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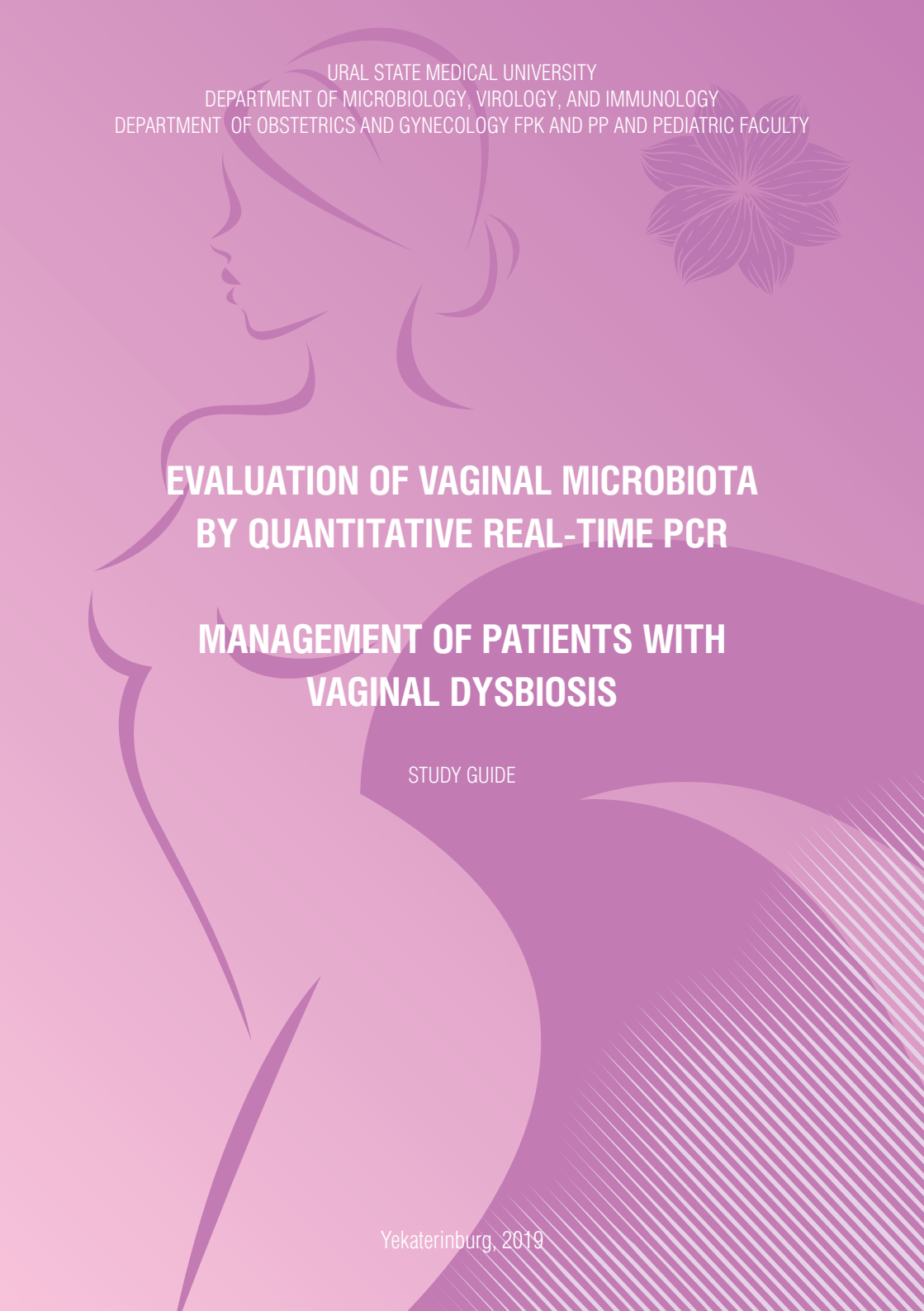

EVALUATION OF VAGINAL MICROBIOTA BY QUANTITATIVE REAL-TIME PCR

MANAGEMENT OF PATIENTS WITH VAGINAL DYSBIOSIS

STUDY GUIDE

Yekaterinburg, 2019

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STUDY GUIDE

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This guide is devoted to problems related to the management of patients with vaginal dysbiosis and the treatment of vaginal infections associated with opportunistic microorganisms. The guide presents the current concepts about vaginal microbiota (taking into account large-scale studies of the last decade), modern techniques for studying this microbiota, and diagnosing dysbiosis. Recommendations for the specific treatment of patients with different variants of vaginal dysbiosis or pathologies caused by opportunistic microflora are presented.

Indications for quantitative analysis of the vaginal microbiota by Femoflor

- Clinical and/or laboratory signs of urogenital tract infection;
- Potencial alteration of vaginal microbiota after:
  - ➤ Treatment by antibiotics (both oral and topical), hormones or cytostatics,
  - ➤ Use of contraceptives, including intrauterine devices (IUDs),
  - ➤ Vaginal douching,
  - ➤ Sex with a new partner,
  - ➤ Other factors affecting vaginal microbiota;
- Planning of non-urgent vaginal or pelvic surgery;
- Bad reproductive and obstetric history (spontaneous abortion and recurrent pregnancy losses, preterm delivery, infertility);
- Preconception care;
- Pregnancy (all the trimesters);
- Discrepancy between clinical and laboratory findings;
- Postmenopausal atrophic vaginitis;
- Screening test for asymptomatic women (together with gynecological smear).

CONTENT

LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
ATP	Adenosine triphosphate
AV	Aerobic vaginitis
BROH	Bad reproductive and obstetric history
BV	Bacterial vaginosis
GE/ml	Genome equivalents per 1 milliliter
HPV	Human papilloma virus
HSV	Herpes simplex virus
II	Inflammation Index
LFU	Low frequency ultrasound
MO	Microorganism
OM	Opportunistic microorganisms
PCR	Polymerase chain reaction
RT-PCR	Real-time PCR
STP	Sexually transmitted pathogens
TBM	Total bacterial mass (load)
TMD	Total microorganisms detected
VC	Vulvovaginal candidiasis
VIR	Vaginal inflammatory response
WBC	White blood cells

1. MODERN VIEWS ON VAGINAL MICROBIOTA

The term **vaginal microbiota** is generally used in reference to all microbial taxa on the surface of the vaginal epithelium. This biotope is of particular interest to doctors and researchers due to its influence on the female reproductive health and its role in the establishment of a new-born baby's microbiome [34]. In addition, the imbalance of the vaginal microbiota – **dysbiosis** – is strongly associated with an increased risk of developing urogenital infections and with a number of pregnancy complications [23, 28, 42, 56, 59, 66].

In recent decades, great advances were made in understanding composition and functioning of the vaginal microbiota in women of reproductive age. This became possible because of the possibility to study microbial communities using **molecular techniques**: genome sequencing, polymerase chain reaction, and DNA hybridization. These methods allowed researchers to overcome the limitations of the culture-based method, which for many years was the «gold standard» in the study of human microbiome (Figure 1.1).

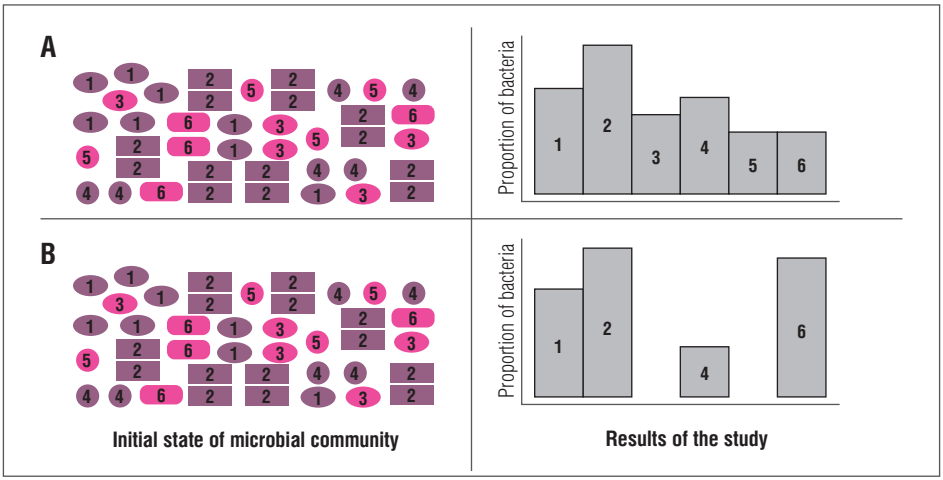


Figure 1.1. Results of microbiota-analysis dependent on the technique used

A. *The use of molecular techniques makes it possible to identify and determine the number of all microbiota species in the studied material.*

B. *The use of culture-based techniques leads to the «loss» of non-culturable microbes 3 and 5. Microbe 6 multiplied in the transport medium; therefore, too high concentrations are measured not reflecting the status at the time of sampling. For microbe 4, however, the selected growth conditions are sub-optimal leading to too low values.*

The introduction of molecular genetic research methods in the study of human microbiota culminated in the **«Human Microbiome Project»**, launched in 2008 [65].

As a result, new microorganisms were discovered, the taxonomy of many commensal microbes were revised. Moreover, new microbes associated with various pathological conditions were identified, and the interpretation of the «normal» vaginal microbial community was revised.

1.1. The composition of resident microbiota, detected in the vagina of women of reproductive age

The female vagina is colonized by a variety of microbes, among which bacteria are prevalent. Interestingly, compared to other body sites, such as those in the gastrointestinal tract, fewer bacterial species have been reported to inhabit the vagina [47]. Another peculiarity is that representatives of a particular genus (genus *Lactobacilli*) or even of a particular species are often clearly predominant (Figure 1.2). Apparently, the macroorganism has certain selection mechanisms, leading to the formation of such a specific microbial community [47].

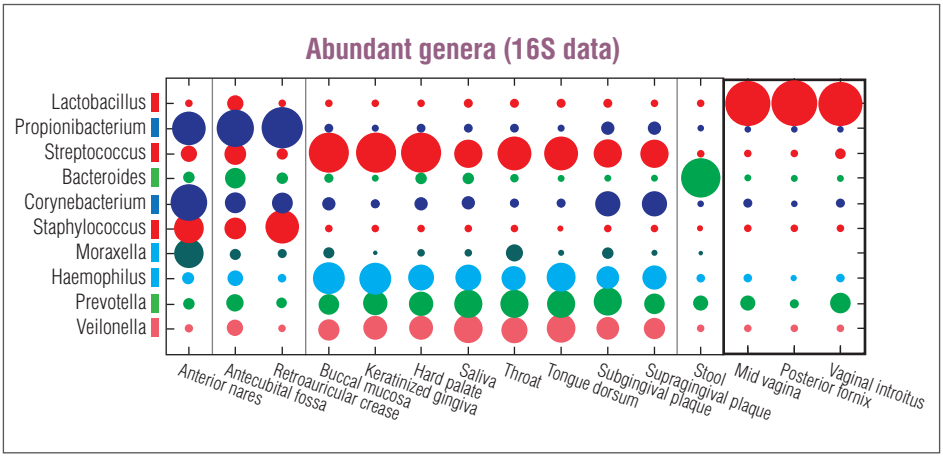


Figure 1.2. Prevalence of individual groups of bacteria in human biotopes.

Vaginal microbiota (marked by a rectangle in bold) is characterized by reduced species diversity and total prevalence of g. Lactobacillus bacteria. Figure adopted from the «Human microbiome» project [47].

More often than others, four bacterial phyla[1]: *Firmicutes, Actinobacteria, Bacteroidetes,* and *Fusobacteria*, are identified in the vagina (Figure 1.3). Enterobacteriaceae (phylum Proteobacteria) species can get to the reproductive tract from the colon, and their increased abundance is often regarded as a causative factor of inflammation in the urogenital tract.

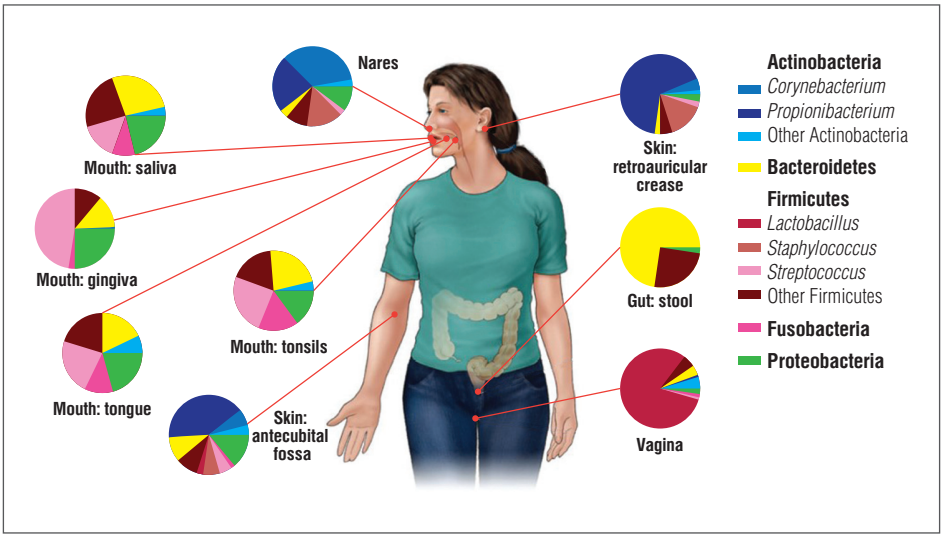


Figure 1.3. The main phyla and genera of bacteria colonizing human skin and mucous membranes.

In healthy women, usually, the bacteria of the Lactobacilli genus, Firmicutes phylum prevail. Bacteria of Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria phyla are often part of vaginal microbiota of women of reproductive age. Figure adopted from the «Human microbiome» project [41].

[1] Phylum is one of the highest taxonomic ranks used in the classification of bacteria. To date, 29 bacterial phyla have been identified.

Lactobacilli

Lactobacilli, which prevail in most healthy women of reproductive age, make up from 80 to 100 % of all bacteria detected in the lower urogenital tract. The proportion of lactobacilli is the main criterion for the determination of the vaginal microbiocenosis variant (Figure 1.4) [1]. Bacteria of the *Lactobacilli* genus are large, gram-positive, non-spore-forming, rod-shape bacteria producing lactic acid during the fermentation of glucose and formed oligosaccharides (Figure 1.5). As a rule, only a few species of lactobacilli are identified in the vagina: *L. iners, L. crispatus, L. gasseri, L. jensenii* [7, 47, 53, 68].

