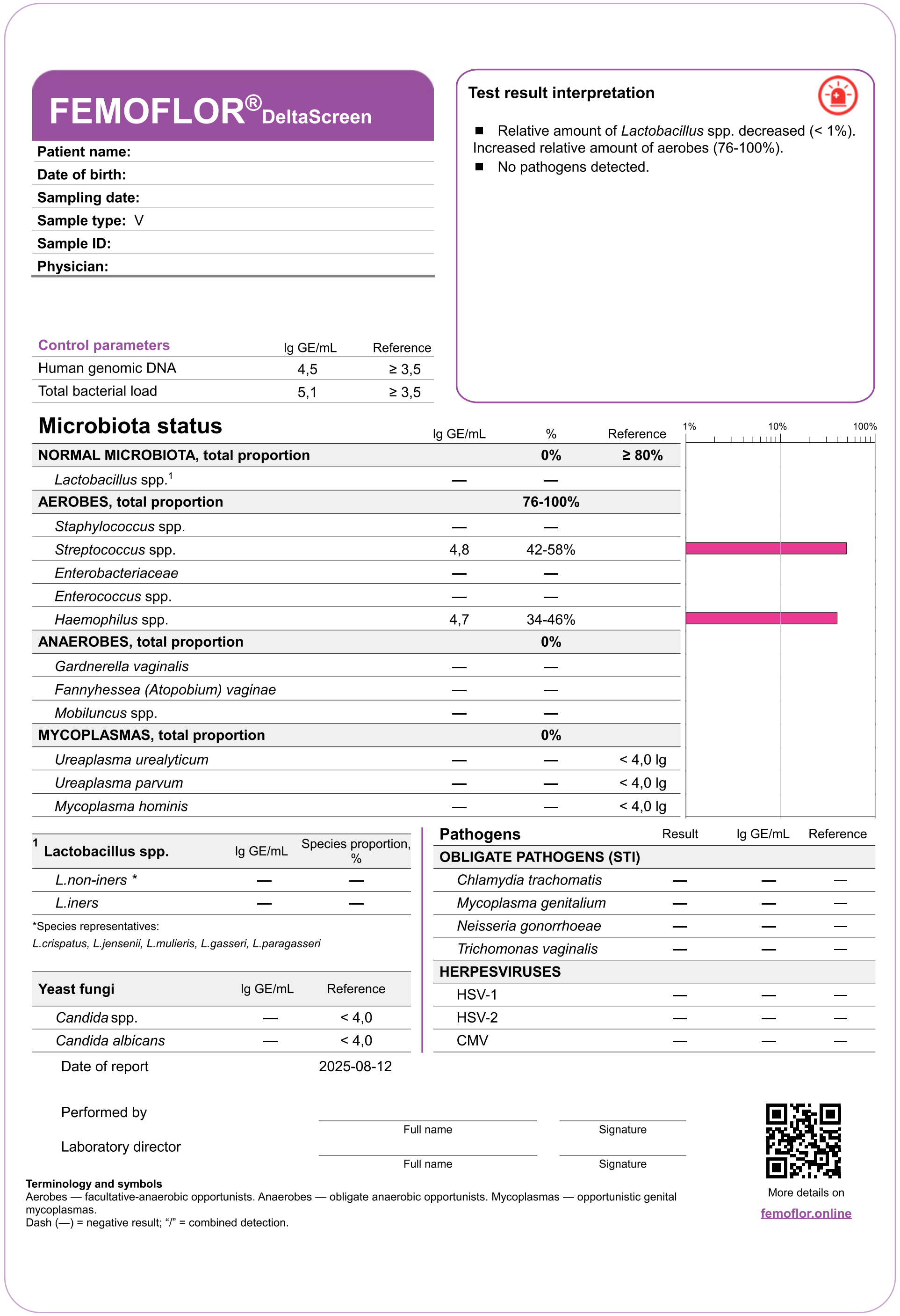
- HPV 31–68 — combined detection without genotyping among HPV31/33/35/39/51/52/56/58/59/66/68.
- Reference for *Streptococcus agalactiae*:
gestational age ≥37–38 weeks — negative result; all other cases — <4.0 lg GE/mL.

Types of infographics on the report form
- colored indicator icon — overall assessment of microbiota status (eubiosis, moderate or pronounced dysbiosis);
- stacked bar chart — display of the proportions of normal microbiota, aerobes, anaerobes, and mycoplasmas within the microbiota;
- bar chart — individual microorganism profile.

Terminology and symbols
Aerobes — facultative-anaerobic opportunists. Anaerobes — obligate anaerobic opportunists. Mycoplasmas — opportunistic genital mycoplasmas.
Dash (—) = negative result; "/" = combined detection.

Test result
in interactive
format on
femoflor.online

2/2



FEMOFLOR®DeltaScreen

Patient name:	
Date of birth:	
Sampling date:	
Sample type:	V
Sample ID:	
Physician:	

Test result interpretation

- Relative amount of *Lactobacillus* spp. decreased (< 1%). Increased relative amount of aerobes (76-100%).
- No pathogens detected.

Control parameters	lg GE/mL	Reference
Human genomic DNA	4,5	≥ 3,5
Total bacterial load	5,1	≥ 3,5

Microbiota status

	lg GE/mL	%	Reference
NORMAL MICROBIOTA, total proportion		**0%**	**≥ 80%**
Lactobacillus spp.[1]	—	—	
AEROBES, total proportion		**76-100%**	
Staphylococcus spp.	—	—	
Streptococcus spp.	4,8	42-58%	
Enterobacteriaceae	—	—	
Enterococcus spp.	—	—	
Haemophilus spp.	4,7	34-46%	
ANAEROBES, total proportion		**0%**	
Gardnerella vaginalis	—	—	
Fannyhessea (Atopobium) vaginae	—	—	
Mobiluncus spp.	—	—	
MYCOPLASMAS, total proportion		**0%**	
Ureaplasma urealyticum	—	—	< 4,0 lg
Ureaplasma parvum	—	—	< 4,0 lg
Mycoplasma hominis	—	—	< 4,0 lg

[1] **Lactobacillus spp.**	lg GE/mL	Species proportion, %
L.non-iners *	—	—
L.iners	—	—

*Species representatives:
L.crispatus, L.jensenii, L.mulieris, L.gasseri, L.paragasseri

Yeast fungi	lg GE/mL	Reference
Candida spp.	—	< 4,0
Candida albicans	—	< 4,0

Pathogens	Result	lg GE/mL	Reference
OBLIGATE PATHOGENS (STI)			
Chlamydia trachomatis	—	—	—
Mycoplasma genitalium	—	—	—
Neisseria gonorrhoeae	—	—	—
Trichomonas vaginalis	—	—	—
HERPESVIRUSES			
HSV-1	—	—	—
HSV-2	—	—	—
CMV	—	—	—

