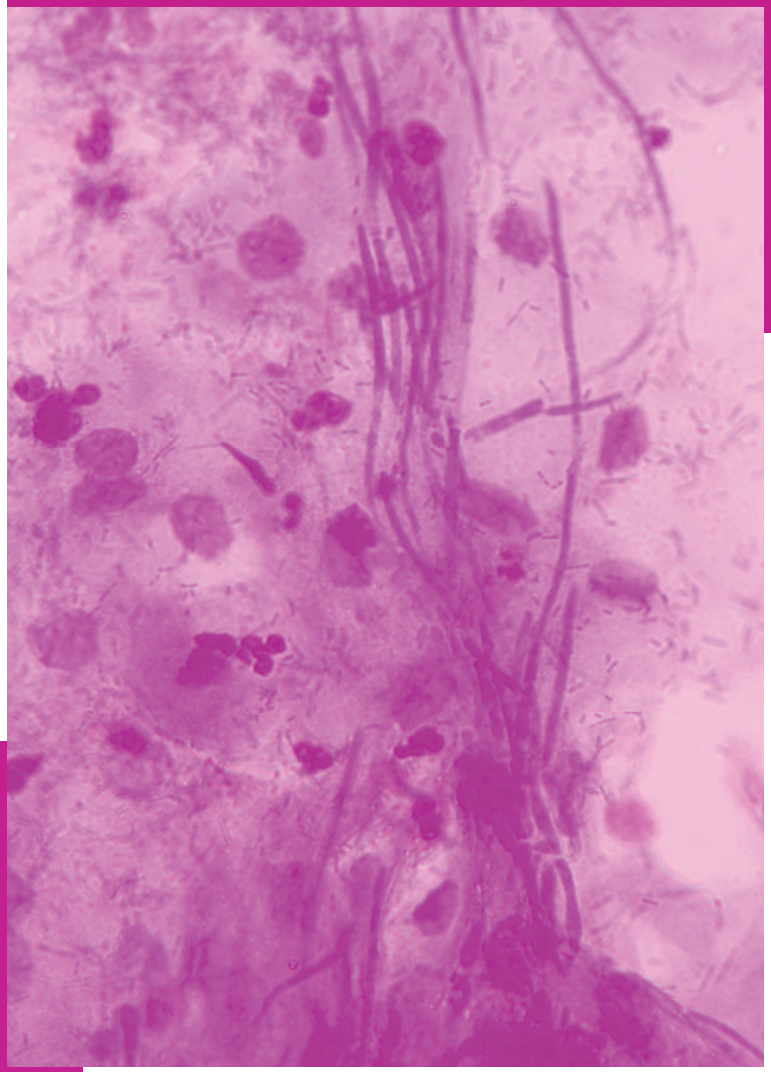
FEMOFLOR®: BRIEF INTRODUCTION

Answers to basic doctors' questions regarding the diagnosis of reproductive tract infections



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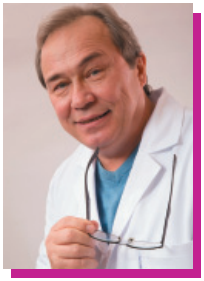

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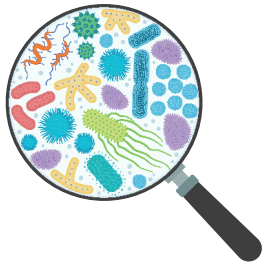
CONTENT

01

WHY DOES FEMOFLOR® INCLUDE SO MANY MICROBES? WHY IS TRADITIONAL STD-SCREENING NOT SUFFICIENT?



According to the results of international Vaginal Human Microbiome Project, it was found that there are more than one thousand types of microorganisms in vaginal biota of healthy women — representatives of normal and opportunistic flora. In most cases, dominance of lactobacilli is the main criteria of normal microbiome composition in reproductive-age women. Therefore, the purpose of diagnostics has changed: there is a need not only for qualitative detection of STDs causative agents, but also for evaluation of microbiome composition, including quantitative testing for pathogens and opportunistic microflora. Statistically more than 80% of women visit a gynecologist not because of STDs (less than 10%); usually they seek medical advice with symptoms of imbalance in microbiota.
Femoflor® was created for diagnosis of such conditions.

02

WHY IS PCR USED? WHAT IS THE DIFFERENCE BETWEEN CONVENTIONAL AND REAL-TIME PCR?