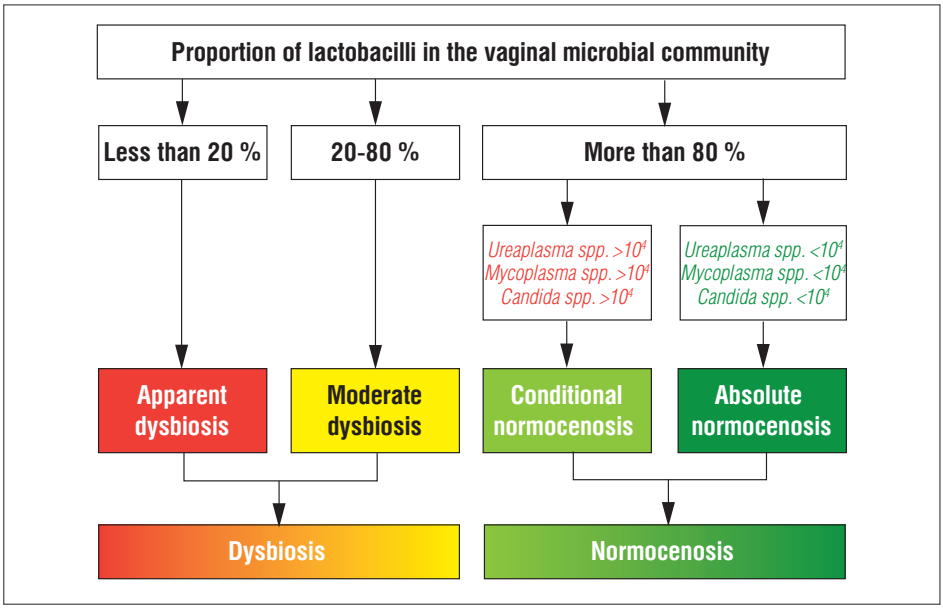


Figure 1.4. Vaginal microbiocenosis variants analyzed using RT-PCR [1].

The key criterion is the proportion of lactobacilli in the microbiocenosis. If it exceeds 80 % of all the identified bacteria, the microbiocenosis is considered as normal or otherwise, as dysbiosis. In normocenosis, in addition to the proportion of lactobacilli, absolute amounts of microbial associates are taken into account. Normocenosis are subdivided into absolute and conditional, depending on the number of the latter.

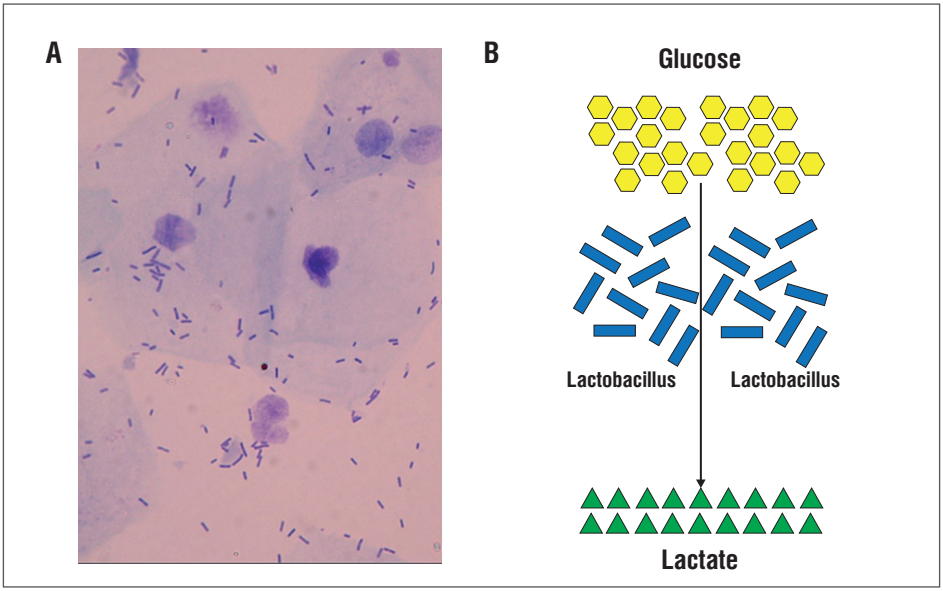


Figure 1.5. Vaginal lactobacilli.

A. *Microscopy image of a vaginal smear. «Lactobacilli» morphotype are large non-spore-forming rod-shape bacteria.*

B. *Lactobacilli form lactic acid as a result of the fermentation of glucose.*

Facultative anaerobic opportunistic microorganisms

This group includes microorganisms capable of growth in nutrient media in presence as well as absence of oxygen. In the lower urogenital tract, they are mainly represented by *Enterobacteriaceae spp., Staphylococcus spp.* and *Streptococcus spp.* (Figure 1.6). For technical reasons these microorganisms are detected especially frequently when using culture-based techniques. However, dysbiosis mainly associated with this group of microorganisms is rare.

Obligate-anaerobic opportunistic pathogens

This group includes the bacteria cultured under anaerobic conditions, difficult to culture bacteria, and non-culturable bacteria. *In vivo* this group can often compete with lactobacilli for a dominant place in a biotope. Usually, with dysbiotic disorders, obligate-anaerobic bacteria colonize the vaginal epithelium [5]. This group includes many representatives of the *Actinobacteria, Firmicutes, Bacteroidetes* and *Fusobacteria* phyla (Figure 1.6).

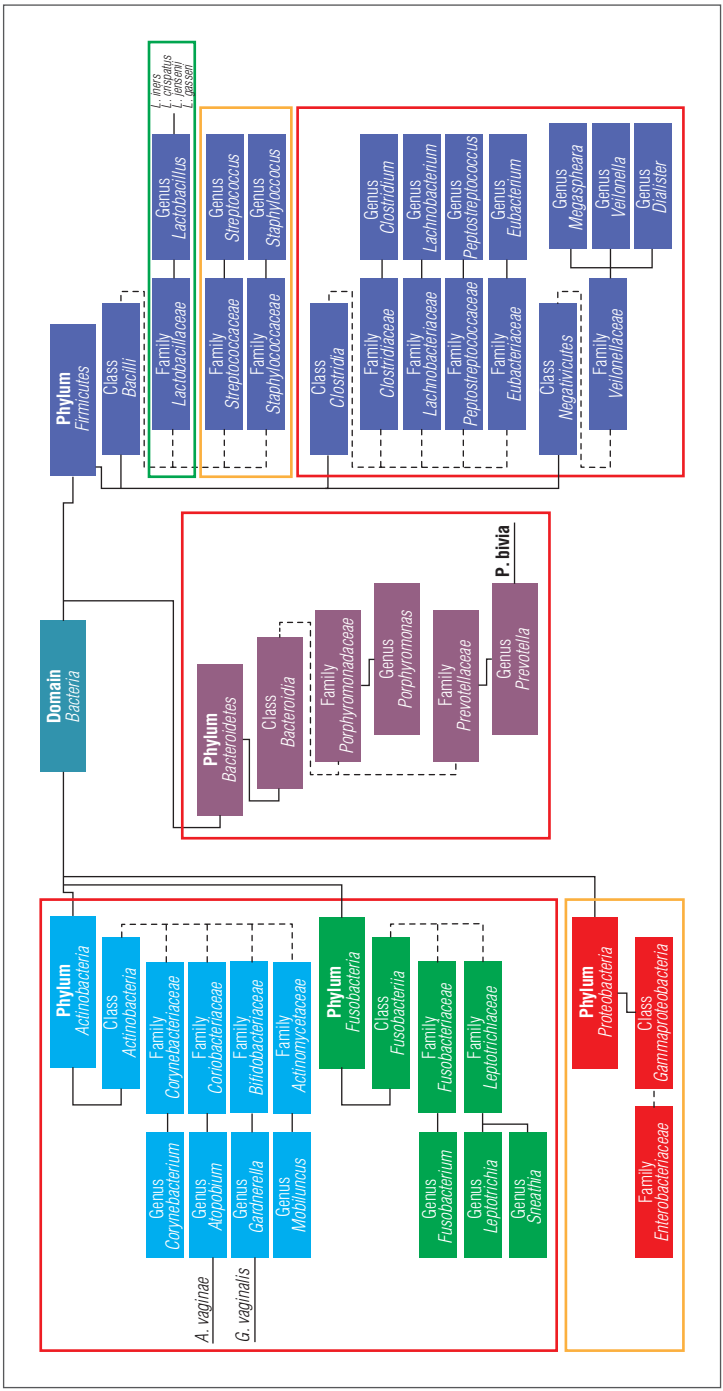


Figure 1.6. The main groups of bacteria detected in the lower urogenital tract using «Femoflor 16» test.

The test system for RT-PCR- «Femoflor 16», which is widely used in Russia, allows to identify the most common species of vaginal microbiota. Target groups of bacteria for this system were selected based on global studies. These are bacteria of five phyla: Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria and Proteobacteria. Identification is usually done up to the genus level. The most clinically important microorganisms are identified to the species level. Normal vaginal microbiota is marked with the Green frame, the facultative anaerobic OM is marked with the Yellow frame, and the obligatory anaerobic OM is marked with the Red frame.

Associated microorganisms

In addition to the above-mentioned bacteria, *Ureaplasmas* and *Mycoplasmas* (*Tenericutes phylum*) – both are small polymorphic bacteria missing a peptidoglycan wall – are often present in the lower urogenital tract of women of the reproductive age. Some eukaryotic microorganisms can also be identified, including the yeast-like fungi of the *Candida* genus. The main feature of associated microorganisms is the lack of competition with the main symbionts for a biotope (their amount is always disproportionately smaller than the amount of the prevailing microorganisms). However, under certain conditions, mycoplasmas, ureaplasmas, and candida can trigger the development of an inflammation.

1.2. The effect of the macroorganism on the composition and functioning of the vaginal microbiota

The composition of vaginal microbiota and their age-related changes are mainly determined by the macroorganism. One of the key factors responsible for the prevalence of lactobacilli at a reproductive age is the woman's level of estrogens (Figure 1.7) [36]. Estrogens stimulate production of glycogen in the vaginal epithelium cells. Glycogen is decomposed by α-amylase of the cervicovaginal fluid to glucose-containing oligosaccharides (maltose, maltotriose), metabolized by lactobacilli to lactate, which leads to a decreased pH of the vaginal discharge [71]. Estrogen deficiency in postmenopausal patients leads to a decrease in the number of vaginal lactobacilli, their substitution with other bacteria, and an increased pH of the vaginal discharge [44, 46, 64, 88]. In this case, the prescription of post-menopausal estrogen replacement therapy leads to the restoration of the vaginal lactobacilli population [44].

The influence of the menstrual cycle on the composition of the vaginal microbial community is disputable. Some researchers argue that microbiota of the lower urogenital tract does not undergo significant changes during the menstrual cycle [48]. At the same time, other authors note that there is evidence of changes in the vaginal microbial community during menstruation. In a prospective study, Srinivasan et al. noted a transient decrease in the amount of lactobacilli and increased abundance of *Gardnerella vaginalis* during menstruation. The growth of *G. vaginalis*, according to the authors, could be stimulated by iron ions present in menstrual blood [73].

Pregnancy is also a factor affecting the composition of vaginal microbiota. During the gestation period, there is an increase in the proportion of lactobacilli and a decrease in the number of opportunistic microorganisms [20, 52, 69]. That being said, changes depend on the species composition of lactobacilli [83]. In a recent study, British researchers demonstrated a marked increase in the diversity of vaginal microbiota in the postnatal

period, while during pregnancy it decreased and mainly lactobacilli were present in the lower urogenital tract [52]. Apparently, an increase in microbial diversity and a decrease in the proportion of lactobacilli in the postnatal period are associated with changes in the vaginal microbial community, triggered by a sharp drop in estrogen level [52].

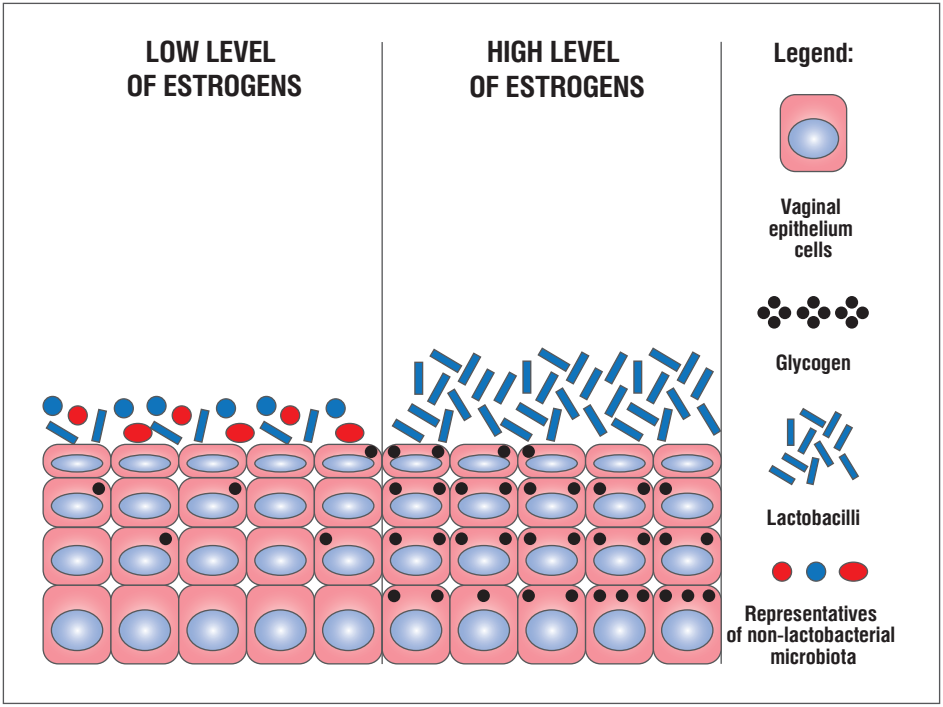


Figure 1.7. The effect of the estrogen level on the composition of vaginal microbiota.

An increase in the level of estrogens at a reproductive age leads to an increase in the production of glycogen by the cells of the vaginal epithelium. The products of glycogen degradation are a nutrient substrate for lactobacilli, and it leads to an increase in their population and their prevalence in the biotope.

1.3. The role of lactate producing bacteria in maintaining colonization resistance of the vaginal epithelium

The bacteria of the *Lactobacilli, Streptococcus*, and *Leptotrichia* [90, 91] genera are the producers of lactic acid in the vagina, which is one of the main factors providing biotope resistance to pathogenic and opportunistic microbes. In healthy women of reproductive age, lactobacilli are usually prevalent. Bacteria of this genus help maintain colonization resistance of the vagina, inhibiting the growth and reproduction of other groups of microorganisms through the following mechanisms:

- Competition for adhesion receptors on the surface of the vaginal epithelium;
- Competition for nutrient substrate;
- Production of lactate in the process of glucose fermentation;
- Production of active oxygen species;
- Production of bacteriocins.