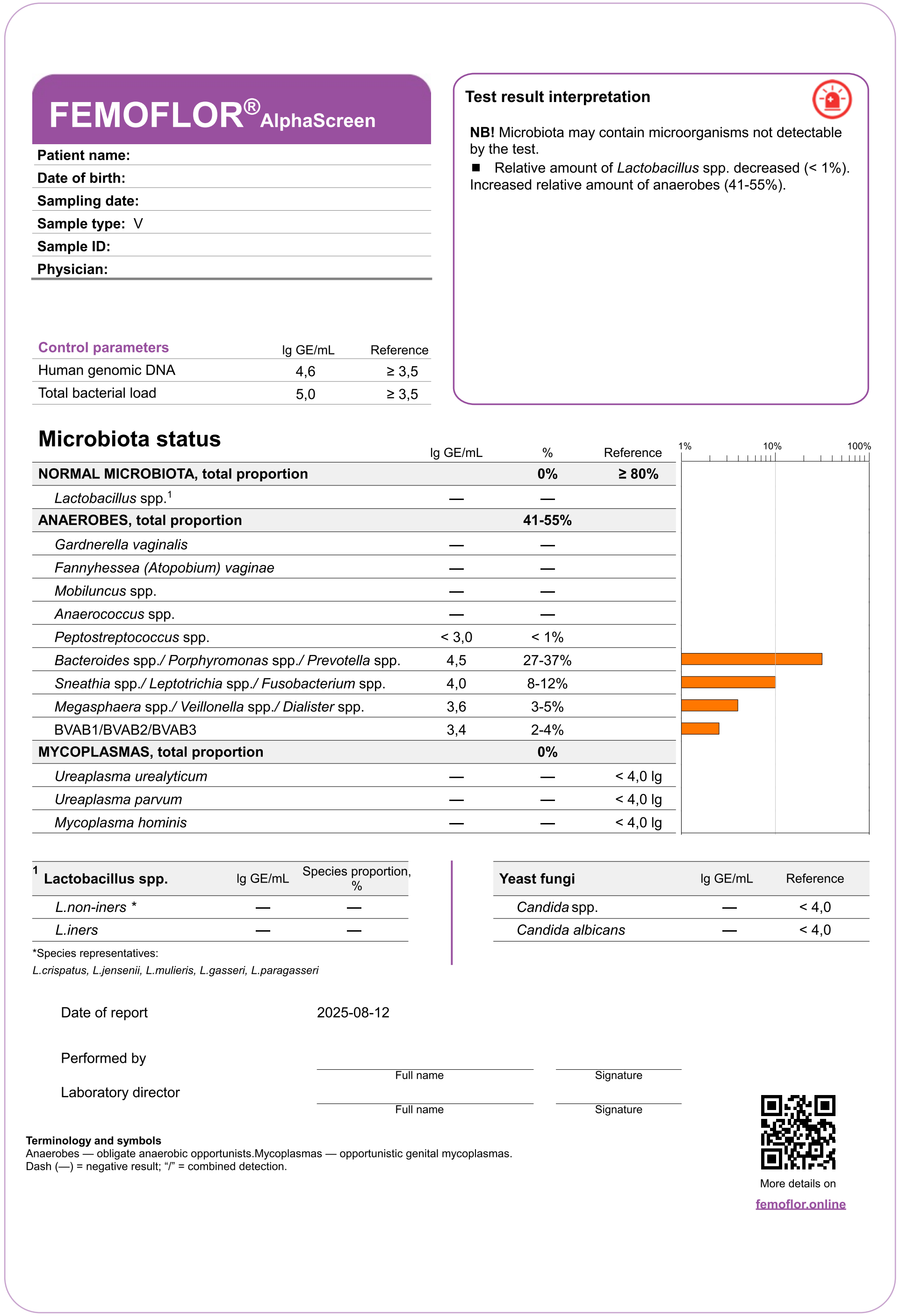
Date of report 2025-08-12

Performed by ____________ Full name ____________ Signature

Laboratory director ____________ Full name ____________ Signature

More details on
femoflor.online

Terminology and symbols
Aerobes — facultative-anaerobic opportunists. Anaerobes — obligate anaerobic opportunists. Mycoplasmas — opportunistic genital mycoplasmas.
Dash (—) = negative result; "/" = combined detection.



FEMOFLOR®AlphaScreen

Patient name:	
Date of birth:	
Sampling date:	
Sample type:	V
Sample ID:	
Physician:	

Test result interpretation

NB! Microbiota may contain microorganisms not detectable by the test.

- Relative amount of *Lactobacillus* spp. decreased (< 1%). Increased relative amount of anaerobes (41-55%).

Control parameters	lg GE/mL	Reference
Human genomic DNA	4,6	≥ 3,5
Total bacterial load	5,0	≥ 3,5

Microbiota status

	lg GE/mL	%	Reference
NORMAL MICROBIOTA, total proportion		**0%**	**≥ 80%**
Lactobacillus spp.[1]	—	—	
ANAEROBES, total proportion		**41-55%**	
Gardnerella vaginalis	—	—	
Fannyhessea (Atopobium) vaginae	—	—	
Mobiluncus spp.	—	—	
Anaerococcus spp.	—	—	
Peptostreptococcus spp.	< 3,0	< 1%	
Bacteroides spp./ *Porphyromonas* spp./ *Prevotella* spp.	4,5	27-37%	
Sneathia spp./ *Leptotrichia* spp./ *Fusobacterium* spp.	4,0	8-12%	
Megasphaera spp./ *Veillonella* spp./ *Dialister* spp.	3,6	3-5%	
BVAB1/BVAB2/BVAB3	3,4	2-4%	
MYCOPLASMAS, total proportion		**0%**	
Ureaplasma urealyticum	—	—	< 4,0 lg
Ureaplasma parvum	—	—	< 4,0 lg
Mycoplasma hominis	—	—	< 4,0 lg

[1] **Lactobacillus spp.**	lg GE/mL	Species proportion, %
L.non-iners *	—	—
L.iners	—	—

*Species representatives:
L.crispatus, L.jensenii, L.mulieris, L.gasseri, L.paragasseri

Yeast fungi	lg GE/mL	Reference
Candida spp.	—	< 4,0
Candida albicans	—	< 4,0

Date of report 2025-08-12

Performed by ____________ Full name ____________ Signature

Laboratory director ____________ Full name ____________ Signature

More details on
femoflor.online

Terminology and symbols
Anaerobes — obligate anaerobic opportunists.Mycoplasmas — opportunistic genital mycoplasmas.
Dash (—) = negative result; "/" = combined detection.

MICROBIOTA STATE

Normal microbiota

The microbiota of the female reproductive system is normally represented by lactobacilli, most commonly, by *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners* and *Lactobacillus jensenii*. However, the dominant groups of lactobacilli may differ at various points in life. Thus, the most commonly observed predominance of *L. crispatus* in the vagina may shift towards *L. iners* during menstruation (11).

One of the internationally used microbiome classification includes 5 vaginal community state types (CSTs). Four of them are dominated by *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus gasseri* (recently reclassified as *L. gasseri* and *L.paragasseri*), or *Lactobacillus jensenii* (recently reclassified as *L.jensenii* and *L.mulieris*). The remaining CST is characterized by a decrease in *Lactobacillus* spp. quantities and high diversity of bacterial species (12,13).

The microbiota in CST I is dominated by *L. crispatus*. This type is the most common and the most stable. CST II and CST V types dominated, respectively, by *L. gasseri* and *L. jensenii* are less common. CST III dominated by *L. iners* is considered transitional: *L. iners* are unable to maintain low pH values, making the microbiome conditions more suitable for the reproduction of opportunistic microorganisms. In CST IV, anaerobic bacteria are the dominant group (12). CST IV can be categorized into two subtypes based on the state of microbiota. In CST IV-A, the microbiome is dominated by anaerobes of the genera *Anaerococcus* spp., *Prevotella* spp., *Streptococcus* spp., with *L. iners* also present in small amounts, while in CST IV-B a predominance of BV-associated bacteria: *Atopobium* spp. and *Megasphaera* spp. is observed (1,14).

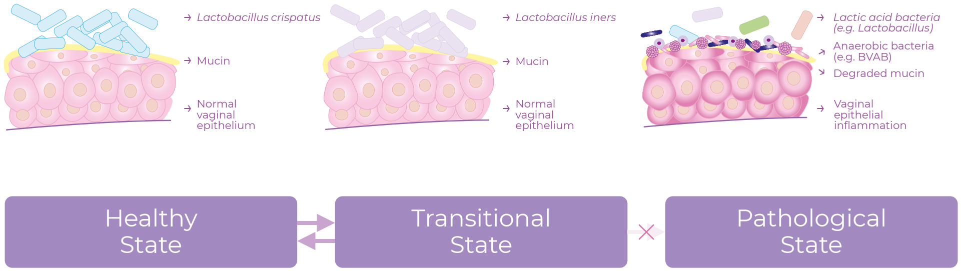
Each vaginal CST is associated with a specific pH range: the pH of CST I ranges from 3.7 to 4.3; CST II is between 4.3 to 5.7; CST III is from pH 3.8 to pH 5.0; CST IV is from 4.7 to 5.9, and CST V from 4.3 to 5.1(15).