PCR is the most sensitive and specific method for detection of microorganisms and viruses based on the identification and quantification of genome's fragments. It is a technique employing detection instruments and software, so the personnel risk of incorrect or subjective evaluation of the test results is minimal. Because PCR is not a cultivation technique, strict requirements for preservation of the microbial viability during transport to the laboratory and for the technical capabilities of the bacterial laboratory for cultivation of strict anaerobic microorganisms are irrelevant.

Conventional PCR (end-point PCR, «qualitative» PCR) made it possible to obtain a result only in «detected/ not detected» format. It is clinically significant only for detection of obligate pathogens, because normally they should not be present in microbiome composition of urogenital tract. Real-time PCR technology is intended for qualitative but also for quantitative assessment. Its results make it possible to establish whether the microflora balance is disturbed or not, and in case of imbalance, to identify the dominant group of opportunistic bacteria (Fig. 1).

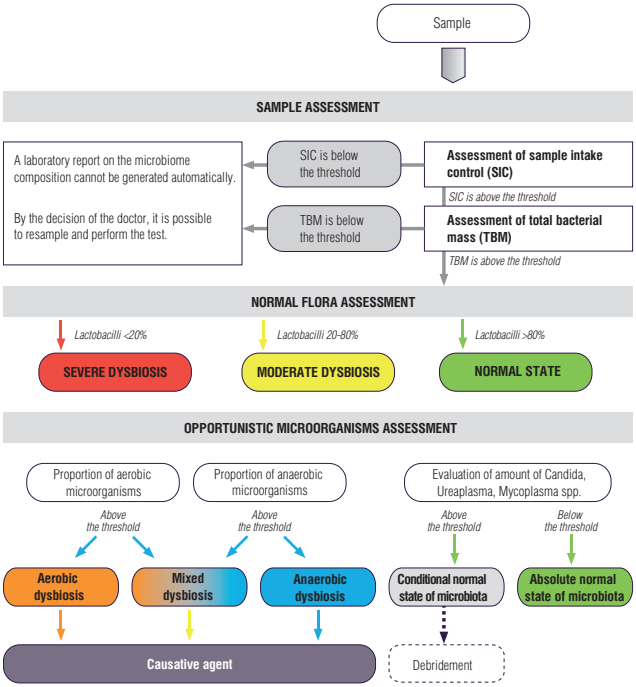


Fig. 1. Algorithm for the interpretation of Femoflor®

03

WHY DO YOU NEED FEMOFLOR®, IF YOU HAVE A MICROSCOPY?

Nobody calls to completely replace traditional diagnostic methods of infectious diseases with Femoflor®.

It is relevant to start the examination with pH-measurement. In many countries it is the first line of screening. If normal pH values are detected in the absence of clinical symptoms, further examination is not required. In case of a shift of the acidity index to the alkaline side from 4.5 to 7.5, further differential diagnostics using Femoflor® is recommended.

Microscopy is a fast, familiar and inexpensive way of evaluation of microbiome composition and presence/absence of inflammation (based on the leucocytes count). However, microscopy describes only morphological grounds, without quantitative assessment and microbial typing. This method is subjective and the terms “predominant bacillus flora” or “lactobacilli dominate” mean the presence of a wide group of microorganisms that look like lactobacilli in the sample (lactobacilli are mainly anaerobic microorganisms).

In the hands of experienced, specially trained pathologist the microscopic method is irreplaceable for assessing the vaginal microbiome composition, which can be used in conjunction with Femoflor® test. The assessment of microbiocenosis is more complex than the detection of leukocytes and the determination of the bacterial morphotype; it is also the ratio of leukocytes to epithelial cells, the presence or prevalence of lactobacilli, the presence of basal / parabasal cells, “clue” cells, their differentiation from pseudo “clue” cells and many other parameters.

ARE MICROBIOLOGICAL CULTURES GOOD ENOUGH? IS IT SUFFICIENT TO DETERMINE PH?

It should be noted that «clue» cells can be a sign of bacterial films. This fact is important for the choice of therapy when identifying opportunistic bacteria.

Microscopic criteria are widely used to diagnose aerobic vaginitis and bacterial vaginosis (Donders, Nugent, Ison-Hay criteria). For a long time, bacteriological (cultural) methods remained the gold standard of diagnostics, and they are still widespread among doctors, primarily because of the ability not only to determine the types of microorganisms, but also the antibiotic sensitivity.

However, all this is true for microorganisms, if it is possible to:

- preserve their viability at the stages of specimen collection, storing and transporting;
- cultivate them on growth media.

Therefore, viruses in general and usually also strict anaerobes remain outside the scope of bacteriological (cultural) method.