Competition for adhesion receptors on the surface of the vaginal epithelium

Lactobacilli block the adhesion of various urogenital pathogens: group B streptococcus, *Staphylococcus aureus* [89], *Gardnerella vaginalis* [25], *Neisseria gonorrhoeae* [84], *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [63]. In *in vitro* experiments, it was demonstrated that *Lactobacilli crispatus* living cells, as well as its exopolysaccharide, reduced the adhesion of *Candida albicans*, while the exopolysaccharide itself increased the expression of beta-defensin 2 through vaginal epithelial cells [35], which can also protect the vagina from colonization by pathogenic and opportunistic microorganisms.

Competition for nutrition substrate and production of lactate

Maltose and maltotriose that form during the degradation of glycogen from vaginal epithelial cells are a nutrient substrate for many microorganisms. Due to the rapid utilization of these glucose-containing oligosaccharides, lactobacilli do not leave the required amount of nutrient substrate to other less prevalent bacteria in the biotope. In addition, as a result of lactic fermentation, lactobacilli **form α-hydroxypropionic acid (lactic acid or lactate)**. The increase in the lactate concentration leads to a decrease in the pH of the vaginal discharge to 3.8–4.5. Many pathogenic and opportunistic microorganisms are not capable of reproduction at the given pH values. However, recent *in vitro* studies have shown that lactate itself is more critical for pathogens than a decrease in pH, since lactate has a much more significant microbicidal effect on opportunistic microorganisms than other organic acids [62].

Production of reactive oxygen species

Vaginal lactobacilli are capable of producing hydrogen peroxide, which is detrimental to many anaerobic bacteria. It was demonstrated that the presence of H_2O_2-producing lactobacilli in the vagina is associated with a reduced risk of bacterial vaginosis (BV), a pathology characterized by increased bacterial diversity, elevated pH, vaginal discharge and associated with vaginal dysbiosis [22, 31, 87]. Wilks et al. demonstrated that the presence of lactobacilli that produce large amounts of hydrogen peroxide in the vagina at the 20th week of gestation is associated with a lower incidence of BV at the time of the study and with a lower risk of developing chorioamnionitis by the time of birth [87]. However, a 2010 study by O'Hanlon et al. demonstrated that the antimicrobial activity of hydrogen peroxide is inhibited by cervical, vaginal and seminal fluids [61]. At the same time, it was previously noted that the vagina contrains low concentrations of oxygen, which is a metabolic precursor of H_2O_2 [45]. In another study O'Hanlon et al. noted that microbicidal concentrations of H_2O_2 during cultivation under anaerobic conditions *in vitro* inhibit the growth of lactobacilli much stronger than BV-associated bacteria [62]. The data from the study calls into question the statement that production of H_2O_2 by lactobacilli is a significant antimicrobial factor *in vivo*.

Bacteriocin production

Bacteriocins are proteins produced by certain bacteria inhibiting the growth and reproduction or even killing other bacteria. At least two bacteriocins produced by lactobacilli have been described to date. For example, lactocin 160 damages the membrane of *Gardnerella vaginalis*, a microbe associated with BV. The pores formed by lactic acid in the Gardnerella membrane are specific for ATP channels through which the cell loses ATP causing the death of the microbe [79].

2. THE IMPACT OF OPPORTUNISTIC VAGINAL MICROBIOTA IN THE DEVELOPMENT OF GENITAL INFECTION PATHOLOGY

Opportunistic microorganisms (OM) colonizing the vaginal epithelium can be the etiological (causative) agents of vaginal inflammatory diseases. In addition, dysbiosis of the vaginal microbiota is a predisposing factor for the development of an upper genital tract infection [28, 42, 59, 66], and it increases the risk of HIV infection [23, 56].

2.1. Microorganisms associated with bacterial vaginosis

BV is a clinical and laboratory syndrome that involves major changes in the balance of microbes in the vagina and is based on the vaginal anaerobic dysbiosis [5]. BV is a polymicrobial condition, characterized by a decrease in the number of Lactobacilli and a one-thousand-fold increase in the number of opportunistic obligate anaerobic microorganisms (Figure 2.1a). The most studied markers of BV are *Gardnerella vaginalis* and *Atopobium vaginae* [27, 82]. However, many other groups of microorganisms, including representatives of the *Prevotella, Porphyromonas, Megasphaera, Sneathia, Mobiluncus* genera and representatives of the *Clostridiales* order, are also involved in the development of dysbiosis [38, 39, 51]. At the same time, *A. vaginae* is **resistant to metronidozole**, the main antimicrobial drug used for the treatment of BV [37]. Therefore, an alternative metronidazole drug, clindamycin, is used for the treatment of BV associated with *A. vaginae*.

Despite the increased number of these opportunistic microorganisms usually no apparent vaginal inflammatory response (VIR) is observed. Partially, this can be explained by the anti-inflammatory effect of butyrate produced by these bacteria [52]. These bacteria do not produce lactic acid, as a result, there is an increase in the vaginal discharge pH. During the process of protein decomposition and the subsequent decarboxylation of amino acids, anaerobic bacteria associated with the development of dysbiosis produce diamines: putrescine, cadaverine, trimethylamine. Diamines also contribute to an increase the pH of the vaginal discharge. Furthermore they are responsible for the specific «fishy» odor of vaginal discharge after the addition of KOH (or after intercourse).

2.2. Microorganisms associated with inflammatory vaginal infection

Facultative anaerobic OM, the **bacteria of the *Streptococcus, Staphylococcus, Enterococcus*, and *Enterobacteriaceae* genera**, can cause a vaginal inflammatory infection – aerobic vaginitis (AV) [78]. Like BV, this condition is associated with dysbiosis:

the number of lactobacilli decreases and the number of these microbial groups increases.

In contrast to BV, associated with anaerobes, AV is characterized by the **development of a pronounced VIR**. There is a local increase in the levels of pro-inflammatory cytokines: interleukin-1beta, interleukin-6, interleukin-8 [55], which can be partly explained by the depletion of the lactobacilli population. It was previously noted that the presence of lactobacilli, in particular *L. crispatus* and *L. jensenii*, reduces the production of interleukin-1beta, which prevents the inflammation [57]. At the same time, it should be noted that the presence of *L. iners* does not have the same effect [57].

Inflammation in the lower urogenital tract can be also associated with microbial associates like bacteria of the *Ureaplasma* and *Mycoplasma* genera, and the yeast-like fungi of the *Candida* genus.

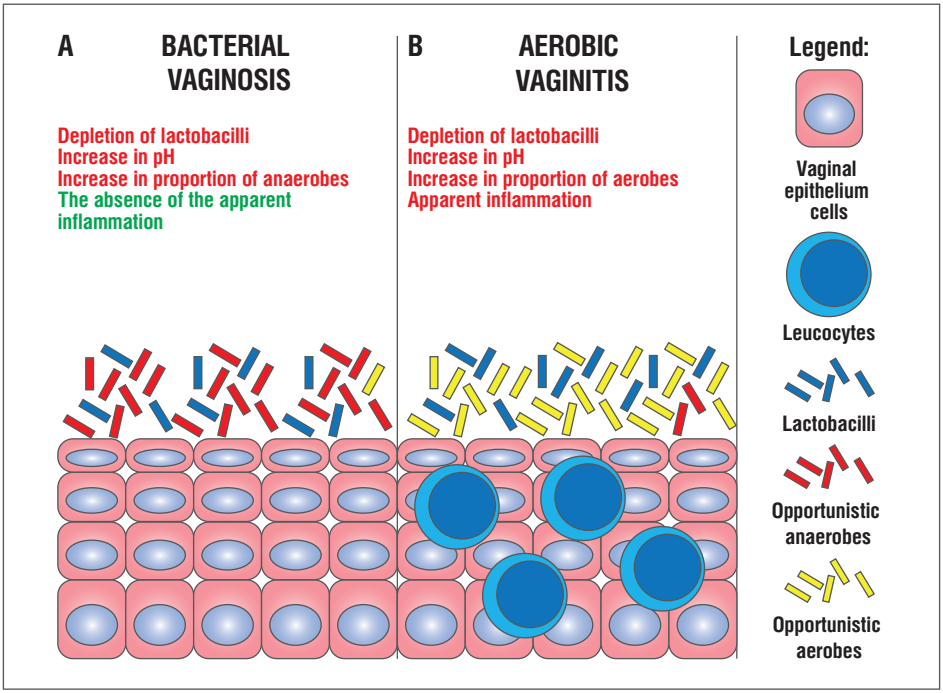


Figure 2.1. The correlation between the pathological reaction with the microorganisms prevailing in the vagina.

A. *In case of anaerobic OM population overgrowth and deficiency of lactobacilli, bacterial vaginosis develops. It is characterized by and the absence of an inflammatory response.*

B. *In case of facultative anaerobic OM population overgrowth and a decrease in the amount of lactobacilli, aerobic vaginitis develops. It is characterized by increased levels of pro-inflammatory cytokines in the vagina and, as a result, by an inflammatory response.*

3. THE DIFFICULTIES OF VAGINAL DYSBIOSIS TREATMENT

The search for new methods for the treatment of vaginal dysbiosis is of high priority in the global scientific community, as well as in practical health care. The drugs that are currently used for this purpose, metronidazole and clindamycin, do not always provide a long-term therapeutic and microbiological effect. For example, recurrent episodes of BV (condition caused by the vaginal anaerobic dysbiosis) within six months after treatment with metronidazole were observed in 50 % of patients [26, 27].

3.1. Bacterial biofilms on the surface of the vaginal epithelium

Biofilms are microbial communities localized on a dense surface and consisting of closely spaced microbial cells embedded in an extracellular polysaccharide matrix [32]. Lactobacilli are capable of forming biofilms on the surface of the vaginal epithelium (Figure 3.1) [80, 81], thereby protecting the vagina from colonization by opportunistic microorganisms. Swidsinski et al. demonstrated that with BV opportunistic microorganisms are present on the surface of the epithelium in the form of biofilms [73]. These biofilms consist primarily of cohesive form of *G. vaginalis* and *A. vaginae* [76], and could be transmitted sexually [75]. *In vitro* studies have shown that probiotic lactobacilli strains can destroy the biofilms of some pathogenic microorganisms [53].

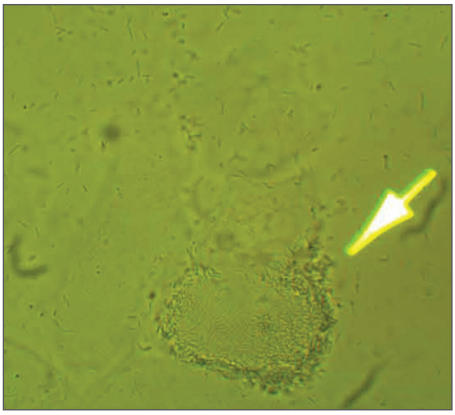


Figure 3.1. Lactobacilli biofilms formed on the surface of vaginal epithelium *in vivo*.
In the wet mount of vaginal discharge, there is a dense accumulation of lactobacilli embedded in an extracellular matrix (marked by the arrow). Microphotograph is adopted from a publication by G. Ventolini [81].

3.2. Bacterial biofilms resistance to antimicrobial drugs

Bacteria inside biofilms are characterized by their high resistance to antimicrobial and antiseptic drugs. On the example of *Pseudomonas aeruginosa* biofilms, it was demonstrated that a minor population of bacteria in a biofilm survives even after 10-fold increased therapeutic doses of antimicrobial drugs effective against planktonic forms of this microbe [29]. In the future, this population of resistant cells can multiply again to reach its original quantities, thereby leading to a recurrence of the disease. It is possible that such a mechanism causes the recurrence of BV. It was demonstrated that **neither metronidazole nor moxifloxacin is able to destroy the biofilms of *G. vaginalis*** in women with BV (Figure 3.2) [74, 76]. The study of the vaginal microbiome after the use of metronidazole has shown that metronidazole treatment leads to a pronounced decrease in the number of anaerobic BV-associated bacteria. However, after the end of the treatment it increases again [67, 73].

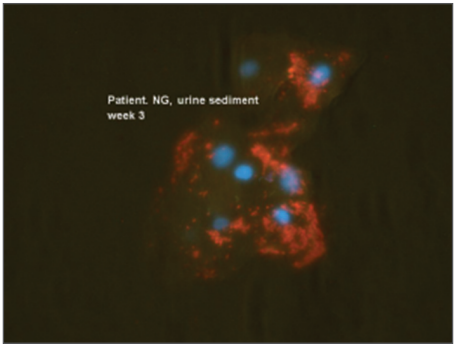


Figure 3.2. *G. vaginalis* on the surface of cast-off vaginal epithelium cells 3 weeks after a course of moxifloxacin.
G. vaginalis cells were stained with red. Microphotograph is adopted from a publication by A. Swidsinski et al. [74].

4. LABORATORY TECHNIQUES FOR ANALYZING VAGINAL MICROBIOTA

Currently, there are several approaches to analyzing vaginal microbiota. Vaginal discharge is used as a test material for different microbiological tests: microscopic, bacteriological (culture-based techniques), or molecular techniques (polymerase chain reaction (PCR), for example). It should be noted that each of the presented methods has both a number of advantages and some limitations.

4.1. Microscopic method

Traditionally microscopy is the easiest and the cheapest method for assessing the state of the vaginal microflora.

Microscopy of vaginal smears assesses the following parameters:

- epithelium cell type – predominating type of epithelial cells (squamous or parabasal), their number counted in Gram smears or wet mount per the microscope's field of view;
- vaginal inflammatory response – WBC (predominantly polymorphonuclear leucocytes) number counted in Gram smears or wet mount per the microscope's field of view;
- total bacterial mass (load) – TBM, the morphological composition of the microflora and the approximate quantitative ratio of microbial morphotypes, the presence of pseudohyphae or budding spores of yeast-like fungi, trichomonads;
- presence of «clue» cells (squamous epithelium cells coated with the anaerobic gram-variable coccobacilli).

Microscopy is practically the only method of laboratory diagnostics that allows estimating the intensity of the VIR. A clinically healthy woman has no more than 10 WBC in the field of view. At the same time, this parameter can vary considerably depending on the technique of sampling and applying vaginal discharge on the glass slide. Since there are no clearly defined standards as to the WBC content in the vaginal discharge, the inflammation index (II) – ratio of WBC to vaginal epithelial cells – should be considered more informative. In most healthy women II is less than 1 [17].

Modern microscopy is able to identify up to 10 morphotypes of microorganisms that inhabit the vagina, including *Lactobacilli spp., Gardnerella vaginalis, Bacteroides spp., Mobiluncus spp., Leptotrichia spp., Fusobacterium spp., Veillonella spp., Candida spp.* gram-positive and gram-negative cocci, gram-negative rod-shape bacteria.