Classification into cervicotypes (CTs) is another common method of cervical canal microbiome analysis. It relies on ectocervical swabs to determine the microbiota community structure (16). CT1 is mainly dominated by *Lactobacillus* spp. (except *L. iners*), CT2 is dominated by *L. iners*, CT3 — by *Gardnerella* spp. and CT4 is characterized by a predominance of BV-associated anaerobes.

Lactobacilli produce lactate during metabolism. *L. crispatus, L. gasseri* and *L. jensenii* are able to produce both D- and L- isomers of lactic acid, while *L. iners* can only produce L-lactic acid (17, 18). The D-isomer of lactic acid has a more pronounced inhibitory effect on exogenous bacteria, and therefore plays a greater protective role than L-lactic acid (19). In addition, *L. crispatus, L. jensenii, and L. gasseri* are major producers of H_2O_2 and bacteriocins that protect the biotope from opportunists, such as *S. aureus, Klebsiella spp.*, *E. faecalis*, and *E. coli* (20). This may explain why *L. crispatus, L. gasseri, and L. jensenii*, compared with *L. iners*, protect better against opportunists and are less frequently associated with adverse pregnancy outcomes (3, 19, 21, 22).

The predominance of ***L. crispatus*** in microbiota composition is a sign of stable microbiome in healthy women of reproductive age (CST 1) (23). *L. crispatus* have the highest capacity to produce lactic acid among vaginal lactobacilli and they, consequently, maintain the lowest vaginal pH of all CSTs (12, 25). A vaginal microbiome dominated by *L. crispatus* is linked to a lower prevalence of STIs (17). Also, *L. crispatus* can reduce the virulence of *Candida albicans* and enhance the local immune response of vaginal epithelial cells by increasing the concentration of IL-2, IL-6, IL-17 and decreasing IL-8 (23).

CSTI: *L. crispatus*-dominated

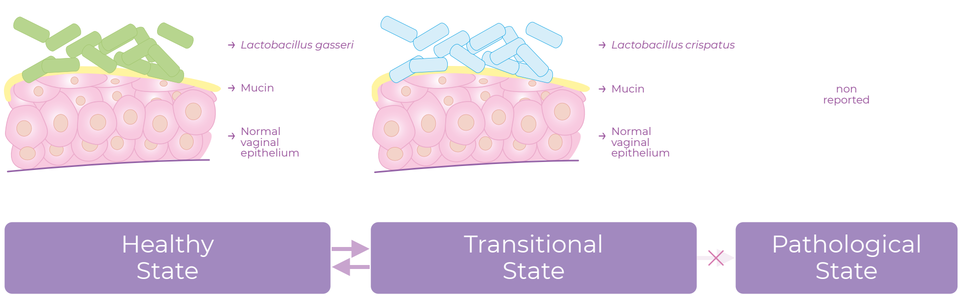


- This CST is relatively stable and transition to diseased state in a very rare occasion
- Low vaginal pH (< pH 4.0) contributed by the lactic acid and organic acids production from *Lactobacillus*
- CST I can undergo temporal transition to L. iners-dominated CST or mixed species *Lactobacillus* due to pregnancy, menses, and sexual practice

Adapted from Chee et al. (3)

L. gasseri-dominated normal microbiota is found in healthy women of reproductive age and is considered more dynamic than the one with *L. crispatus* predominance. Communities dominated by *L. gasseri* (CST II) have the highest pH among all communities characterized by a prevalence of *Lactobacillus* spp. (26, 27, 32).

CST II: *L. gasseri*-dominated



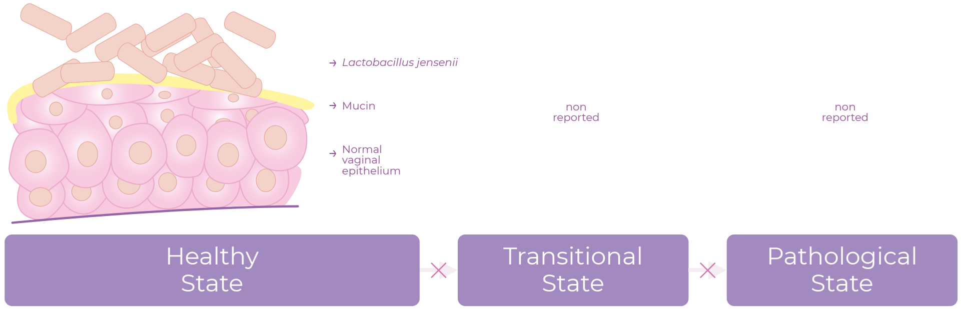
- This CST is dynamic, but the transition to diseased state CST is not actively reported
- The transition to CST I (*L. crispatus*-dominated) may occur rarely during pregnancy
- Low vaginal pH (pH 4.4) contributed by the lactic acid and organic acids production from *Lactobacillus*

Adapted from Chee et al. (3)

Normal microbiota dominated by ***L. jensenii*** is a rare occurrence, mostly found in healthy women of reproductive age. *L. jensenii* exhibit protective properties such as immunomodulation and competitive binding to epithelial cells (3, 28, 29, 30). A study by S. Navarro et al. found that *L. jensenii* are capable of glycogen metabolism across a wider pH range (4.0-5.0), while, for example, *L. crispatus* are more sensitive to pH values (25).

Although L. jensenii and L. gasseri are widely present in the vaginal microbiota, key strains of *L. gasseri* and *L. jensenii* frequently mentioned in various studies of the vaginal microbiome have recently been classified as separate species: *L. paragasseri* and *L. mulieri*s, respectively (31).

CST V: *L. jensenii*-dominated



- This CST is stable and no transition to other CST was reported
- The average vaginal pH is pH 4.2
- Less detailed reports available for the characteristics of this CST

Adapted from Chee et al. (3)

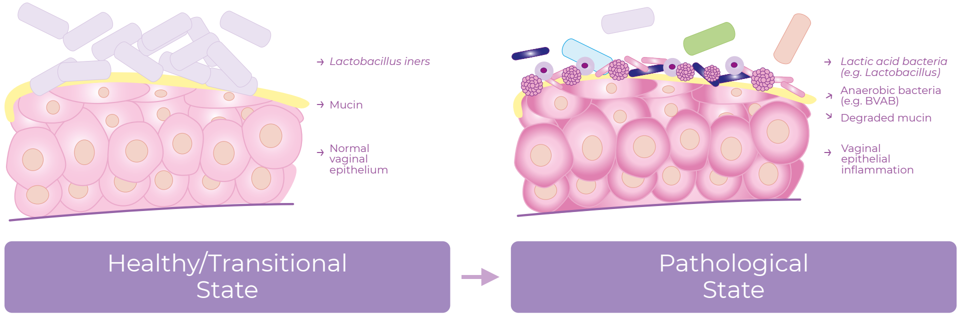
L. iners as one of the main representatives of vaginal normal microbiota has a Gram-positive morphology, but often stains as a Gram-negative bacterium, resulting in a high Nugent score and false positive results for BV (1, 32). Also, some isolates have coccobacillary rather than rod-like morphology (33, 34). *L. iners* is not cultured on Man-Rogosa-Sharpe agar, a selective growth medium for lactobacilli, and the nutrient requirements of *L. iners* are more complex than those of other vaginal lactobacilli (35, 36).