Femoflor® can be considered as a fast and accurate tool for identifying non-cultivatable microorganisms, including possible pathogens of the infectious-inflammatory process, both for diagnosis and for therapy monitoring.

04

WHO INVENTED FEMOFLOR®? WHERE IS IT USED?

The inventors of Femoflor® are a group of Russian doctors and scientists. The test was developed, tested and approved for use in the practice of obstetrician-gynecologist, dermatovenerologist, although it is often used in scientific research as well.

Most of the work, especially determining the form of report and interpretation of the results (Fig. 2-3), was carried out together with the doctors of National Medical Research Center of Obstetrics, Gynecology and Perinatal Medicine named after V.I. Kulakov and Research Institute of Obstetrics and Gynecology named after D.O. Ott. Now Femoflor® is actively used in Russia and 46 countries worldwide for an accurate diagnosis of reproductively significant infections.

National Medical Research Center of Obstetrics, Gynecology and Perinatology named after V.I. Kulakov

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

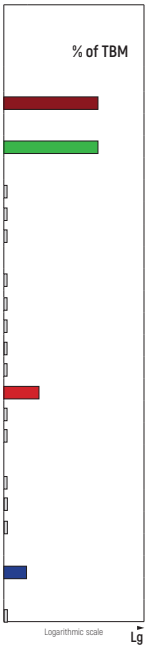


Fig. 2. Normal state of vaginal microbiome composition of conditionally healthy reproductive-age woman

№	Test title	Results	
		Quantitative	Relative, Lg (X/TBM)
1	Sample intake control	$10^{5,0}$	
2	Total Bacterial Mass	$10^{6,0}$	
	NORMAL FLORA		
3	Lactobacillus spp.	$10^{0,0}$	-6,0 (<0,1%)
	FACULTATIVE ANAEROBIC MICROORGANISMS		
4	Enterobacterium spp.	$10^{5,4}$	-0,6 (19-26%)
5	Streptococcus spp.	not detected	
6	Staphylococcus spp.	$10^{4,8}$	-1,2 (5-7%)
	OBLIGATE ANAEROBIC MICROORGANISMS		
7	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	
8	Eubacterium spp.	$10^{5,8}$	-0,2 (50-67%)
9	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	
10	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{5,4}$	-1,8 (1,3-1,8%)
11	Lachnobacterium spp. + Clostridium spp.	not detected	
12	Mobiluncus spp. + Corynebacterium spp.	not detected	-3,1 (<0,1%)
13	Peptostreptococcus spp.	not detected	
14	Atopobium vaginae	$10^{0,0}$	-6,0 (<0,1%)
	MYCOPLASMA		
15	Ureaplasma urealyticum	$10^{0,0}$	
16	Ureaplasma parvum	$10^{0,0}$	
17	Mycoplasma hominis	$10^{0,0}$	
	YEAST-LIKE FUNGI		
18	Candida spp.	$10^{3,4}$	
	PATHOGENIC MICROORGANISMS		
19	Mycoplasma genitalium	not detected	

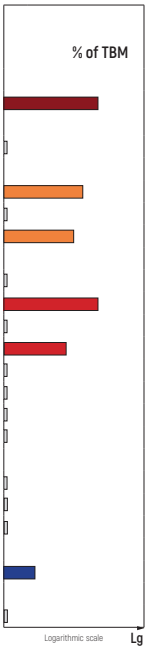


Fig. 3. Imbalance in vaginal microbiome in reproductive-age woman with nonspecific vulvovaginitis

05

WHAT ARE THE INDICATIONS FOR FEMOFLOR® SCREEN AND FEMOFLOR®-16 TESTS?

The recommendations for gynecologists to choose the right test are based mainly on the list of determined parameters.

Femoflor® Screen tests for main microflora parameters (STI pathogens, normal flora and the most common opportunistic pathogens). It can be prescribed to verify the diagnosis during the initial treatment of patients with complaints, during a routine examination even in the absence of complaints (low- and asymptomatic forms of diseases, which are widespread), preparation for ART (Assisted reproductive technology) programs.

Femoflor®-16 offers an extended profile for detailed characterization of microbiome composition. Most often, it is used by gynecologists to examine patients with complaints after ineffective treatment, relapses of diseases, when planning pregnancy and surgeries on the pelvic organs. This test does not include a complete list of pathogens. The study can be complimented with PCR tests for Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamydia trachomatis, viruses — HSV-1,2, CMV. All tests can be performed starting out from the same sample-tube with biomaterial at the physician's discretion.