A number of algorithms were used for assessment of vaginal smears until consensus was reached to define the diagnosis of BV using the criteria described by Amsel et al. [21]. Later, the Nugent scoring method was introduced as the gold standard for laboratory-based BV diagnosis [59]. Both of these methods score the smears by quantification of the different vaginal morphotypes, taking into account the presence or absence of WBC. In 2002 aerobic vaginitis was characterised by Donders et al. For an accurate diagnosis of AV, it was recommended to consider Lactobacillus grade, number of WBC, proportion of toxic WBC, background flora, and proportion of parabasal epitheliocytes in wet mount smear.

In 2001, Russian scientist E. F. Kira proposed a classification of vaginal microbiota based on the results of microscopic examination [8]:

1. Normal microbiota smear – characterized by the prevalence of lactobacilli, the absence of gram-negative microflora, pseudohyphae or budding spores of yeast-like fungi, the presence of single WBC and squamous epithelial cells corresponding to the phase of the menstrual cycle, or II that is less than1 (Microscopic images 1, 2).

2. Intermediate type smear – characterized by a moderate or reduced number of lactobacilli and the presence of cocci. WBC, macrophages, epithelial cells can be identified. It is a borderline type, often seen in healthy women, rarely accompanied by subjective complaints and clinical manifestations (Microscopic images 3, 4).

3. Dysbiotic type smear – characterized by an insignificant amount or the complete absence of lactobacilli, the presence of abundant polymorphic gram-negative and gram-positive rod-shape bacteria and coccal microflora; the presence of «clue» cells. The number of WBC varies; phagocytosis is either absent or incomplete (Microscopic image 9).

4. Inflammatory type smear – characterized by a large number of WBC, macrophages, parabasal epithelium cells, and marked phagocytosis. If gonococci, trichomonas, pseudohyphae or budding spores of yeast-like fungi are identified, an appropriate etiological diagnosis is made. In the absence of etiologically significant microorganisms with such a microscopic pattern, patients are diagnosed with nonspecific vaginitis (Microscopic images 5-8).

The microscopic method has several limitations. It is impossible to identify a number of pathogens significant in pathology, due to their small size (*Chlamydia trachomatis*), or the absence of a cell wall (*Mycoplasma spp.* and *Ureaplasma spp.*) or pronounced polymorphism (*Atopobium vaginae*).

The sensitivity of a microscopic study in BV diagnosis (identification of «clue» cells) ranges from 40 to 90 %, specificity is about 80 % [70]. While microscopic methods facilitate quick diagnosing and are relatively simple and cheap, they also suffer from sub-

jectivism, raised requirements for sampling, transporting material and preparing smears, which, in turn, requires both clinical and laboratory doctors to have high qualifications and experience [70].

4.2. Culture-based technique

Cell culture is the process where individual cells (or a single cell) of prokaryotes or eukaryotes are grown under controlled conditions *in vitro*.

Culture-based (or bacteriological) technique still remains the «gold» standard in the diagnosis of many infectious diseases, since it allows not only to isolate pathogenic microorganisms from the clinical samples, but also to identify the microorganisms' species (strain), estimate their approximate number, and determine their sensitivity to antimicrobial agents. This method can also be used to analyze vaginal microbiota.

Microbiological analysis involves determining both the species composition and the quantitative composition of the vaginal bacterial flora. In most laboratories, bacteriological examination results have an accuracy of 1–2 lg, e.g. the «scatter» of data is 10–100 times, which does not allow for the precise assessment of the microorganisms' quantitative relationships.

The main advantage of the culture-based method is the possibility to determine the sensitivity of the potentially clinically important microorganisms to antibacterial drugs.

At the same time, it is important to remember that, in accordance with modern views on the vaginal microbiota composition, some of the significant sexually transmitted pathogens (*Chlamydia trachomatis, Mycoplasma genitalium*) and the majority of vaginal OM inhabiting the vagina are difficult to culture or non-culturable, including *A. vaginae* and obligate anaerobes. As a result, bacteriological method reveals a smaller part of microorganisms significant for urogenital pathology, predominantly the representatives of the *Streptococcaceae, Staphylococcaceae* and *Enterobacteriaceae* families, which distorts the results of the study and reduces its value to a physician.

Additional difficulties are caused by the potencial loss of the microorganisms' viability during storage or transport of the patient-sample to the laboratory. Moreover, the use of transport media may alter the quantitative relationships between representatives of the vaginal microbiota.

It should also be noted that the method of sampling urogenital material, commonly used in clinical practice, is not quantitative. Thus, the bacteriological laboratory cannot accurately evaluate the quantity of the identified microorganisms. In some cases, a semiquantitative assessment of the growth of microorganisms is performed by a streak plate method [9]. However, for the reliable quantitative assessment, it is necessary to sample

the biomaterial with a calibrated loop.

The sensitivity of the culture-based technique in BV diagnosis is 25–60 %, specificity is about 90 % [70].

In contrast to molecular techniques culture-based techniques are quite demanding, as well as time-consuming (7–10 days), requires highly qualified clinical microbiologists, and a strict methodical approach to the laboratory organization, storage and transportation of the biomaterial.

4.3. Qualitative analysis by polymerase chain reaction (PCR)

The introduction of PCR significantly expanded our knowledge of vaginal microbiota. This method allows one to identify also difficult to culture or non-culturable microorganisms by amplifying the nucleic acid of a microorganism without need of propagation. Qualitative PCR answers the question of whether a particular microorganism is present in a given sample. This is usually sufficient for identifying microorganisms in sterile biological fluids (urine, blood, cerebrospinal fluid) or for the detection of obligate pathogenic microorganisms. Thus, the use of a qualitative PCR is absolutely justified for finding significant obligate pathogens [19].

Using qualitative PCR to analyze the balance of microorganisms inhabiting the vagina is not possible. Recent studies have shown that clinically healthy women can have a variety of opportunistic microorganisms in the normal vaginal microbiota [38, 68, 90, 91]. Researchers are currently finding a correlation between different types of vaginal polymicrobial communities and clinical manifestations. [49]. For the assessment of a microorganism's etiological role, it is necessary to take into account the number of microbial cells and its proportion in the total bacterial mass (load) – TBM.

4.4. Quantitative analysis by real-time PCR

Fast, efficient and reliable determination of the number o microbial cells in a given sample became possible due to the development of real-time PCR (RT-PCR). This method allows not only to identify the majority of participants in the vaginal microbiocenosis, but also to estimate their absolute number with high accuracy. Thus, for the first time, it became possible to calculate the amount of a particular species in relation to the TBM. This allows a comprehensive assessment of the microbiocenosis and a more accurate evaluation of the etiological role of the opportunistic agent in the development of a pathology in a given patient.

In contrast to the traditional culture-based technique, RT-PCR does not require special conditions for the transportation and storage of clinical samples (without compromising the quality of the test) and has high analytical sensitivity and specificity. Moreover, the analysis time is much shorter than that of the culture-based method: less than one working day is required to carry out the test. This, in turn, provides an opportunity to work out a more targeted treatment and the time-frame for it as soon as possible.

To date, only RT-PCR kit «Femoflor» (DNA-Technology) has been designed for complex evaluation of vaginal microbiota by real-time PCR method in Russia. Criteria for interpreting results of «Femoflor» have been developed in several clinical trials with more than 2000 participants conducted in 2008–2011 [1]. The method has been certified and is successfully in routine use for the last 10 years.

Depending on the proportion of lactobacilli and opportunistic microorganisms in the TBM, three basic types of vaginal microbiocenosis can be discriminated:

1. **«Normocenosis».** This variant of vaginal microbiocenosis is predominated by lactobacilli. The proportion of lactobacilli is more than 80 % of the TBM, and the proportion of opportunistic microorganisms (specifically obligate anaerobes) is less 20 % of the TBM. Depending on the quantity of the associated bacteria (*Mycoplasma hominis, Ureaplasma spp.*) and yeast-like fungi (*Candida spp.*), «normocenosis» is further divided into two groups:
   a. Vaginal microbial community is considered as **Absolute normocenosis (AN)** when the quantity of associated microorganisms (*Ureaplasma spp., M. hominis, Candida spp.*) is less than 10^4 genome equivalent per 1 ml – GE/ml (hereinafter all the quantities of microorganisms are shown in this units).
   b. Vaginal microbial community is considered as **Conditional normocenosis (CN)** when the quantity of associated microorganisms (*Ureaplasma spp., M. hominis, Candida spp.*) is more than 10^4 GE/ml.
2. **«Moderate dysbiosis» (MD)** is an intermediate state of vaginal microbial community when the proportion of lactobacilli decreases and constitutes less than 80 % but more than 20 % of the TBM. Thus, the proportion of opportunistic microorganisms is more than 20 % but less than 80 % of the TBM. Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of MD can be identified:
   a. **«Moderate aerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is less than 10 % of the TBM.
   b. **«Moderate anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBM.

   c. **«Moderate mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBM.
3. **«Apparent dysbiosis» (AD)** – this variant of vaginal microbiocenosis is predominated with various opportunistic bacteria: the proportion of lactobacilli is less than 20 % of the TBM, and the diverse microbial community (specifically strictly anaerobic bacteria) constitutes more than 80 % of the TBM (Figure 1.4). Depending on the prevalence of obligate anaerobes or facultative anaerobes, three variants of AD can be identified:
   a. **«Apparent aerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is less than 10 % of the TBM.
   b. **«Apparent anaerobic dysbiosis»** – when the proportion of facultative anaerobes is less than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBM.
   c. **«Apparent mixed aerobic-anaerobic dysbiosis»** – when the proportion of facultative anaerobes is more than 10 %, and the proportion of obligate anaerobes is more than 10 % of the TBM.

For many years this method is successfully used in the clinical practice in Russia and other countries.

5. CLINICAL INTERPRETATION OF LABORATORY RESULTS AND RECOMMENDATIONS FOR PATIENT MANAGEMENT

In this chapter, both normal and pathological variants of vaginal microbiocenosis will be considered in the context of RT-PCR diagnostics (using the Femoflor test). In subsequent chapters, different options for treating dysbiotic disorders of the vaginal microflora will be presented. Only a physician is qualified to make decisions regarding the appropriate course of treatment, taking into account all the available data: anamnesis, clinical presentation, microscopy data and the results of the Femoflor test. It should be noted that firstly the patient should be tested for sexually transmitted pathogens (STP) (*Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium*). If one of the above pathogens is detected, it is absolutely necessary to prescribe antibiotics. Only in the absence of STP, a physician can consider correction of vaginal dysbiosis. Possible variants of clinical conditions depending on the state of vaginal microbiocenosis according to the «Femoflor» test, microscopy (in accordance with E. F. Kira classification, 2001 [8]) and clinical data are presented in table 5.1.

Laboratory criteria for normocenosis and dysbiosis

The criterion for normocenosis is the predominance of lactobacilli whose proportion should be higher than 80 % of the TBM in the vaginal microbiocenosis.

«Absolute normocenosis» (AN) – is a condition characterized by the dominance of lactobacilli (more than 80 % of TBM), whereas *Ureaplasma spp., Mycoplasma hominis, Candida spp*. are not detectable or less than 10^4 GE/ml (Figure 5.1). This pattern reflects the typical state of the normal vaginal biotope. This variant is typical for clinically healthy women with normal vaginal microscopy (predominance of lactobacilli, absence of pseudohyphae or budding spores of yeast-like fungi, presence of single WBC and squamous epithelial cells (see figures in the appendix) [2].

The combination of **AN** with an intermediate smear type (see figures in the appendix) can be considered as a normal variant in the absence of clinical manifestation and does not require any correction. If there are complaints or objective signs of an infection or inflammation process in the lower urogenital tract, it is recommended to further examine the patient to exclude STP, viruses, or extragenital pathology.

Table 5.1. *Clinical and laboratory data as criteria for diagnostics and management of gynecological patients*

Lacto-bacilli proportion	OM proportion/ predominant group of micro-organisms	The number of associates of microbio-cenocis	Femoflor test result	Microscopy data	Clinical manifestation, BROH, reproductive plans, special situations (No[1], Yes[2])	Clinical diagnosis	Need for treatment
80–100 %	0–20 %	Absent or $<10^4$ GE/ml	Absolute normocenosis	Normocenosis	No	Physiological standard	Not required
80–100 %	0–20 %	*Ureaplasma spp* $>10^4$ GE/ml	Conditional normocenosis with *Ureaplasma spp.*	Normocenosis	No	Within the physiological range	Not required
					Yes	Chronic or subacute inflammatory process	Required
				Inflammatory or intermediate smear type	Yes or no	Vaginitis associated with *Ureaplasma spp.*	Required
		M. hominis $>10^4$ GE/ml	*Conditional normocenosis with M. hominis*	Normocenosis	No	Within the physiological range	Not required
				Inflammatory or intermediate smear type	Yes or no	Vaginitis associated with *M. hominis*, acute of subacute type	Required
		Candida spp. $>10^4$ GE/ml	Conditional normocenosis with *Candida spp.*	Normal	No	Within the physiological range	Not required
				Inflammatory smear type	Yes or no	Vulvovaginal candidiasis, acute type	Required
				Normal or intermediate smear type	Yes	Vulvovaginal candidiasis, subacute type or chronicity	Required

Lacto-bacilli proportion	OM proportion/ predominant group of micro-organisms	The number of associates of microbio-cenocis	Femoflor test result	Microscopy data	Clinical mani-festation, BROH, reproductive plans, special situations (No[1], Yes[2])	Clinical diagnosis	Need for treatment
20–80 %	20–80 % / obligate anaerobes prevail (anaerobes)	Any	Moderate anaerobic dysbiosis	Normal or inter-mediate smear type	No	Within the physiological range	Case follow-up is recommended
				Dysbiotic smear type	No	Bacterial vaginosis, chronicity or asymptomatic course[3]	Required
				Dysbiotic or inter-mediate smear type	Yes	Bacterial vaginosis	Required
				Inflammatory smear type	Yes or no	Non-specific vaginitis, associated mainly with anaerobic or mixed microflora[4]	Required
20–80 %	20–80 % / facultative anaerobes prevail (aerobes)	Any	Moderate aerobic dysbiosis	Normal or inter-mediate smear type	No	Within the physiological range	Not required
				Normal or inter-mediate smear type	Yes	Aerobic vaginitis, subacute type	Required
				Inflammatory smear type	Yes or no	Aerobic vaginitis, acute type	Required
20–80 %	20–80 % / anaerobes >10 % aerobes > 10 %	Any	Moderate mixed (aerobicana-erobic) dysbiosis	Normal or inter-mediate smear type	No	Variant of physiological range	Not required
				Dysbiotic smear type	No	Bacterial vaginosis, chronicity or asymptomatic course[3]	Required

Lacto-bacilli proportion	OM proportion/ predominant group of micro-organisms	The number of associates of microbio-cenocis	Femoflor test result	Microscopy data	Clinical manifestation, BROH, reproductive plans, special situations (No[1], Yes[2])	Clinical diagnosis	Need for treatment
20–80 %	20–80 % / anaerobes >10 % aerobes > 10 %	Any	Moderate mixed (aerobicanaerobic) dysbiosis	Dysbiotic or intermediate smear type	Yes	Bacterial vaginosis	Required
				Inflammatory smear type	Yes or no	Non-specific vaginitis, determined by mixed aerobic-anaerobic microflora	Required
0–20 %	80–100 % anaerobes prevail	Any	Apparent anaerobic dysbiosis	Dysbiotic or intermediate smear type	Yes or no	Bacterial vaginosis	Required
				Inflammatory smear type	Yes or no	Non-specific vaginitis, determined mainly by anaerobic or mixed microflora[4]	Required
0–20 %	80–100 % aerobes prevail	Any	Apparent aerobic dysbiosis	Intermediate, dysbiotic or inflammatory smear type	Yes or no	Aerobic vaginitis	Required
0–20 %	80–100 % anaerobes >10% aerobes > 10 %	Any	Apparent mixed (aerobic-anaerobic) dysbiosis	Dysbiotic or intermediate smear type	Yes or no	Bacterial vaginosis	Required
				Inflammatory smear type	Yes or no	Non-specific vaginitis, determined by mixed aerobicmicroflora	Required

[1] – «No» means the absence of all the listed criteria.