When the acidity of the biotope decreases, most lactobacilli lose viability (17). However, *L. iners* are tolerant to low acidity levels (37, 38). The small genome size of *L. iners* is thought to be the result of large-scale gene loss and genome reduction during rapid evolution to allow specific adaptation to the vaginal econiche (36, 39). According to a study by Macklaim et al., *L. iners* genome reduction included the loss of genes involved in carbohydrate transport and energy metabolism (39). In addition, *L. iners* lack a molecular and cellular mechanism for H_2O_2 production via pyruvate oxidation (17). The cell envelope of *L. iners* is thinner, allowing better adaptation to rapid changes in the vaginal environment (17, 38). Unlike other lactobacilli, *L. iners* produce pore-forming cholesterol-dependent cytolysins (inerolysins) commonly found in Gram-positive opportunistic bacteria, making them highly adhesive (35). Also, *L. iners* may be involved in the cleavage of BV-associated pathogenic biofilms (38, 40).

All these features suggest that *L. iners* are better adapted to the dynamic vaginal biotope than other vaginal lactobacilli. *L. iners* may predominate in the vagina of healthy women during menstruation as well as during inflammatory processes such as bacterial vaginosis. In addition, *L. iners* may also be found in patients receiving antibiotic therapy, given their resistance to metronidazole (40 - 42).

However, the predominance of *L. iners* is also associated with higher levels of proinflammatory factors such as MIF, IL-1a, IL18 and TNF-a, which are responsible for activating inflammatory responses in the vagina (3, 43). Vaginal microbiome dominated by *L. iners* is believed to be an adverse factor for pregnancy. A study by Petricevic et al. examined *Lactobacillus* spp. diversity in a subset of women who delivered at term versus those who experienced preterm labor and found that *L. iners* as the sole normal microbiota representative were found in 85% of preterm deliveries and only in 16% of at-term deliveries (44).

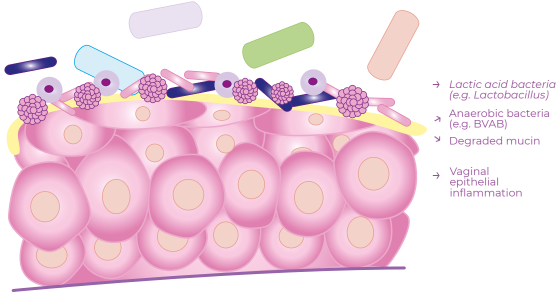
CST III: *L. iners*-dominated



- It is believed that transitional state of CST III (vaginal pH > 4.5) facilitated acquisition of BV as compared to healthy state (vaginal pH ~4.0)
- Associated with the higher baseline in pro-inflammatory factors such as MIF, interleukin-1a, interleukin 18, and TNF-a
- Decreased in the metabolic enzymatic activity of glycolysis (ALDO, DPS, GAPDH, and GPI) associated with higher vaginal pH (>4.5) in diseased state *L. iners*-dominated CST

Adapted from Chee et al. (3)

CST IV- (A and B): Anaerobic bacteria-dominated



- CST IV-A: modest proportion of *L. iners* along with BVAB
- CST IV-B: mostly dominated by BVAB, such as *Atopobium, Gardnerella, Mobiluncus, Prevotella*, and *Sneathia*
- This CST is commonly found in BV patients and healthy African women
- Low lactic acid production due to low number of *Lactobacillus*
- Disruption of mucin layer by BVAB compromising the binding site for LAB adhesion and ability to protect mucosal layer from BVAB
- High Nugent score (≥7) along with the formation of polymicrobial biofilm by BVAB in vaginal epithelium
- Increased the risk of STIs, preterm labour, miscarriage, and pelvic inflammatory diseases

Adapted from Chee et al. (3)

Although lactobacilli are considered the key representatives of normal microbiota, several studies have shown that healthy vaginal microbiome in 5 to 10% of women of reproductive age can be dominated by ***Bifidobacterium* spp.** (1, 45). *Bifidobacterium* spp. are also capable of producing lactic acid and H_2O_2 and tolerating low pH environments (46 - 48). In a study by Jun et al., a surge in *Bifidobacteriaceae* populations was observed in cases of persistent HPV infection (48).

NORMAL MICROBIOTA

Key biomarker of vaginal health. In women of reproductive age, normobiota should account for at least 80% of the total bacterial load. Lactic acid, hydrogen peroxide, and bacteriocins are the main defense factors synthesized by Lactobacilli and Bifidobacteria.

Opportunistic microbiota

When the population of normal microbiota representatives is reduced, the microbiota balance is disturbed, leading to conditions, such as bacterial vaginosis (BV), aerobic vaginitis (AV), mixed vaginitis, vulvovaginal candidiasis (VVC) and others. Although BV and AV share some common manifestations, such as low lactobacilli levels or lack thereof, increased intensity of discharge, and elevated pH levels (often more pronounced in AV), there are also significant differences between them. Women with BV do not develop inflammatory processes, whereas women with AV commonly show signs of inflammation such as swelling, redness and erosions. The discharge in BV patients is usually white or gray and watery, whereas in AV the discharge is thick and sticky, ranging in color from yellow to green. Women with BV do not present with dyspareunia, while some patients with AV may develop dyspareunia in severe cases of disease. The microscopic appearance of the two conditions also differs in various aspects, such as the presence of leukocytes and parabasal or immature epithelial cells in AV and the absence of granular microflora typical of BV. Despite these differences, AV and BV cannot always be properly differentiated, leading to incomplete or inaccurate diagnosis and inappropriate patient management.

Mixed vaginitis is characterized by simultaneous presence of two or more pathogens or opportunistic microorganisms in the vagina, which leads to imbalances in the vaginal biotope and causes a variety of symptoms (48 - 51). Depending on the particular microorganisms, these infections can be further categorized into more than 10 types, the most common of which are bacterial vaginosis, vaginal trichomoniasis, vulvovaginal candidiasis and aerobic vaginitis. Mixed vaginitis is becoming increasingly common worldwide, with incidence rates ranging from 4% to 56% (52, 53).

Both BV and AB can lead to severe health complications in women. In addition to the general symptoms of vaginitis, they can also increase the likelihood of pelvic inflammatory disease (PID) and HPV infection. When they occur during pregnancy, the risks of miscarriage, preterm labor, chorioamnionitis, neonatal infections, and other adverse outcomes significantly increase (54 - 56). Mixed infections often result in mixed biofilm formation, which increases pathogen resistance to therapy and to the host immune response (51).

Obligate anaerobes

Bacterial vaginosis (BV) is a dysbiotic condition of vaginal microbiota characterized by a decrease in lactobacilli producing lactic acid and an increase in quantities of anaerobic bacteria. The diversity of anaerobes includes ***Gardnerella* spp., *Fannyhessea vaginae*** (formerly known as *Atopobium vaginae*), ***Mycoplasma* spp., *Anaerococcus* spp., *Porphyromonas* spp., *Prevotella* spp., *Megasphaera* spp., *Leptotrichia* spp., *Sneathia* spp., *Dialister* spp., *Mobiluncus* spp., *Clostridium* spp.** and **BV-associated bacteria (BVAB1, 2 and 3)**. In addition, BV causes a change in the composition of Lactobacilli, replacing *L. crispatus* with *L. iners.*

BV is common in women of reproductive age. It can either be asymptomatic or accompanied by profuse vaginal discharge, itching, burning sensations and foul odor. BV is associated with an increased risk of STIs and pregnancy complications, including miscarriage, preterm labor, and postpartum endometritis.

BV-causing bacteria have the ability to form polymicrobial biofilms on the surface of vaginal epithelium. This is one of the main factors that complicates treatment and leads to chronic infection.