WHAT SPECIMENS TO CHOOSE FOR FEMOFLOR® SCREEN AND FEMOFLOR®-16?

06

You can collect samples from different localizations; it all depends on the purpose of diagnosis and the patient's medical history. Sample from vagina is the most convenient, popular and informative kind for both tests, due to anatomical features of female reproductive tract and high sensitivity of PCR method. Even microorganisms with tissue tropism to columnar epithelium of the cervical canal (obligate pathogens, viruses) are reliably detected in the vagina.

It is not recommended to mix specimens from C (cervix) and V (vagina), since the vagina and the cervical canal are completely different ecological niches with different types of epithelial tissue and biochemical parameters of the internal environment. Consequently, both the composition of microorganisms and the absolute amounts of microbiota components differ.
The sample taken from vagina is more informative, and by its microbiome composition a physician can indirectly assess the microbiome composition in the cervix, but not vice versa. Devices designed for self-collection at home are especially relevant and useful. Subsequently, the sample is delivered to the laboratory for Femoflor® Screen, Femoflor®-16 or Quant-21 (HPV testing).

Recently, also specimens of the endometrium, collected with a pipelle biopsy or brushing technique are subjected to analysis. In this case, the attending physician should carry out the results interpretation himself without paying attention to the automated interpretation of the data, because the algorithm was developed to assess the vaginal microflora of reproductive-age women.

Table 1. Recommendations for the collection of clinical specimens for Femoflor®

CONTRINDICATIONS	THE CORRECT WAY
The use of PCR inhibitors (ultrasound coupling gel, heparin, chlorhexidine and other chlorine-containing drugs)	No earlier than 24 hours after the use
Colposcopy	No earlier than 24-48 hours after the colposcopy
Transvaginal ultrasound	No earlier than 24 hours after the transvaginal ultrasound
Vaginal douche	Do not make a vaginal douche or deep genital washing on the sample collection day
Use of tampons	Do not use them on the sample collection day
Patients after unprotected sexual intercourse	No earlier than 48-72 hours after unprotected sexual intercourse
Patients after protected sexual intercourse	Do not have a protected sexual intercourse before the visit to physician
Antibiotic usage	No earlier than 2 weeks after taking antimicrobial drugs
Probiotics and eubiotics usage	No earlier than 2 weeks after taking drugs containing microorganisms

07

WHAT IS THE SECRET OF SPECIMEN COLLECTION FOR FEMOFLOR®?

There are no secrets of specimen collection for Femoflor®. General requirements are like for any direct method for infections diagnosis (conventional PCR, real-time PCR, microscopy, bacteriological methods) — to take scrapings of the surface epithelial layers together with the bacterial biofilm attached to them, and not to take the contents of the lumen. The area of sample collection should be as close as possible to the supposed localization of the infectious and inflammatory process. We are often asked: why is it always possible to obtain a result (detected/not detected) with monoplex tests for STD pathogens, regardless of how the sample was taken, how many cells and mucus were in it. Why does a SIC (sample intake control) value sometimes has a red marker and why is there a recommendation to recollect the sample in Femoflor® report?

In Femoflor® — in order to reduce the risk of obtaining a false negative and/or incorrect result due to errors in specimen collection — there is a quantitative indicator of sample collection quality called sample intake control (SIC). The interpretation of the SIC value allows us to answer the question: are there enough epithelial cells with microbial biofilm in the specimen to obtain a reliable result?

Monoplex PCR tests for STDs do not control preanalytical phase and correctness of the report. The main practical recommendations for preanalytical phase are given in the Annex (Table 1).

08

HOW TO READ THE REPORT?