[2] – «Yes» means the presence of any of the listed criteria.

[3] – This condition (mild dysbiosis and the presence of «clue» cells detected by microscopy) may correspond to asymptomatic BV (given the fulfillment of Amsel's criteria).

[4] – Mixed microflora means the presence of genital mycoplasmas, yeast-like fungi of the *Candida* genus >10^4 GE/ml, HSV or HPV.

BROH – bad reproductive and obstetric history

Vaginal dysbiosis is a condition characterized by an imbalance of the qualitative and quantitative composition of the microbiota. In accordance with the criteria proposed for the interpretation of the RT-PCR results [1], dysbiotic disorders are differentiated according to their severity into moderate dysbiosis, where the proportion of lactobacilli is within the range of 20–80 % of TBM, and apparent dysbiosis, a condition characterized by a decrease in the proportion of lactobacilli below 20 % of the TBM. Depending on the prevailing group of the opportunistic microorganisms, aerobic, anaerobic, and mixed aerobic-anaerobic dysbiosis are distinguished.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{5.4}$ ■	
1	Total Bacterial Mass	$10^{6.8}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{5.8}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	□
7	Eubacterium spp.	not detected	□
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	not detected	□
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected □	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	$10^{1.6}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

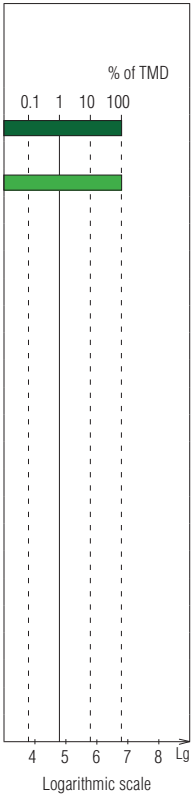


Figure 5.1. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: absolute normocenosis.

6. MANAGEMENT OF PATIENTS WITH APPARENT VAGINAL DYSBIOSIS

Treatment is recommended for women diagnosed with all variants of apparent dysbiosis.

The purpose of treatment is the relief of vaginal symptoms and normalization of vaginal microbiota.

Dysbiosis treatment should be based on the following principles:

- Specificity and selectivity – the choice of the appropriate antibacterial or antiseptic agent should be made taking into account the dominant group of opportunistic microflora;
- Safety – medication used for the dysbiosis treatment should be as safe as possible with respect to the native vaginal lactobacilli;
- Locality – it is preferable to use local antibacterial or antiseptic agents;
- Restoration of normal microbiota at the final stage of treatment (which is defined as a return to the assayed microbial species or profile taken from a healthy individual);
- Compliance – the drug and the way of its administration should be chosen, taking into account the individual needs of a woman.

6.1. Treatment of apparent anaerobic dysbiosis

The clinical syndrome corresponding to apparent anaerobic dysbiosis according to RT-PCR data (Femoflor test) is BV [10]. An example of a vaginal microbiocenosis analysis lab report in a patient with apparent anaerobic dysbiosis is presented in Figure 6.1.

Indications for treatment – apparent anaerobic dysbiosis is an indication for prescribing treatment to women of reproductive age, regardless of clinical manifestations.

The purpose of treatment is the relief of vaginal symptoms and normalization of vaginal microbiota.

The treatment of apparent anaerobic dysbiosis and related clinical manifestations is **carried out in two stages**:

- At first stage, it is necessary to achieve a significant reduction in the number and proportion of obligate anaerobes in the vaginal microbiocenosis.
- At the second stage, the population of normal microbiota (lactobacilli) should be restored. It is a prerequisite for the prevention of BV recurrence [26].

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4.5}$	
1	Total Bacterial Mass	$10^{7.4}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	not detected	
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.6}$	-0.5 (25–34 %)
7	Eubacterium spp.	$10^{6.7}$	-0.5 (27–37 %)
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{6.4}$	-0.8 (14–19 %)
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{6.4}$	-0.7 (16–22 %)
10	Lachnobacterium spp. + Clostridium spp.	$10^{5.2}$	-2.0 (0.9–1.2 %)
11	Mobiluncus spp. + Corynebacterium spp.	$10^{5.4}$	-1.8 (1.5–2.0 %)
12	Peptostreptococcus spp.	$10^{4.7}$	-2.4 (0.3–0.4 %)
13	Atopobium vaginae	не not detected	
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{3.9}$	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{1.9}$	
16	Ureaplasma (urealyticum + parvum)*	$10^{2.5}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

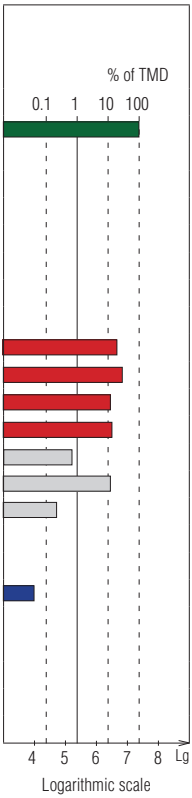


Figure 6.1. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: apparent anaerobic dysbiosis.

Recommended regimens (one of the described variants):

At the first stage, it is recommended to use one of the regimens in accordance with the Federal Clinical Recommendations for Managing Patients with Bacterial Vaginosis of the Russian Society of Dermatovenereologists and Cosmetologists, the Russian Society of Obstetricians and Gynecologists (Moscow 2015) [18], 2015 Sexually Transmitted Disease (STD) Treatment Guidelines, STI treatment European guidlines 2018 [72].

- Clindamycin, cream 2 %, one full applicator (5 g) intravaginally at bedtime for 7 days (grade of recommendation A);
- Metronidazole, gel 0.75 %, one full applicator (5 g) intravaginally, once a day for 5 days (grade of recommendation A);
- Metronidazole 500 mg orally 2 times a day for 7 days (grade of recommendation A);
- Tinidazole 2.0 g orally once per day for 3 days (grade of recommendation A).

Alternative regimens (one of these options):

- Clindamycin, ovules 100 mg intravaginally once at bedtime for 3 days (grade of recommendation B);
- Clindamycin 300 mg orally twice daily for 7 days (grade of recommendation B);
- Tinidazole 1.0 g orally once daily for 5 days (grade of recommendation A).

Prescription of clindamycin is justified with the increasing etiological role of metronidazole-resistant *Atopobium vaginae* in the development of recurrent BV [12]. Moreover, *A.vaginae* can only be detected by RT-PCR.

Apparent anaerobic dysbiosis associated with *A. vaginae* (Figure 6.2) is a specific condition, which is often identified in patients with recurrent BV and requires the prescription of selective antibacterial drugs.

The drug of choice for this form of dysbiosis is Clindamycin, administered as a cream or vaginal suppository according to one of the schedules:

- Clindamycin, cream 2 %, one full applicator 5.0 g intravaginally once at bedtime for 7 days (grade of recommendation A);
- Clindamycin, ovules 100 mg intravaginally once at bedtime for 3 days (grade of recommendation B).

The drug is also active against a wide range of microorganisms detected both in patients with BV and with aerobic vaginitis. (*Fusobacterium spp., Streptococcus spp. (excluding Enterococcus faecalis), Staphylococcus spp., Mobiluncus spp., Bacteroides spp., Peptostreptococcus spp., Mycoplasma hominis, Peptococcus spp.* and *Peptostreptococcus spp., Clostridium perfringens, Clostridium tetani, Propionibacterium spp., Eubacterium spp.* and *Actinomyces israelii*).

NB! The use of Metronidazole and other derivatives of nitroimidazoles is considered bad practice if *A. vaginae* was detected.

At the second stage it is advisable to use probiotics containing lactobacilli, or drugs that acidify the vaginal environment in order to restore native microbiota. Probiotic therapy begins 2–3 days after the end of antibiotic treatment. This period of time is necessary for antibacterial agents to be eliminated from the organism [16].

Currently, a wide range of probiotics is offered for topical and oral administration. The choice of a particular drug depends on the individual needs of the woman, her lifestyle, and it and must be coordinated with her.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4.9}$	
1	Total Bacterial Mass	$10^{8.7}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{4.9}$	-3.3 (<0.1 %)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	$10^{3.0}$	-5.1 (<0.1 %)
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	$10^{3.8}$	-4.4 (<0.1 %)
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{7.2}$	-1.0 (9–13 %)
7	Eubacterium spp.	$10^{7.7}$	-0.5 (26–35 %)
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{4.1}$	-4.0 (<0.1 %)
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{6.3}$	-1.9 (1.1–1.5 %)
10	Lachnobacterium spp. + Clostridium spp.	$10^{7.7}$	-0.5 (29–39 %)
11	Mobiluncus spp. + Corynebacterium spp.	$10^{4.5}$	-3.6 (<0.1 %)
12	Peptostreptococcus spp.	$10^{4.7}$	-3.5 (<0.1 %)
13	Atopobium vaginae	$10^{7.5}$	-0.6 (20–27 %)
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{1.2}$	
16	Ureaplasma (urealyticum + parvum)*	$10^{3.7}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

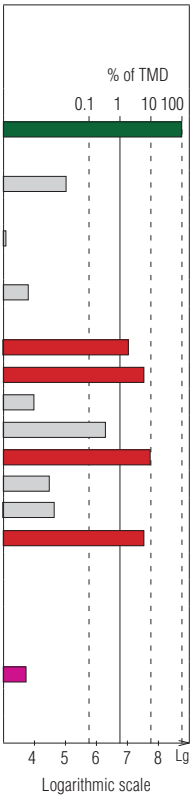


Figure 6.2. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»

Conclusion: apparent anaerobic dysbiosis, Atopobium vaginae present in significant amounts.

Topical (intravaginal) probiotics forms:

- «Ecofemin» – contains *Lactobacilli acidophilus* 10^9 CFU.
  - ➤ Treatment dosage: 1 capsule intravaginally twice daily (morning and evening) for 6 days.
  - ➤ Preventive dosage: 1 capsule intravaginally once at bedtime for 3–7 days.
- «Lactoginal» – contains *Lactobacilli casei rhamnosus* 10^8 CFU.
  - ➤ Treatment dosage: 1 capsule intravaginally twice daily (morning and evening) for 7 days or 1 capsule daily for 14 days.
  - ➤ Preventive dosage: 1 capsule intravaginally daily for no more than 21 days.

- «Lactonorm» – contains *Lactobacilli acidophilus* 10^8 CFU.
  - ➤ Treatment dosage: 1 capsule intravaginally twice daily (morning and evening) for 7 days.
  - ➤ Preventive dosage: 1 capsule intravaginally at bedtime for 7–14 days.

It is possible to use a coformulated drug containing probiotic bacteria and microdoses of estrogen for the topical restoration of the normal flora. This group of drugs has a complex effect on the vaginal microbiocenosis: they restore the normal proportion of lactobacilli and repair vaginal epithelium due to estriol microdose [15].

- «Gynoflor E» – contains *Lactobacilli acidophilus* 10^8 CFU, estriol (0.3 mg):
  - ➤ Treatment dosage: 1–2 tablets intravaginally daily for 6–12 days.
  - ➤ Preventive dosage: for the treatment of estrogen-dependent atrophic vaginitis in postmenopausal women – 1 tablet intravaginally daily for 6–12 days, then, the maintenance dose – 1 tablet intravaginally 1–2 times a week.
- «Trioginal» – contains *Lactobacilli casei rhamnosus* 10^8 CFU, estriol (0.2 mg) and progesterone (2 mg):
  - ➤ Treatment dosage: 2 capsules intravaginally daily for 20 days until symptoms are reversed, then 1 capsule/day for a period of up to 3 months.

Probiotic drugs for oral administration

In recent years, oral administration of lactobacillus capsules has been actively recommended for the indirect restoration of vaginal microflora. Apart from the convenience factor for women, an additional bonus of using such drugs is the simultaneous recovery of the colon microbiota and the rectal ampulla, which is a reservoir for obligatory anaerobic flora and gram-negative bacteria.

Recommended drugs:

- «Vagilac» – contains *Lactobacilli reuteri, Lactobacilli rhamnosus* in a total amount of at least 10^9 CFU of bacteria.
  - ➤ Treatment dosage: 1 capsule orally twice daily for 2 weeks.
  - ➤ Preventive dosage: 1 capsule orally daily for 2–4 weeks.
- «EcofeminFloravag» – contains *Lactobacilli crispatus, Lactobacilli acidophilus, Lactobacilli brevis* in a total amount of at least 10^9 CFU of bacteria.
  - ➤ Treatment dosage: 1 capsule orally twice daily for 2 weeks.
  - ➤ Preventive dosage: 1 capsule orally daily for 4 weeks.

6.2. Treatment of apparent aerobic dysbiosis

Aerobic dysbiosis is clinically manifested as AV, characterized by the classic symptoms of inflammation of the vaginal mucosa, exocervix, and vulva (hyperemia, erosion of mucous membranes, pathological leucorrhoea, discomfort, itching, and discharge from the genital tract). Microscopy identifies the inflammatory type of the smear (see figures in the appendix). Recommendations of The International Union against Sexually Transmitted Infections (IUSTI) for treating AV with clindamycin do not take into account the individual characteristics of vaginal microbiocenosis (http://www.iusti.org/regions/Europe/pdf/2018/IUSTIvaginalDischargeGuidelines2018.pdf). A detailed analysis of vaginal microbiota using the Femoflor test allows one to prescribe individualised therapy, taking into account the dominant aerobic microflora.