F. vaginae and ***G. vaginalis*** are the main constituents of microbial biofilms (57). *G. vaginalis* are highly tolerant to the acidic environment in the vagina and are present in 95-100% of BV cases. However, their presence does not always lead to the development of the disease (58, 59). The sialidase produced by some strains of *G. vaginalis* modifies the glycoproteins of the vaginal epithelial mucosa and allows the bacteria to attach to the vaginal wall. The biofilm formed by *G. vaginalis* promotes colonization by other opportunistic and pathogenic microorganisms and increases the bacterium's resistance to antibiotics, as well as to lactic acid and to H_2O_2 produced by lactobacilli (60). Presumably, some bacterial species, such as *Bacteroides* spp., *Enterococcus faecium, G. vaginalis, Mobiluncus* spp., *Peptostreptococcus* spp. can inhibit the growth of lactobacilli (61).

After *G. vaginalis* biofilm is formed, secondary colonizers such as *F. vaginae*, ***Megasphaera* spp.** and others attach to it. The microscopic appearance of epithelial cells with a bacterial biofilm (so-called clue cells) is a specific symptom of BV according to Amsel criteria (62).

In addition to its involvement in biofilm formation, sialidase can reduce the body's immune response to BV pathogens by degrading IgA (63). *G. vaginalis* also produce enzymes called vaginolysin and prolidase. The cytolytic activity of vaginolysin destroys vaginal epithelial cells and increases nutrient availability for *G. vaginalis*, while prolidase cleaves proline-specific dipeptides, causing distinctive odor associated with BV (64, 65). *Fannyhessea vaginae* (formerly belonged to the genus *Atopobium*) are often a major contributor to BV, and their co-presence with *G. vaginalis* is associated with recurrent BV (67). *F. vaginae* activate the key proinflammatory transcription factor NF-kB in cervical and vaginal cells and promote the production of β-defensin and proinflammatory chemokines IL-6, IL-8, and TNF-α. The ability of *F. vaginae* to activate signaling pathways through the transcription factor NF-kB may play a role in inducing HIV transcription (68). In addition, this species can secrete lactic acid, similarly to *Leptotrichia* spp. and *Megasphaera* spp., which may mask symptoms of BV leading to asymptomatic disease course, which complicates the diagnostics (69, 70).

Species of the genus ***Porphyromonas*** secrete broad-spectrum proteases that can diffuse freely into the cervical canal. These proteases cleave fibrinogen and type I and IV collagens found in the cervix, placenta, and fetal membranes. *P. asaccharolytica, P. uenonis, P. bennonis*, and *P. somerae* species have been found in the vaginal biotope. Recent studies have linked *P. asaccharolytica* and *P. uenonis* to preterm labor, pelvic inflammatory disease and HPV infection that progresses to neoplasia and cervical cancer. *P. gingivalis* have also been isolated from placenta and amniotic fluid of women who experienced preterm labor. This species also secretes gingipains that degrade immunoglobulins as well as components of the extracellular matrix and the complement system. Gingipains also activate matrix metalloproteases of epithelial cells (MMPs), which subsequently degrade collagen. The ability of *Porphyromonas* spp. to degrade fibrinogen may be associated with impaired blood clotting, which may lead to pregnancy loss, placental abruption and increase the risk of postpartum hemorrhage (71).

Species belonging to ***Prevotella*** are among the most common bacteria in the human microbiome. *P. bivia* trigger the production of cytokines associated with pregnancy complications (NF-κB, MIP-3α, RANTES, IL-8). In addition, they can suppress the immune response to G. vaginalis (72). Synergetic relationships between *Prevotella* spp. and other microorganisms have also been described: *P. bivia* produce ammonia, enhancing the proliferation of *G. vaginalis*, and metabolize amino acids produced by *G. vaginalis*. At the same time, amino acids synthesized by *P. bivia* enhance the growth of *Peptostreptococcus anaerobius*. *P. bivia* and *P. disiens* produce collagenase, fibrinolysins, as well as sialidase and prolidase, which can degrade the mucosal surface and lead to detachment of vaginal epithelial cells (72).

Bacteria of the genus ***Mobiluncus*** produce malic acid and trimethylamine, which cause irritation of the vaginal mucosa and the distinct foul odor characteristic of BV. *Mobiluncus* spp. also possess sialidase activity (73). *Mobiluncus* spp. and BVAB1 contain flagellin, which activates Toll-like receptor 5 (TLR5) and increases expression of the inflammatory factors IL-6, IL-8, and TNF-α in the cervical canal, which may explain their ability to colonize the upper urogenital tract (74,75).

BV-associated bacteria (**BVAB1, 2, 3**), have been described as causative agents of BV belonging to the order Clostridiales in 2005 based on 16S RNA amplification (76). These bacteria are unculturable, their detection is only possible using molecular genetic techniques, and their presence is highly specific to BV. Recently, the species of this group of bacteria has been identified: BVAB1 has been classified as *Candidatus Lachnocurva vaginae*, BVAB2 as *Oscillospiraceae* bacterium strain CHIC02, and BVAB3 as *Mageeibacillus indolicus* (77).

The presence of these pathogens in HPV-infected women has been linked to the progression of CIN (78). BVAB1 produce polyamines, including putrescine, cadaverine and trimethylamine, which leads to an increase in vaginal pH the and promotes the colonization of the vagina by other anaerobic bacteria. BVAB1 frequently co-occurs with BVAB3 and *Prevotella* spp. which may indicate a synergic relationship between these bacteria (78). *Dialister* spp. and *Veillonella* spp. also produce putrescine (79).

***Sneathia* spp.** are strict anaerobes capable of producing cytotoxic hemolysin. They are difficult to cultivate and undetectable by cultural method. *S. amnii* and *S. sanguinegens* promote the production of the proinflammatory factors IL-1α, IL-β, TNF-α, and IL-8 (16, 80).

Compared to other BV-associated bacteria, *Sneathia* spp., ***Leptotrichia* spp.**, ***Peptostreptococcus anaerobius***, and ***Anaerococcus tetradius*** are more common in HPV-infected women and in women with precancerous lesions and cervical cancer (81, 82). This observation indicates that these species may play a role in disease progression. In addition, there is evidence to suggest that *Leptotrichia amnionii* and *Sneathia sanguinegens* are associated with spontaneous abortion (83).

Bacteria of the genus ***Fusobacterium*** are common inhabitants of the oral cavity and can be transmitted through unprotected oral-genital contact. *F. nucleatum* are associated with fetal infection and preterm labor. Although this species does not express its own sialidase, it is involved in a symbiotic relationship with other species such as *G. vaginalis* (78).

***Bacteroides* spp.** can inhibit the growth of lactobacilli and express sialidase (85, 86).

Members of ***Clostridium* spp.** are found in the vaginal microbiota of 4-8% of healthy women. However, some species, most notably *C. sordellii*, can cause postpartum and postsurgical complications (87).

Changes in the vaginal microbiota composition lead to alterations in immune and metabolic processes, which may influence the development of other gynecopathies. Women with endometriosis, chronic endometritis, endometrial polyps, dysfunctional uterine bleeding, endometrial cancer/hyperplasia or infertility have an altered vaginal microbiota composition (88).

Mobiluncus curtsii, Mobiluncus mulieris, Sneathia sanguinegens, Fannyhessea vaginae and *Megasphaera* spp. are associated with preterm labor. Evidently, the enzymes produced by these bacteria (proteases, mucinases, hemolysin and others) can degrade the microstructures of fetal membranes, leading to the synthesis of cytokines and prostaglandin and the release of arachidonic acid, thereby triggering myometrial contraction and the initiation of labor (89).

ANAEROBES

They can be found in the reproductive system in small quantities under normal conditions, when the normal microbiota predominates. Excessive proliferation is associated with the development of bacterial vaginosis, mixed infections. Anaerobes participate in biofilm formation, which reduces sensitivity to antibacterial drugs.

Facultative anaerobes

Facultative anaerobes (aerobes) in increased quantities lead to an imbalanced vaginal microbiota, which often causes AV. The incidence of AV ranges from 12% to 23.7% in non-pregnant women and from 4 to 8% during pregnancy (90). AV can occur in association with other related conditions such as BV and VVC. AV also increase the risk of contracting STIs (91, 92).

The microbiota in AV consists of opportunistic aerobic microorganisms of predominantly intestinal origin (93). The most common ones include ***Staphylococcus* spp.** *(S. aureus)*, ***Streptococcus* spp.** *(S. agalactiae)*, ***Enterobacteriaceae*** (*E. coli* and *Klebsiella spp.*), ***Enterococcus* spp.** (*E. faecalis*) and ***Haemophilus* spp.** (94 - 97).

Purulent vaginal discharge in AV (especially in the presence of *S. agalactiae, E. coli, S. aureus, K. pneumoniae*, and *E. faecalis*) can cause complications predisposing the patient to an ascending infection and leading to premature discharge of amniotic fluid, preterm labor, and spontaneous abortion in pregnant women (98-101).

Among staphylococci, ***S. aureus*** are notable opportunistic bacteria that are detected in the vaginal microbiota of 30% of women, 4 - 22% of which are during pregnancy (12, 102 - 104). A study investigating the colonization of methicillin-resistant *S. aureus* (MRSA) showed that 3.5% out of 5732 mothers had MRSA in the vaginal biotope during pregnancy (24). S. aureus produces TSS-toxin-1 leading to the development of toxic shock syndrome, an acute life-threatening condition characterized by sudden onset of fever, skin manifestations, hypotension and multi-organ failure (105).

Group B streptococci (GBS), or ***S. agalactiae***, are β-hemolytic Gram-positive bacteria that are a major cause of neonatal infections. *S. agalactiae* usually colonize the lower gastrointestinal and urogenital tracts. *S. agalactiae* are detected in the vaginal biotope of nearly 25% of women (106). Carriage of these bacteria is usually asymptomatic.

Women who have been infected vaginally during pregnancy are at risk of developing an ascending infection or transmitting *S. agalactiae* to the newborn during labor. If *S. agalactiae* penetrate the amniotic cavity or contact with the placenta, chorioamnionitis or inflammation of the placental membranes may develop (107, 108). Although the introduction of antibiotic prophylaxis during labor has reduced the incidence of neonatal infections, this measure does not prevent ascending infections, which can occur earlier in pregnancy and lead to preterm labor, stillbirth, or late-onset neonatal infections (108-110). *S. agalactiae* is largely present together with *S. aureus* in pregnant women, which can trigger toxin production by *S. aureus* (105, 111). Around 2.9% of pregnant women are infected with group G or C streptococci (111, 112), putting them at an increased risk of postpartum endometritis.

***Enterococcus* spp.** and *Enterobacteriaceae* are representatives of the gastrointestinal microbiota, known as one of the most common causes of nosocomial urinary tract infections (113, 114). *E. coli* are a major contributor to maternal and neonatal morbidity and mortality (115, 116). *E. coli* accounts for 80 to 90% of AV infections in pregnant women and is often isolated as the sole microorganism in these cases (117 - 119). *E. coli* were more frequently detected in women who delivered prematurely compared to those who delivered at term (119 - 121). Preterm neonates are more likely to develop neonatal sepsis and meningitis caused by *E. coli* (119, 122, 123). Evidence also suggests that the presence of *E. coli* in the biotope increases the pathogenicity of *Candida* spp.

Studies have shown that asymptomatic carriage of *Enterococcus* spp. in the genital tract is prevalent in pregnant women (119, 124). Enterococci can cause life-threatening infections in premature infants (119, 125). *E. faecalis* and *E. faecium* are considered the most significant human pathogens among enterococci. *E. faecalis* can boost TSST-1 toxin production and increase the virulence of *S. aureus*. Also, *E. faecalis* can cause inflammation and elevate IL-6, IL-8, and TNF levels in AV patients, elevating the risk of HPV infection (126, 127). An association between increased enterococcal colonization and prematurity has been found, with premature neonates carrying *E. faecalis* more likely to suffer from necrotizing enterocolitis, compared to neonates born at term (119, 125, 128, 129).

Vaginal carriers of ***Haemophilus* spp.** represent around 10% of women (130). *H. influenzae* is a potential cause of invasive infections during pregnancy accounting for 4.4% of women (131-133). It is also important to consider the possibility of vertical (mother-to-child) transmission of bacteria in pregnant women, which can result in neonatal sepsis (130).

AEROBES

They can be found in the reproductive system in small quantities under normal conditions, when the normal microbiota predominates. Excessive proliferation is associated with the development of infectious and inflammatory diseases of the reproductive system (vaginitis, cervicitis), the risk of pregnancy and postpartum complications, and neonatal infections.

Mycoplasmas

Mycoplasma hominis, Ureaplasma urealyticum and ***Ureaplasma parvum*** are opportunistic bacteria that can cause cervicitis, urethritis, cystitis under certain conditions, leading to pregnancy, postpartum and post-abortion complications.

M. hominis live and replicate not only in human cells but also in *Trichomonas vaginalis* cells, which are capable of spreading bacterial infection to both mycoplasma-free protozoan isolates and human epithelial cells (160). *M. hominis* synergistically enhance the proinflammatory response to *T. vaginalis*. *M. hominis* lipoproteins can stimulate IL-23 production by dendritic cells and macrophages, contributing to a strong proinflammatory response (160). Infection caused by *M. hominis* is usually asymptomatic and is sometimes associated with vaginal microbiota alterations and BV (208). The presence of *M. hominis* in the vagina of women with BV correlates with high levels of amines and short-chain fatty acids (209). Short-chain fatty acids play an important role in a wide range of immune responses by inhibiting the production of proinflammatory cytokines, affecting migration and phagocytosis of immune cells, and inducing apoptosis in various cell types, including neutrophils (204). These data support the hypothesis of the prominent role of *M. hominis* in the development of BV.

M. hominis can colonize the uterus and placenta of pregnant women, causing severe complications such as preterm labor and chorioamnionitis (210). Intrauterine infection is a rare occurrence, except when the fetal membranes are ruptured. Colonization with genital mycoplasmas, most commonly, with *M. hominis* and/or ureaplasmas, usually happens through contact with the cervix and vagina during childbirth. Babies born by cesarean section are infected much less frequently. Neonatal colonization with *M. hominis* and ureaplasmas usually does not persist (208). Some studies suggest that ureaplasmas may influence premature discharge of amniotic fluid and may be associated with placental inflammation and neonatal respiratory distress syndrome (211).

U. urealyticum may increase the risk of infection with HPV of high oncogenic risk (211), and ***U. parvum*** may play a synergetic role in the initial stage of cervical lesions caused by papillomavirus infection (184).

MYCOPLASMAS

They can be found in the reproductive tract in small quantities under normal conditions, when the normoflora predominates. Excessive proliferation and the absence of timely treatment are associated with the development of urinary tract diseases, pelvic inflammatory disease (PID), and an increased risk of pregnancy complications, as well as postpartum, and neonatal complications.

Yeast fungi

Candida albicans, along with other closely related *Candida* species, are the primary cause of VVC – a multifactorial infectious disease of the female lower genital tract. Unlike other forms of candidiasis, VVC is a disease of immunocompetent and healthy women, prevalent mainly in reproductive age. As representatives of the opportunistic human microbiota, *C. albicans* usually colonize the vagina asymptomatically. However, symptomatic infection can occur as a result of intense inflammation of the mucosa, which is caused primarily by excessive proliferation of microorganisms in the vagina, subsequent invasion of the epithelium and production of virulence factors. Risk factors for VVC include antibiotic use, immunosuppressive therapy, use of estrogen-rich oral contraceptives, sexual activity, pregnancy, use of sodium-glucose cotransporter 2 (SGLT2) inhibitors, and uncontrolled diabetes mellitus (194 - 196).