Apparent aerobic dysbiosis associated primarily with streptococci (Figure 6.3)

Since the etiological structure of urogenital diseases is caused by various microorganisms in various combinations, it is possible to use a coformulated drug with a broad spectrum of antimicrobial, antifungal, and antiprotozoal activity for effective treatment of AV caused by mixed flora.

These criteria are fulfilled by **«Tergynan»** vaginal ovules, which consist of:

- Ternidazole (200 mg), an antimicrobial agent selective for anaerobic bacteria and protozoa;
- Nystatin (100000 IU), an antimycotic inhibiting the growth of yeast-like fungi;
- Neomycin sulfate (100 mg), a broad-spectrum antibiotic active against gram-positive and gram-negative bacteria;
- Prednisolon metasulphobenzoate sodium (3 mg), corticosteroid agent with local anti-inflammatory action.

Dosage – 1 ovule intravaginally daily for 10 days.

NB! «Tergynan» is not approved for use in the 1st trimester of pregnancy.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4.4}$	
1	Total Bacterial Mass	$10^{5.6}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	not detected	
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	$10^{5.6}$	0.0 (84–100 %)
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	
7	Eubacterium spp.	not detected	
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{3.4}$	-2.2 (0.5–0.7 %)
10	Lachnobacterium spp. + Clostridium spp.	not detected	
11	Mobiluncus spp. + Corynebacterium spp.	not detected	
12	Peptostreptococcus spp.	not detected	
13	Atopobium vaginae	not detected	
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{1.8}$	
16	Ureaplasma (urealyticum + parvum)*	$10^{1.8}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

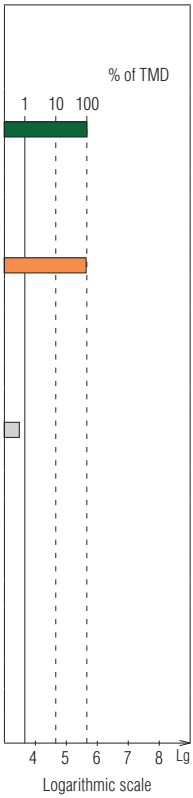


Figure 6.3. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»

Conclusion: apparent aerobic dysbiosis, due to the predominance of Streptococcus spp.

Apparent aerobic dysbiosis, primarily associated with enterobacteria (Figure 6.4)

When opportunistic microflora is mainly presented by gram-negative bacteria, *Enterobacteriaceae spp*., it may be a good alternative to use the coformulated drug **«Polygynax»**, containing:

- Neomycin sulfate (35000 IU), a broad-spectrum antibiotic active against gram-positive and gram-negative bacteria;
- Polymyxin B sulfate (35000 IU), an antibiotic active mainly against gram-negative bacteria;
- Nystatin (100,000 IU), an antimycotic inhibiting the growth of yeast-like fungi.

Dosage – 1 ovule intravaginally daily for 10 days.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4.6}$ ■	
1	Total Bacterial Mass	$10^{6.1}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	not detected	□
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	$10^{6.1}$	0.0 (85–100 %) ■
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	□
7	Eubacterium spp.	not detected	□
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	not detected	□
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected □	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	not detected □	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

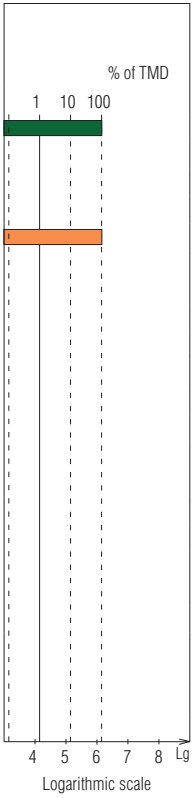


Рисунок 6.4. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: apparent aerobic dysbiosis, due to the predominance of Enterobacteriaceae spp.

NB! «Polygynax» is not approved for use during pregnancy and lactation.

If there are no lactobacilli, it is advisable to carry out a second stage which involves the use of probiotic drugs containing lactobacilli to restore the vaginal microbiota.

6.3. Treatment of apparent mixed dysbiosis

Clinically apparent mixed (aerobic-anaerobic) dysbiosis can manifest as both BV and AV. As with other dysbiosis variants, treatment in this case is carried out **in two stages**: elimination of OM during the first stage and restoration of native lactobacilli during the second stage. In the absence of *A. vaginae*, one of the complex drugs containing nitroimidazole derivatives («**Tergynan**») can be recommended to the patient.

If *A. vaginae* is detected, **Clindamycin** (see the chapter on the treatment of anaerobic dysbiosis) or **Nifuratel** are the drugs of choice.

Recommended regimen for nifuratel drugs

- «***Macmiror***» tablets contain nifuratel (200 mg), a nitrofuran derivative. It has antibacterial, antiprotozoal and antifungal effects.
  ➤ ***Dosage*** – 1 tablet 3 times daily after meals for 7 days.
- «***Macmiror Complex***» is either a suppository or a cream for vaginal use that contains nifuratel (500 mg in 1 suppository or 10 g per 100 g of cream) and nystatine (200000 IU in 1 suppository or 4 million IU in 100 g of cream) and has antibacterial, antiprotozoal and antifungal effects.
  ➤ ***Dosage*** – 1 suppository or 2.5 cream once or twice daily for 8 days.

6.4. The use of topical antiseptic drugs for the treatment of vaginal dysbiosis

At present, taking into account the continually increasing antibiotic resistance, it is advisable to use products from the antiseptics group. Currently, the topical antiseptics that have been thoroughly studied are chlorhexidine («**Hexicon**»), povidone-iodine («**Betadine**») and dequalinium chloride («**Fluomesin**»).

«**Hexicon**» (chlorhexidine) affects a wide range of microorganisms that cause aerobic and anaerobic vaginal dysbiosis. It does not have an adverse effect on lactobacilli and bifidobacteria and does not change vaginal pH.

Dosage – 1 suppository, twice daily, for 7–10 days.

«**Fluomizin**» (dequalinium chloride) is an antiseptic with antibacterial and antimycotic activity. One vaginal tablet dissolved in the vagina provides the level of antiseptic concentration that is 3–4 times higher than the minimum inhibitory dosage for the majority of pathogens.

Dosage – 1 suppository, twice daily, for 7–10 days.

NB! It is important to note that «Hexicon» and «Fluomizin» are allowed for use in all trimesters of pregnancy and lactation.

«**Betadine**» is an antiseptic with povidone-iodine as active ingredient. It is active against gram-positive and gram-negative aerobic and anaerobic bacteria, viruses, protozoa, fungi.

Dosage – in case of acute vaginitis, 1–2 suppositories daily 7 days; for chronic and subacute vaginitis, 1 suppository daily 14 days.

NB! The drug is not recommended after 3 months of pregnancy and during lactation.

6.5. Drug-free treatment of vaginal dysbiosis with chlorhexidine solution activated by low-frequency ultrasound treatment

The global spread of bacterial resistance requires the search for alternative treatment approaches to fight bacterial diseases. Resistance to antibiotics and antiseptics is mediated by the appearance of genetically resistant bacteria clones and by the formation of biofilms whose exopolysaccharide matrix protects bacteria from the antimicrobial drug penetrating the biofilm [29]. At the same time, bacteria associated with vaginal dysbiosis form biofilms on the surface of the vaginal epithelium [75, 76, 77].

Moreover, antibiotics used for the correction of vaginal dysbiosis are contraindicated to certain categories of patients, for example, pregnant women. In addition, regular use of imidazoles and clindamycin can lead to the development of intestinal dysbiotic disorders.

Low-frequency ultrasound (LFU) is a safe physical alternative. The treatment of liquids with low-frequency ultrasound leads to the formation of numerous micro-bubbles (this phenomenon is called cavitation). When bubbles collapse, at the boundary of the media kinetic energy is released. Properly dosed it creates a micro-massage effect on the epithelium, destroys the extracellular matrix of bacterial biofilms, and provides an

immunomodulatory effect. In addition, as a result of the water molecule decomposition, reactive oxygen species, destructive for many anaerobic bacteria, are formed [14]. It was showed that LFU has a promising synergistic bactericidal effect against both planktonic and biofilm bacteria [30].

As an effective alternative to antibiotics for the correction of dysbiosis, it was proposed to use a cavitated solution of chlorhexidine or other antiseptics for vaginal irrigation [13]. This procedure does not eliminate certain vaginal lactobacilli types and significantly increases the relative proportion of lactobacilli in the microbiocenosis [4, 13].

Recommended regimen for dysbiosis treatment using chlorhexidine solution cavitated with low-frequency ultrasound (LFU).

A 0.05 % chlorhexidine solution can be used as an antiseptic. Vaginal irrigation with a 0.05 % chlorhexidine solution, cavitated with LFU, is carried out using AUZH-100 cavitation apparatus (Fotek, Russia) in accordance with the guidelines [11]: exposure time is 1–2 minutes, the power is 6–8 units, and the amount of the solution used is 150–200 ml. The number of irrigations depends on the initial state of the microbiocenosis and on the clinical manifestation (vaginal symptoms) of the disease and usually ranges from 3 to 7 procedures (1 irrigation per day, duration 3–7 days). After a series of irrigations, it is possible to prescribe a course of probiotic drugs in order to restore normal flora.

7. MANAGEMENT OF PATIENTS WITH MODERATE VAGINAL DYSBIOSIS

In some cases, moderate dysbiosis can be considered as a variant of the normal state of vaginal microbial community or as a transient condition that does not require treatment [3]. However, in a number of cases (namely, patients with clinical manifestations, BROH or those preparing for gynecological surgery) moderate dysbiosis has to be treated. In this case it is preferable to use topical antiseptics, drug-free methods (irrigation with solutions cavitated by LFU). One should take into account the necessity of restoring vaginal pH, the lactobacilli population, function and trophism of the vaginal epithelium.

Drugs containing ascorbic acid (**«Vaginorm C»**) and lactic acid (**«Femilex»**, **«Lactagel»**) are used to maintain the acidic environment in the lower urogenital tract.

Dosage:

- ***Vaginorm C*** (250 mg of ascorbic acid in 1 vaginal tablet) – 1 tablet daily for 6 days;
- ***Femilex*** (100 mg of lactic acid in 1 vaginal suppository) – 1 suppository daily for 10 days;
- ***Lactagel*** (225 mg of lactic acid + glycogen in 1 gel tube) – 1 tube daily for 7 days.

NB! Drugs acidifying vaginal environment are not recommended for infections associated with *Candida spp.* and when planning natural conception.

Intravaginal or oral probiotics are used for the restoration of the native lactobacilli population (see the section on the treatment of apparent anaerobic dysbiosis).

In women with low estrogen levels (perimenopause), local administration of estrogen has been shown to restore function and trophism of the vaginal epithelium. Complex drugs containing estriol (**«Trioginal»**, **«Gynoflor E»**) maintain maturation and shedding of the vaginal epithelium, facilitating the vaginal colonization by lactobacilli [15].

8. MANAGEMENT OF PATIENTS WITH UROGENITAL INFECTIONS ASSOCIATED WITH OPPORTUNISTIC GENITAL MYCOPLASMAS OTHER THAN *M. GENITALIUM*

Genital mycoplasmas (*Ureaplasma parvum, Ureaplasma urealyticum, M. hominis*) are opportunistic microorganisms that may be present as part of the normal microflora of the urogenital tract. Asymptomatic carriage of these bacteria is common, and the majority of individuals do not develop a disease. An exception is *M. genitalium*, which is considered to be an obligate STP.

Urogenital diseases associated with mycoplasmas have no specific pathognomonic symptoms, but include pelvic inflammatory disease, cervicitis, miscarriage, infertility, chorionamnionitis, post-partum fever and stillbirth. [58, 85, 86].

On the other hand, genital mycoplasma infection can be asymptomatic in many cases. Ureaplasmas and mycoplasmas persist in the vagina both during normal and dysbiotic conditions. Moreover, *Ureaplasma spp.* in quantity >10^4 GE/ml is significantly more often detected in normal microbiota (when the proportion of lactobacilli is high), and *M. hominis* often associated with anaerobic dysbiosis [6].

Indications for treatment

Asymptomatic carriage of these bacteria is common and the majority of individuals do not need any treatment. If *M. hominis* and/or *Ureaplasma spp.* are detected in a quantity lower than 10^4 GE / ml, and there is no clinical and/or laboratory evidence of urogenital inflammation, then treatment is not carried out.

Only symptomatic patients with BROH, negative tests for STP (*C. trachomatis, N. gonorrhoeae, T. vaginalis, M. genitalium*) and bacterial load > 10^4 GE/ml should be considered for specific antimicrobial therapy.

Sexual partners of a patient infected with *Ureaplasma spp.* and/or *M. hominis*, are subject to treatment if they have clinical and/or laboratory signs of the urogenital tract inflammation (provided that any other possible etiology of this inflammatory process was ruled out).

8.1. Treatment of urogenital inflammatory diseases, associated with Ureaplasma spp., when conditional vaginal normocenosis was detected

Conditional normocenosis is a variant of the vaginal microbial community with predominance of lactobacilli (more than 80 % of the TBM) and with high bacterial load (more than 10^4 GE/ml) of associated MO (Figure 8.1 and Figure 8.2) [1].

The purpose of the treatment is the elimination of laboratory signs of inflammation and the recovery of the patient. Eradication of genital mycoplasmas is not a requirement for the successful treatment. (Federal clinical guidelines for the management of patients with urogenital diseases caused by *Ureaplasma spp., Mycoplasma hominis*, Moscow 2015 [18]).

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{6.1}$ ■	
1	Total Bacterial Mass	$10^{7.6}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{7.3}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	□
7	Eubacterium spp.	not detected	□
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	$10^{3.1}$	-4.1 (<0,1 %) □
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected □	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	$10^{5.0}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

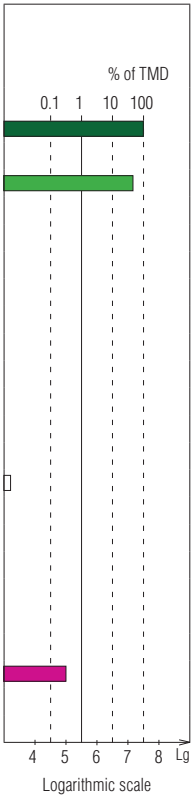


Figure 8.1. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: conditional normocenosis, associated with Ureaplasma spp.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{4.6}$ ■	
1	Total Bacterial Mass	$10^{7.3}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{7.3}$	0.0 (82–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{4.4}$	-2.9 (<0.1 %) □
7	Eubacterium spp.	$10^{3.7}$	-3.6 (<0.1 %) □
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{4.6}$	-2.5 (0.3–0.4 %) □
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	$10^{3.2}$	-4.1 (<0,1 %) □
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected □	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{5.8}$ ■	
16	Ureaplasma (urealyticum + parvum)*	$10^{4.8}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

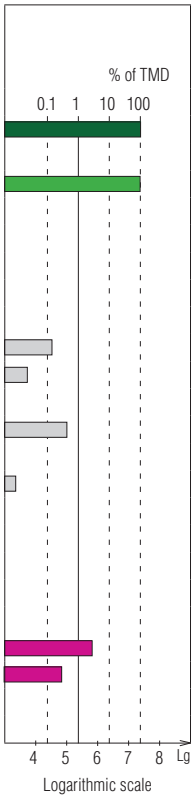


Figure 8.2. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: conditional normocenosis, associated with Ureaplasma spp. and Mycoplasma hominis.

Recommended treatment schemes:

- Doxycycline monohydrate 100 mg orally twice daily for 10 days (grade of recommendation B);
- Josamycin 500 mg orally 3 times a day for 10 days (grade of recommendation B).

Special situations: According to evidence-based medicine, systemic enzyme therapy, immunomodulatory therapy, and therapy exclusively with local antiseptic preparations are not recommended.

8.2. Management of patients with dysbiosis in the presence of high bacterial load of Ureaplasma spp. and Mycoplasma hominis

The number of *M. hominis* is usually increased in vaginal microbial community predominated with strict anaerobes – anaerobic dysbiosis (Figure 8.3), but the contribution of mycoplasmas to the development of the pathological process has not yet been clarified. High bacterial load of *M. hominis* in BV increases the risk of developing pelvic inflammatory disease and post-partum infections. In case of mixed infection the goal of the therapy is the control of BV [24]. In case of a mixed infection associated with obligate anaerobes and genital mycoplasmas, at first stage the patient should be treated according to recommended regimens for BV with a subsequent assessment of clinical efficacy and evaluation of *Ureaplasma spp*. and/or *M. hominis* bacterial load at follow-up. If the signs of the inflammation persist and mycoplasmas bacterial load is still high, systemic treatment could be prescribed (according to one of the regimens).

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{6.2}$	
1	Total Bacterial Mass	$10^{8.4}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{4.4}$	-4.1 (<0.1 %)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	
4	Streptococcus spp.	$10^{6.1}$	-2.4 (0.4-0.5 %)
5	Staphylococcus spp.	$10^{3.3}$	-5.1 (<0.1 %)
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{8.3}$	-0.2 (56-75 %)
7	Eubacterium spp.	$10^{7.5}$	-1.0 (9-13 %)
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{7.5}$	-1.0 (9-12 %)
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{7.4}$	-1.1 (7–10 %)
10	Lachnobacterium spp. + Clostridium spp.	$10^{4.9}$	-3.5 (<0.1 %)
11	Mobiluncus spp. + Corynebacterium spp.	not detected	
12	Peptostreptococcus spp.	$10^{7.0}$	-1.4 (3-4 %)
13	Atopobium vaginae	not detected	
	YEAST-LIKE FUNGI		
14	Candida spp.*	not detected	
	MYCOPLASMAS		
15	Mycoplasma hominis*	$10^{1.6}$	
16	Ureaplasma (urealyticum + parvum)*	$10^{5.3}$	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

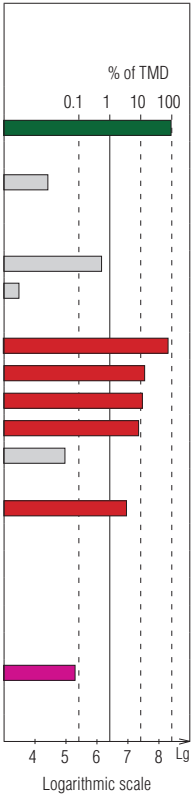


Figure 8.3. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»

Conclusion: apparent anaerobic dysbiosis with A. vaginae, Ureaplasma spp., Mycoplasma hominis and Candida spp. in clinically significant amounts.

9. MANAGEMENT OF PATIENTS WITH UROGENITAL INFECTIONS ASSOCIATED WITH *CANDIDA SPP*.

Currently, vulvovaginal candidiasis (VVC) can be classified as either uncomplicated or complicated (CDC, 2015 – Centers for Disease Control and Prevention, USA). Uncomplicated VVC is diagnosed when the woman either is newly diagnosed with VVC or has less than 4 episodes a year. Usually uncomplicated VVC is caused by *C. albicans*, develops in patients without co-morbidities leading to immunosuppression (diabetes mellitus, treatment with cytotoxic drugs, glucocorticoids, etc.) and has moderate clinical manifestation.

The diagnosis of complicated VVC should be established in the presence of severe vaginal symptoms of vulvovaginal candidiasis (redness, swelling, ulceration, cracks of the mucous membranes and skin of the perianal area). Recurrent VVC defined as 4 or more episodes of symptomatic VVC occur within one year. VVC is also considered as being complicated if *C. non-albicans* is detected and risk factors associated with possible immunosuppression (diabetes mellitus, treatment with cytotoxic drugs, glucocorticoids) are present.

Indications for treatment: a diagnosis of urogenital candidiasis determined based on the clinical picture and laboratory studies.

9.1. Treatment of patients with conditional normocenosis associated with Candida spp.

The lab report generated for an RT-PCR test of vaginal microbiocenosis, typical of vulvovaginal candidiasis, is shown in Figure 9.1. The microscopic pattern usually meets the criteria for specific vaginitis (WBC >10 or II >1, pseudohyphae or budding spores of yeast-like fungi are identified) (see Figure in the Appendix).

The purpose of the treatment is clinical recovery, normalization of laboratory test results (the decrease of WBC count in wet mount or Gram stain and the absence of or a significant reduction in the number of fungi in the Femoflor test), and the prevention of post-partum and postoperative complications.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{6.2}$ ■	
1	Total Bacterial Mass	$10^{8.8}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{8.8}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{4.1}$	-4.6 (<0.1 %) □
7	Eubacterium spp.	$10^{3.1}$	-5.7 (<0.1 %) □
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	not detected	□
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	$10^{2.3}$	-6.5 (<0.1 %) □
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{4.9}$ ■	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	not detected □	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

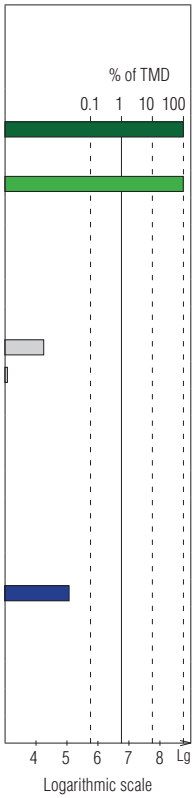


Figure 9.1. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: conditional normocenosis, associated with Candida spp.

Recommended regimen for VVC treatment (one of suggested variants):

- Natamycin, vaginal suppositories 100 mg once daily for 6 days;
- Clotrimazole, vaginal tablet 200 mg once daily at bedtime for 3 days or 100 mg once daily at bedtime for 7 days;
- Clotrimazole, cream 1 % 5 g intravaginally once daily at bedtime for 7-14 days;
- Itraconazole, vaginal tablet 200 mg once daily at bedtime for 10 days;
- Miconazole, vaginal suppositories 100 mg once daily at bedtime for 7 days;
- Butoconazole, 2 % cream 5 g intravaginally in a single application;
- Fluconazole 150 mg orally in a single dose;
- Itraconazole 200 mg, once a day for 3 days.

In case of severe vaginal symptoms of VVC, it is recommended to increase the duration of intravaginal therapy with drugs of the azole group up to 10–14 days or to increase the fluconazole dose: 150 mg orally twice within 72 hours.

Treatment of complicated recurrent vulvovaginal candidiasis

Treatment of chronic recurrent urogenital candidiasis is carried out in two stages. The purpose of the first stage is to stop the recurrence of the disease. For this, the upper recommended regimens are used. In case of chronic recurrent VVC caused by *Candida spp.* sensitive to azoles, the use of topical azole agents up to 14 days or 150 mg fluconazole every third day for a total of 3 doses is recommended. In cases of nonalbicans Candida VVC treatment with natamycin is recommended: 100 mg intravaginally daily for 6–12 days.

After achieving the clinical and microbiological effect, the second stage of therapy is recommended – supportive treatment, for 6 months with **one of the following drugs**:

- Natamycin, 100 mg vaginal suppositories once a week;
- Clotrimazole, 500 mg vaginal tablet once a week;
- Fluconazole 150 mg orally, in a single dose dose once a week.

If the disease recurs after the end of the supporting treatment less than 4 times a year, the treatment is carried out according to the regimens for a separate episode. If relapses develop more than 4 times a year, the course of supporting treatment should be prescribed again.

Special situation – treatment of pregnant women.

For the treatment of pregnant women, **topical antifungals are used (one of the indicated regimens)**:

- Natamycin, 100 mg vaginal suppository, one suppository daily for 3–6 days (allowed for use from the first trimester);
- Clotrimazole, 100 mg vaginal tablet, one tablet daily at bedtime for 7 days (allowed for use from the second trimester);
- Clotrimazole, 1 % cream 5 g 1 intravaginally daily at bedtime for 7 days (allowed for use from the second trimester).

9.2. Treatment of patients with conditional vaginal normocenosis associated with Candida spp. and Ureaplasma spp.

Patients with this variant of vaginal microbiocenosis (Figure 9.2), could be treated with a drug combination effective for treating infections associated with *Ureaplasma spp.* or *Candida spp.*

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{5.3}$ ■	
1	Total Bacterial Mass	$10^{8.2}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{8.2}$	0.0 (85–100 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{5.5}$	-2.7 (0.2–0.2 %) □
7	Eubacterium spp.	$10^{4.6}$	-3.6 (<0.1 %) □
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	not detected	□
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{5.3}$ ■	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	$10^{4.8}$ ■	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

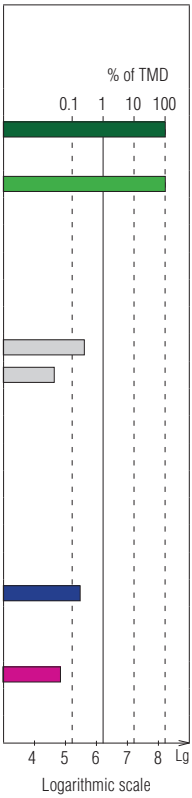


Figure 9.2. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: conditional normocenosis, associated with Ureaplasma spp. and Candida spp.

9.3. Management of patients with dysbiosis and Candida spp.

The treatment of mixed bacterial and fungal vaginal infections presents a particular challenge. The combination of anaerobic dysbiosis (BV) and VVC (when the quantity of *Candida spp.* > 10^4 GE/ml) requires the use of coformulated drugs effective against both groups of MO.

Recommended regimen (one of suggested variants):

- «Neo-Penotran» (containing 500 mg of metronidazole and 100 mg of myco-nazole nitrate): one vaginal suppository twice daily for 7 days;
- «Neo-Penotran Forte» (metronidazole – 750 mg, miconazole nitrate – 200 mg): one suppository intravaginally once daily at bedtime for 7 days
- «Klion D» (metronidazole 100 mg and miconazole nitrate 100 mg): one suppository intravaginally once daily at bedtime for 10 days. This combination enhances the antibacterial efficacy of metronidazole due to the additive effect of miconazole and the prevention of the development of *Candida* infection.

A special case — anaerobic dysbiosis with significant quantities of *A. vaginae* and *Candida* in spp. (Figure 9.3)

«**Macmiror**» with proven activity against bacteria and fungi, including *Candida spp.* and *A. vaginae* is the drug of choice. The presence of *A. vaginae* would require the prescription of **Clindamycin**, effective against a wide range of opportunistic microorganisms. Clindamycin is also effective against lactobacilli, which in turn can lead to the development of VVC.

Recommended regimen (one of suggested variants):

- «Macmiror» tablets (containing nifuratel 200 mg): 1 tablet 3 times daily after meals for 7 days;
- «Macmiror complex» (nifuratel 500 mg and nistatin 200000 ME in 1 suppository) one suppository intravaginally at bedtime or 2.5 g of cream once or twice daily for 8 days.

An **alternative** may be the vaginal cream «Clindacin B prolong», 100 mg of which contains 2 mg of butoconazole with fungicidal activity and 2 mg clindamycin.

Dosage: cream, 5 g intravaginally once daily, for at least 3 days.

Restoration of lactobacilli population at the second stage of treatment could be advised to patients with persistent dysbiosis at follow-up.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{6.1}$	
1	Total Bacterial Mass	$10^{8.3}$	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{5.5}$	-2.5 (0.2–0.3 %)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	$10^{3.6}$	-4.5 (<0.1 %)
4	Streptococcus spp.	not detected	
5	Staphylococcus spp.	not detected	
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{7.3}$	-0.7 (15–21 %)
7	Eubacterium spp.	$10^{6.9}$	-1.1 (6–8 %)
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	$10^{6.7}$	-1.4 (3–5 %)
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{7.3}$	-0.8 (15–20 %)
10	Lachnobacterium spp. + Clostridium spp.	$10^{5.9}$	-2.1 (0.6–0.9 %)
11	Mobiluncus spp. + Corynebacterium spp.	$10^{4.7}$	-3.4 (<0.1 %)
12	Peptostreptococcus spp.	$10^{6.6}$	-1.5 (3–4 %)
13	Atopobium vaginae	$10^{7.7}$	-0.3 (41–56 %)
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{5.9}$	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected	
16	Ureaplasma (urealyticum + parvum)*	not detected	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected	

* Quantitative analysis Lg (X).
** Qualitative analysis.

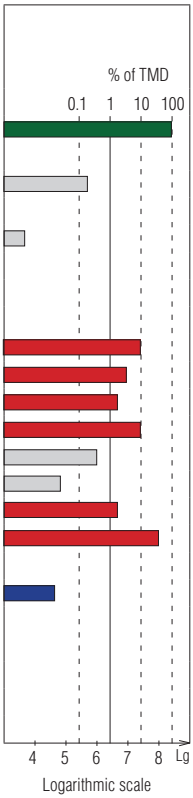


Figure 9.3. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: apparent anaerobic dysbiosis, predominated by A. vaginae; with significant quantity of Candida spp.

A special case is moderate anaerobic dysbiosis with significant amounts of *Candida spp.* (Figure 9.4).

Patients with this variant of vaginal dysbiosis should be treated only in case of clinical manifestation. The predominance of VVC symptoms requires the prescription of antifungals with the subsequent assessment of the state of vaginal microbiocenosis.