C. albicans is the causative agent in more than 90% of VVC cases. However, other *Candida* species (*Candida* non-*albicans*) have also been identified as etiologic agents. Of these, *C. glabrata* is considered the second leading cause of VVC (~8% of cases), while *C. krusei, C. parapsilosis*, and *C. tropicalis* account for the majority of the remaining VVC cases. Symptoms of infection with *Candida* non-*albicans* species are often less severe (194-196).

The highest prevalence of VVC is observed among pregnant women in the second and third trimesters. This is associated with decreased cellular immunity, increased estrogen levels, and increased glycogen production, which promotes vaginal colonization by *Candida* spp. (197-199). Increased estrogen levels initiate the transition of *Candida* spp. from yeast form to invasive filamentous form by activating candida lysin secretion (201). This is the most crucial pathogenicity factor, represented by a toxin with lytic activity that directly damages vaginal epithelial cells (201, 202).

VVC during pregnancy may be associated with adverse birth outcomes, including preterm labor, premature rupture of fetal membranes, chorioamnionitis, low fetal birth weight, and postpartum infections (201, 205).

Almost all clinically significant yeast species of the genus *Candida* form biofilms. The presence of a biofilm, a highly resistant structure that is impermeable to most antimicrobials, on the vaginal mucosa, may explain the ineffectiveness of antifungal drugs. Biofilms can also act as a reservoir of persistent cells causing recurrence of VVC (204, 206, 207).

A specific characteristic of urogenital tract infections is the frequent association of opportunistic and pathogenic microorganisms with each other (mixed infections), the absence of pathognomonic symptoms, vague clinical manifestations with predominance of chronic disease forms, which requires complex diagnostics to establish the diagnosis and prescribe etiological treatment.

YEAST FUNGI

They can be found in the reproductive tract in small quantities under normal conditions, when the normoflora predominates. Excessive proliferation can cause inflammation of the reproductive tract, increase the risk of preterm labor and fungal infections in the newborn.

Pathogens

Obligate pathogens

Chlamydia trachomatis is an obligate intracellular pathogen known as the most common etiological agent of bacterial STIs. Chlamydiae have tropism for cylindrical epithelium which allows them to affect the mucous membrane of the urethra, cervical canal, rectum, oropharynx and conjunctiva of the eyes. Microorganisms are unable to proliferate in the intact multilayer squamous vaginal epithelium of adult women due to their high sensitivity to the acidic environment of its contents. The primary lesion focus is usually the cervical mucosa, and the most frequent clinical manifestation is mucopurulent cervicitis. It is important to note that the course of chlamydial infection is often subjectively asymptomatic, which prevents the disease from being diagnosed in the early stages in its uncomplicated form. In cases of delayed therapy or lack of treatment, the infectious process may become ascending and/or chronic and cause severe complications such as PIDs and infertility (134-138). PIDs increase the risk of adhesions, chronic pelvic pain syndrome and ectopic pregnancy (137 - 139). Chlamydia infection in pregnant women can lead to pregnancy termination, miscarriage, fetoplacental insufficiency, intrauterine and neonatal infections and postpartum inflammatory diseases (141, 146).

Epidemiologic data indicate that chlamydial infection significantly increases the risk of cervical and ovarian cancer. That is presumably due to unresolved chronic inflammation leading to aberrant production of cytokines, chemokines, and growth factors, as well as dysregulated flow of immune cells into tissues. *C. trachomatis* may contribute to cancer development through aberrant activation of the LIF pathway, which regulates multiple hallmarks of cancer, including proliferation, metastasis, and chemoresistance. A substantial decrease in the number of ciliated epithelial cells and an increased population of less differentiated secretory epithelial cells has been noted, which may be associated with an elevated risk of post-infection infertility, ectopic pregnancy, and cancer (138).

C. trachomatis co-infection with HPV is widespread and often asymptomatic. Chlamydia can cause lesions of the mucosa and affect the immune response and virus clearance, which leads to inflammation, decreased population of effector T cells, activation of dendritic cells and production of proinflammatory cytokines/chemokines, and contributes to virual persistence (140, 141).

Women with tubal infertility and chlamydia have unique vaginal microbiota dominated by *Lactobacillus iners* rather than *Lactobacillus crispatus*. This is coupled with an overall reduction in *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacter* spp. and *Streptococcus* spp. quantities as well as decreased levels of cytokines, such as IFN-γ and IL-10. This altered vaginal microbiota can be restored to a certain extent following standard treatment of chlamydial infection (134).

Mycoplasma genitalium is a pathogenic microorganism belonging to the motile species of bacteria. *M. genitalium* has tropism for cylindrical epithelium and an ability to infect the mucous membrane of the urethra and cervical canal. Over 50% of infected women are subjectively asymptomatic (143). *M. genitalium*-associated PIDs are characterized by fewer inflammatory markers and less pronounced clinical manifestations, which complicates timely diagnosis and increases the risk of complications, such as infertility, pregnancy failure, adhesions, chronic pelvic pain syndrome and ectopic pregnancy (140, 144, 145). Urogenital mycoplasma infection is a risk factor for spontaneous preterm labor and low birth weight (145, 146, 147).

Neisseria gonorrhoeae is a Gram-negative, non-motile, non-spore-forming bacterium that causes gonococcal infection. *Neisseria gonorrhoeae* firmly attach to the epithelial cells of the mucous membrane, causing an inflammatory reaction with the release of purulent exudate. Since *Neisseria gonorrhoeae* do not express potent exotoxins, activation of the innate immune response at colonization sites is the main trigger of the infectious process (150). They infect the cylindrical epithelial cells of the urethra and cervical canal and spread through the mucosa and lymphatic vessels to other parts of the urogenital tract (fallopian tubes, ovaries). More than 70% of women have subjectively asymptomatic course of the disease. In menopausal women, vaginal epithelial cells may be affected, which triggers the development of vulvovaginitis (151). An ascending infection may lead to PIDs, while chronic forms of infection can cause infertility (150, 151). Women presenting with gonococcal infection during pregnancy are more likely to experience adverse birth outcomes, including preterm labor, premature rupture of fetal membranes, low birth weight, neonatal ophthalmia, and perinatal mortality (140, 146, 153, 154). Extragenital disease forms may affect the mucous membranes of the oropharynx, rectum, and conjunctiva. Hematogenous dissemination of Neisseria gonorrhoeae with transient gonococcemia or gonococcal sepsis is a rare occurrence (151, 155).

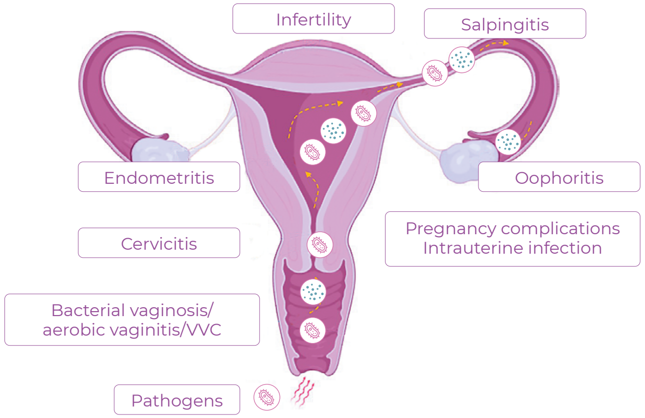
Trichomonas vaginalis are unicellular protozoa with tropism for squamous epithelium. In women, urogenital trichomoniasis occurs predominantly in the form of vulvovaginitis, cervicitis, and, less frequently, urethritis (156). The ascending infection may result in the development of PIDs. In 20-40% of cases urogenital trichomoniasis is subjectively asymptomatic, which complicates diagnosis and treatment at early disease stages, leading to the onset of complications and further spread of the infection. Urogenital trichomoniasis is associated with complications, such as ectopic pregnancy, preterm labor, low birth weight, and infertility (140, 147, 157).