The absence of vaginal symptoms, a decrease in the number of *Candida spp.* and the increase in the proportion of lactobacilli will be the recovery criteria. If dysbiosis persists, probiotics should be recommended.

In patients with predominance of clinical and microscopic signs of BV, it is recommended to use **coformulated drugs** effective against both the anaerobic microflora and fungi of *Candida spp.* («Klion D», «Neopenotran», «Macmiror», «Clindacin B prolong»). Restoration of lactobacilli population using probiotics is required at the second stage of treatment.

No	Test title	Result	
		Quantitative	Relative Lg (X/TMD)
	Sample intake control	$10^{5.1}$ ■	
1	Total Bacterial Mass	$10^{6.6}$ □	
	NORMAL MICROFLORA		
2	Lactobacillus spp.	$10^{6.3}$	-0.3 (40–55 %) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
3	Enterobacteriaceae	not detected	□
4	Streptococcus spp.	not detected	□
5	Staphylococcus spp.	not detected	□
	OBLIGATE ANAEROBIC MICROORGANISMS		
6	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	$10^{6.0}$	-0.7 (18–25 %) ■
7	Eubacterium spp.	$10^{6.1}$	-0.5 (26–36 %) ■
8	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	□
9	Megasphaera spp. + Veillonella spp. + Dialister spp.	not detected	□
10	Lachnobacterium spp. + Clostridium spp.	not detected	□
11	Mobiluncus spp. + Corynebacterium spp.	not detected	□
12	Peptostreptococcus spp.	not detected	□
13	Atopobium vaginae	not detected	□
	YEAST-LIKE FUNGI		
14	Candida spp.*	$10^{4.5}$ ■	
	MYCOPLASMAS		
15	Mycoplasma hominis*	not detected □	
16	Ureaplasma (urealyticum + parvum)*	not detected □	
	PATHOGENIC MICROORGANISMS		
17	Mycoplasma genitalium**	not detected □	

* Quantitative analysis Lg (X).
** Qualitative analysis.

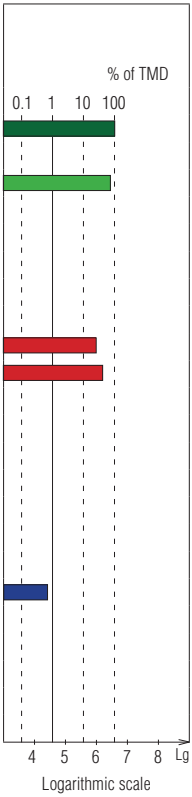


Figure 9.4. Example of a lab report generated after testing the vaginal microbiocenosis using the RT-PCR Test «Femoflor»
Conclusion: moderate anaerobic dysbiosis with significant amounts of Candida spp.

CHECK YOURSELF

MULTIPLE-CHOICE QUESTIONS

1. Interprete the results of a vaginal RT-PCR test for assessment of vaginal microbiota analysis for patient X. (the proportion of lactobacilli is 84 %, the amount of *Ureaplasma spp.* – $10^{1.9}$ GE/ml, *Candida spp.* – $10^{3.1}$ GE/ml):

A. absolute normocenosis
B. conditional normocenosis
C. moderate dysbiosis
D. apparent dysbiosis

2. Select the group of opportunistic microorganisms associated with the development of aerobic vaginitis:

A. *Atopobium vaginae*
B. *Gardnerella vaginalis*
C. *Enterobacteriaceae spp.*
D. *Eubacterium spp.*

3. Select the the most effective drug against *Atopobium vaginae*:

A. Fluconazole
B. Amoxiclav
C. Metronidazole
D. Clindamycin

4. Derivatives of nitroimidazoles are not recommended for the treatment of dysbiosis in presence of:

A. *G. vaginalis / Prevotella bivia / Porphyromonas spp.*
B. *Eubacterium spp.*
C. *Mobiluncus spp./Corynebacterium spp.*
D. *A. vaginae*

5. The azoles could be used for treatment of vaginitis associated with:

A. *M. hominis*
B. *C. albicans*
C. *Ureaplasma spp.*
D. *Enterobacteriaceae spp.*

6. Select the probiotic oral drug containing live *L. crispatus*:

A. «Ecofemin Floravag»
B. «Vagilac»
C. «Lactoginal»
D. «Tergynan»

7. Choose the coformulated antimicrobial drug affecting aerobic and anaerobic bacteria and yeast-like fungi:

A. Clindamycin
B. «Macmiror»
C. «Polygynax»
D. Metronidazole

8. Choose the complex antimicrobial drug that is active against both aerobic and anaerobic bacteria and has a significant anti-inflammatory effect:

A. «Polygynax»
B. «Tergynan»
C. Clindamycin
D. «Macmiror Complex»

9. For the treatment of dysbiosis associated with *A. vaginae*, the following antimicrobial drugs are recommended:

A. Metronidazole and Clindamycin
B. Metronidazole and Nifuratel
C. Clindamycin and Nifuratel
D. Ketronidazole and Fluconazole

10. For the treatment of the vaginitis associated with *Ureaplasma spp.* in pregnant women the following drug should be used:

A. Josamycin
B. Doxycycline
C. Clindamycin
D. Metronidazole

ANSWERS TO CHECK YOURSELF

1. Correct answer A. Absolute normocenosis, since the proportion of lactobacilli is more than 80 %, and the amount of associates (*Ureaplasma spp., Candida spp.*) is $<10^4$ GE/ml.

2. Correct answer C. Aerobic vaginitis can develop due to the excessive growth of the *Enterobacteriaceae spp*., of *Streptococcus spp.* and *Staphylococcus spp*.. Other microorganisms listed in the answers are anaerobes and associated with the development bacterial vaginosis.

3. Correct answer D. Metronidazole and «Amoxiclav» are antibacterial drugs, but they are not effective against *A. vaginae*. Fluconazole is an antifungal drug that does not affect bacteria, including *A. vaginae*.

4. Correct answer D. *A. vaginae* is not sensitive to the derivative of nitroimidazoles: metronidazole, ornidazole, tinidazole, etc. Other groups of bacteria listed are highly sensitive to these drugs.

5. Correct answer B. The azole group drugs (fluconazole, ketoconazole, etc.) block the synthesis of the fungi cytoplasmic membrane components, including *C. albicans*, and do not affect bacteria, which are all other microorganisms listed in the answers.

6. Correct answer A. *L. crispatus* is contained only in the drug «Ecofemin Floravag», intended for oral administration. The oral probiotic «Vagilac» contains *L. reuteri* and *L. rhamnosus* cells, but not *L. crispatus*. The «Lactoginal» contains *L. casei* rhamnosus and is only available for topical administration. «Tergynan» is not a probiotic drug.

7. Correct answer C. «Polygynax» contains an antifungal agent, Nystatin, and two antibacterial agents: Neomycin sulfate, Polymyxin B.

8. Correct answer B. In addition to antimicrobial compounds «Tergynan» contains Prednisolone with pronounced anti-inflammatory effect. «Polygynax» and «Macmiror Complex» consist of combinations of antimicrobial drugs.

9. Correct answer C. *Atopobium vaginae* is resistant to the derivatives of nitroimidazoles, including Metronidazole. Therefore, clindamycin and nifuratel are the drugs of choice used for the treatment of dysbiosis associated with this microorganism. Fluconazole does not affect bacteria.

10. Correct answer A. For the treatment of vaginitis associated with *Ureaplasma spp.* two drugs are recommended: Doxycycline monohydrate and Josamycin. However, Doxycycline is contraindicated during pregnancy.

CASE STUDIES

Case study no. 1.

Patient M., 28 years old, seeks medical advice for pregnancy planning. Obstetric history: surgical abortion at 6–7 weeks of gestation. Chronic recurrent cystitis in remission. No complaints at the moment. Speculum examination: vaginal mucosa is not changed, scanty white thick discharge; the cervix is clean, scanty, mucoid discharge.

Microscopy: the cervix: columnar epithelium 8–12–15 per field of view; WBC 20–26 per field of view; microflora consists mainly of rod-shape bacteria; pseudohyphae or budding spores of yeast-like fungi, trichomonas were not found. Vagina: squamous epithelium 4–6–11 per field of view; WBC 16-18-24 field of view; microflora mixed, represented mainly by rod-shape bacteria; pseudohyphae or budding spores of yeast-like fungi, trichomonas were not found. Conclusion: inflammatory type smear.

Femoflor result: «Moderate anaerobic dysbiosis» (lactobacillus proportion – 64 %), *Ureaplasma spp.* is found in a clinically significant amount (5.6 Lg GE/ml).

1. Diagnose the patient.
2. Assess the need for treatment.
3. Justify the regimen, if needed.

Case study no. 2.

Patient R., 25 years old, came for prophylactic examination. Gynaecological history: sexually active from 16 years, unmarried, seven sexual partners (new partner appeared 2 months ago), claims to have no gynecological diseases. Together with her partner the patient was tested for STP (*Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhoeae*) by PCR method, the tests were negative. The patient does not have any complaints. Objectively, speculum examination: vagina looks moderately inflamed, copious thin white discharge; the cervix is clean, scanty mucoid discharge.

Microscopy. The cervix: columnar epithelium 11–15–19 per field of view; WBC 10–12–18 per field of view; microflora is represented by cocci; pseudohyphae or budding spores of yeast-like fungi, trichomonas were not found. Vagina: squamous epithelium 5–6–8 per field of view; WBC 0–1–2 per field of view; microflora is represented by cocci; pseudohyphae or budding spores of yeast-like fungi, «clue» cells, Trichomonas are not found. Conclusion: inflammatory type smear.

Femoflor. Apparent anaerobic dysbiosis (lactobacillus proportion – 2 %), *A. vaginae* (24 % of TBM) was detected.

1. Diagnose the patient.
2. Assess the need for treatment.
3. Justify the regimen, if needed.

ANSWERS FOR CASE PROBLEMS

Case study no. 1

Correct answer

1. Subacute vaginitis, associated with *Ureaplasma spp*.
2. Treatment is necessary.
3. For the first stage prescribe Doxycycline monohydrate 100 mg orally twice daily for 10 days or Josamycin 500 mg orally 3 times a day for 10 days. For the second stage, topical («Ecofemin», «Lactoginal», «Lactonorm») or oral («Vagilac», «Ecofemin Floravag») probiotics with lactobacilli are recommended.

Comments. The BROH, concomitant inflammatory disease of the urinary tract, reproductive plans of the woman and signs of the inflammatory process according to microscopy require treatment with antibiotics to which *Ureaplasma spp.* is sensitive. Moderate decrease in the proportion of lactobacilli at the beginning of the treatment (moderate anaerobic dysbiosis) is a risk factor for the development of apparent dysbiotic disorders after antibiotic therapy. The use of probiotic drugs is advisable for the restoration of normal vaginal microbiota.

Case study no. 2

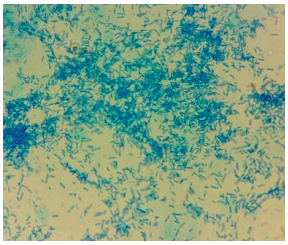
Correct answer.

1. Bacterial vaginosis.
2. Treatment is necessary.
3. For the first stage prescribe Clindamycin suppository 100 mg daily (at bedtime) for 3 days or Clindamycin cream 2 % 5.0 g intravaginally daily (at bedtime) for 7 days. For the second stage, topical («Ecofemin», «Lactoginal», «Lactonorm») or oral («Vagilac», «Ecofemin Floravag») probiotics with lactobacilli are recommended.

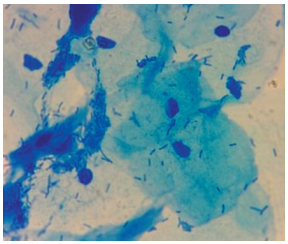
Comments. Bacterial vaginosis is a clinical manifestation of predisposing apparent anaerobic dysbiosis according to RT-PCR data. Frequent change of sexual partners is one of the risk factors for the development of this condition. Treatment is necessary.

A. vaginae is resistant to nitroimidazole derivatives, which requires the prescription of Clindamycin or Nifuratel. Absence of *Candida spp*. points toward selecting Clindamycin. Restoration of lactobacilli population is necessary for the prevention of recurrence of BV.

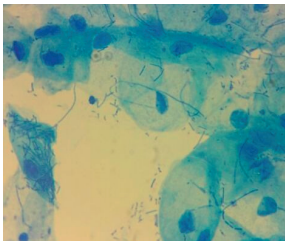
APPENDIX.
MICROSCOPIC IMAGES OF VAGINAL SMEAR SAMPLES



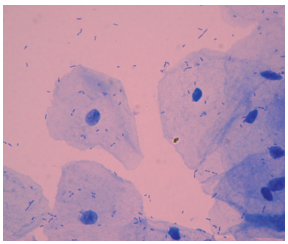
Microscopic image 1. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Normal microbiota smear.



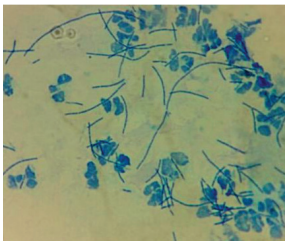
Microscopic image 2. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Normal microbiota smear.



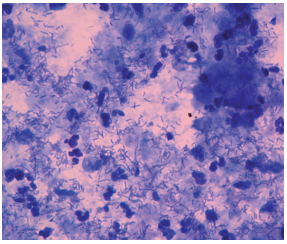
Microscopic image 3. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Intermediate type smear.



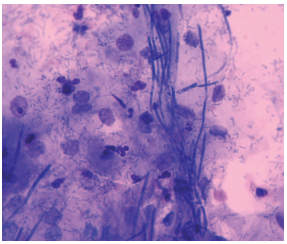
Microscopic image 4. Vaginal discharge sample.. Light microscopy. Methylene blue staining. x1000. Intermediate type smear.



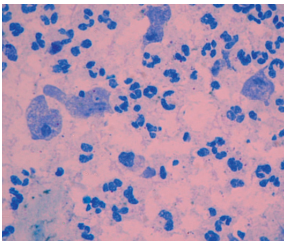
Microscopic image 5. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Inflammatory type smear: non-specific vaginitis.



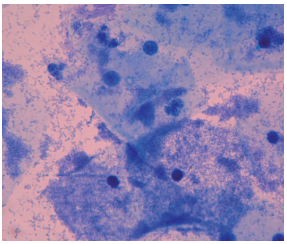
Microscopic image 6. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Inflammatory smear type: non-specific vaginitis.



Microscopic image 7. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Inflammatory type smear: pseudohyphae of yeast-like fungi.



Microscopic image 8. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Inflammatory type smear: Trichomonas.



Microscopic image 9. Vaginal discharge sample. Light microscopy. Methylene blue staining. x1000. Dysbiotic type smear.

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