T. vaginalis produce cytotoxic chemicals, such as cell-detaching factor and N-nitrosamines, which stimulate epithelial atypia and dysplasia (157). The presence of *T. vaginalis* in the urogenital tract is associated with persistence of most carcinogenic HPV types and the risk of cervical dysplasia (157 - 159).

An important pathobiological aspect of urogenital trichomoniasis is the complex relationship between *T. vaginalis* and intracellular symbionts, particularly *M. hominis*, which underlie the high variability in clinical manifestations and complications of this infection (160). The biocommunication between *T. vaginalis* and *M. hominis* significantly influences the pathogenesis of infectious processes caused by both microorganisms. In fact, *M. hominis* gain protection from antibiotics and host immune response due to its intracellular localization in trichomonad cells, while *T. vaginalis*, in turn, increases its cytopathic activity, damages host cells, activates phagocytosis and proinflammatory response (158).

OBLIGATE PATHOGENS

They should normally be absent from the female reproductive system. These microorganisms cause infectious diseases of the reproductive tract with similar clinical presentations and a high prevalence of asymptomatic cases. Untreated infections are a major risk factor for the development of pelvic inflammatory diseases (PID), reproductive issues, chronic pelvic pain syndrome, neonatal infections, and postpartum complications.



Adapted from Yu et al. (163)

Viruses

Herpesvirus infection is a chronic recurrent viral disease caused by herpes simplex virus types 1 **(HSV1)** and 2 **(HSV2)**. The most common cause of genital herpes is HSV2. Clinical forms of urogenital herpes infection in women include vaginitis, cervicitis, urethritis, and cystitis (161). Recurrences of the disease in case of HSV2 infection occur more frequently than with HSV1 infection and are accompanied by more pronounced clinical symptoms.

Herpes infection during pregnancy increases the risk of spontaneous abortion, preterm labor, and stillbirth (161). Vertical transmission can cause spontaneous abortion or fetal infection. Both primary and recurrent herpes infection in pregnant women can lead to intrauterine transmission, resulting in congenital infection (163). Intrauterine transmission is far more prevalent with primary infection during pregnancy (164). Crucially, intrauterine herpes infection is one of the leading causes of congenital malformations (165).

Neonatal herpes infection is more common in children born to mothers with primary herpes infection compared to children of those with recurrent herpes infection (166). The disease can manifest in three clinical forms: 1) local, with recurrent lesions of the skin, eyes, and oral mucosa; 2) local, mainly affecting the nervous system (encephalitis, meningoencephalitis); and 3) disseminated, followed by the development of sepsis (166-168).

Cytomegalovirus infection is a viral disease caused by cytomegalovirus **(CMV)**. It is characterized by diverse clinical manifestations ranging from asymptomatic course to severe generalized forms with damage to internal organs and central nervous system (169). Cytomegalovirus is one of the most clinically significant viral pathogens during pregnancy. CMV infection during pregnancy can have a negative impact on fetal development and pregnancy outcome. CMV increases the risk of spontaneous abortion, preterm labor, and stillbirth (170). Congenital infection results from intrauterine CMV transmission from mother to fetus. This can happen in cases of primary infection during pregnancy, reactivation of infection during pregnancy, or infection of a seropositive pregnant woman with a different CMV strain (171) due to its presence in the vagina and cervix (172). The risk of infection at birth is higher in the case of primary infection during pregnancy, but fetal CMV infection can also result from infection reactivaion during pregnancy (173, 174). Depending on the gestational age at which the infection occurs, fetal lesions range from blasto- and embryopathies, often accompanied by termination of pregnancy, to late fetopathies affecting various organs and systems of the newborn. Congenital CMV infection can cause congenital anomalies leading to fetal death, severe generalized (possibly lethal) disease of the newborn, and irreversible disabling diseases such as sensorineural hearing loss, blindness, cerebral palsy, and retarded neuropsychological developmental (169, 175, 176).

Human papillomaviruses (HPV) of high oncogenic risk have tropism for human epithelial cells, the most sensitive of which located in the cervical transformation zone. They can cause neoplastic transformation of the epithelium and act as the main etiological factor in the development of cervical cancer (177, 178, 179). According to WHO, HPV of high oncogenic risk include types 16, 18, 31, 33, 33, 35, 39, 45, 51, 51, 52, 56, 58, 59, 66, 68 (178). Around 50% of cervical precancerous lesions are caused by HPV types 16 and 18 (178, 180, 181). The virus infects the mucosa and produces viral particles in epithelial cells. Viral oncoproteins E5, E6, and E7 affect cell cycle regulation, leading to uncontrolled cell division and accumulation of genetic damage (182, 183). It is known that in many cases self-elimination of the virus takes place within a few years. The presence of HPV of high oncogenic risk in squamous epithelial cells is a predisposing factor for the development of cervical precancer, and persistent HPV infection is a key risk factor for cervical squamous cell cancer (184, 185).

Research suggests that HPV-associated CIN is accompanied by pronounced dysbiosis with a predominance of obligate anaerobes. Differences in microbiota composition have been observed in women with normal cytology as well as in those with cervical lesions and cancer (191). *L. crispatus* and *L. iners* were the predominant lactobacilli species in women without cytological abnormalities (both in the presence and absence of infection), while *Sneathia* spp. and *Fusobacterium* spp. predominated in those with squamous cell intraepithelial lesions and cervical cancer, respectively. The prevalence of CST IV community (high diversity microbiome lacking *Lactobacillus* spp.) was directly proportional to the severity of cervical pathology: the proportion of CST IV progressively increased in low grade squamous intraepithelial lesions (LSIL), high grade intraepithelial lesions (HSIL) and cervical cancer. Moreover, higher quantities of *Sneathia sanguinegens, Anaerococcus tetradius and Peptostreptococcus anaerobius* were observed in HSIL samples than in LSIL samples, indicating alterations in microbiota composition depending on the disease severity (192). Similarly, *Lactobacillus* dominance declined as the severity of cervical dysplasia increased, while *Sneathia* spp. quantities were elevated in precancerous lesions of low/high malignancy and invasive cervical carcinoma (193). Thus, performing an extended PCR test can assist in the diagnosis and rectification of dysbiotic disorders can reduce the risk of CIN progression.

HPV infection during pregnancy can negatively affect the health of both the mother and the baby, increasing the risk of severe pregnancy complications. These include spontaneous abortion, preterm labor, preeclampsia, delay of fetal developmental, premature rupture of fetal membranes, and stillbirth (185, 186).

Extended genotyping is necessary not only in cases of cervical squamous cell lesions but also for screening cervical glandular intraepithelial neoplasia, given the high prevalence of HPV types 16, 18, and 45 infection in patients with neoplasia and its antecedent changes (187, 188).

Infection with different HPV types varies significantly in cumulative risk and annual rate of progression to precancerous changes. HPV genotyping is an important part of accurate risk assessment and optimal clinical management of patients. HPV type 16 is uniquely carcinogenic and should, therefore, be differentiated individually. HPV types 18 and 45 are associated with an elevated risk of cancer, especially for adenocarcinoma and squamous cell carcinoma. HPV types 31, 33, 35, 52, and 58 are linked a significantly higher risk of cancer compared to HPV types 39, 51, 56, 59, and 68 (189). HPV genotypes 31, 33, and 58, along with HPV 45 and 52, account for nearly 15% of cervical cancers and 11% of all HPV-associated cancers (190).

VIRUSES

Herpesviruses are a risk factor for reproductive disorders; during early pregnancy, they can cross the placental barrier and cause various complications. HPV infection is associated with an increased risk of pregnancy complications, development of dysbiosis and a higher susceptibility to STIs. The role of high-risk (oncogenic) HPV types in the development of reproductive cancers has been proven.

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