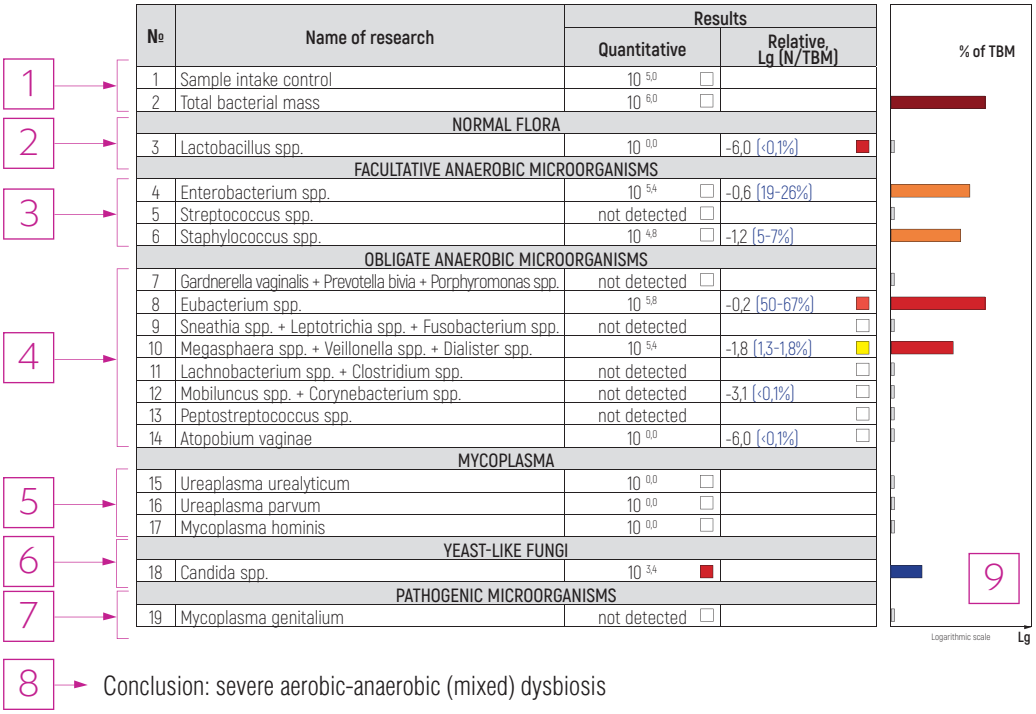
The aim of the test is the division of total bacterial mass (TBM) into components and the establishment of the ratio (proportion) of normal flora to opportunistic microorganisms. To do so, you can review the report in 2 seconds:

1. Pay attention to the color markers near the numerical values in the table (yellow and red marked boxes show the degree of deviation from the norm) or to the colored bars of the histogram (clinically significant deviations are highlighted in color). Table and histogram are 2 ways for results presentation, you can use both of them;
2. Read the conclusion: it shows the type of microbiome composition. The table looks like a “traffic light”: normal values are green (lactobacilli) / transparent (all other indicators, except for normal microflora), deviation from the norm is yellow, pathological values are red.

The length and color of the bar are important: small amounts of opportunistic microorganisms that do not cross the cut-off line (1% of TBM corresponds to the norm) look like short gray bars. Large amounts of opportunistic microorganisms look like long bars, colored in the color of microorganism’s group (for example, facultative anaerobes or obligate anaerobes, mycoplasmas, yeast-like fungi).

More details about color-coding can be found in the explanation on the 2nd page «Description of the results form of microbiome composition of female reproductive tract by Femoflor® real-time PCR test». The page with the explanation is a configurable option in the program; please inform a laboratory if you want to receive a 2-page report (Fig. 4).

Fig. 4. Flowchart of Femoflor® report



№	Name of research	Results: Quantitative	Results: Relative, Lg (N/TBM)
1	Sample intake control	$10^{5,0}$ ☐	
2	Total bacterial mass	$10^{6,0}$ ☐	
	NORMAL FLORA		
3	Lactobacillus spp.	$10^{0,0}$	-6,0 (<0,1%) ■
	FACULTATIVE ANAEROBIC MICROORGANISMS		
4	Enterobacterium spp.	$10^{5,4}$ ☐	-0,6 (19-26%)
5	Streptococcus spp.	not detected ☐	
6	Staphylococcus spp.	$10^{4,8}$ ☐	-1,2 (5-7%)
	OBLIGATE ANAEROBIC MICROORGANISMS		
7	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected ☐	
8	Eubacterium spp.	$10^{5,8}$	-0,2 (50-67%) ■
9	Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.	not detected	☐
10	Megasphaera spp. + Veillonella spp. + Dialister spp.	$10^{5,4}$	-1,8 (1,3-1,8%) ■
11	Lachnobacterium spp. + Clostridium spp.	not detected	☐
12	Mobiluncus spp. + Corynebacterium spp.	not detected	-3,1 (<0,1%) ☐
13	Peptostreptococcus spp.	not detected	☐
14	Atopobium vaginae	$10^{0,0}$	-6,0 (<0,1%) ☐
	MYCOPLASMA		
15	Ureaplasma urealyticum	$10^{0,0}$ ☐	
16	Ureaplasma parvum	$10^{0,0}$ ☐	
17	Mycoplasma hominis	$10^{0,0}$ ☐	
	YEAST-LIKE FUNGI		
18	Candida spp.	$10^{3,4}$ ■	
	PATHOGENIC MICROORGANISMS		
19	Mycoplasma genitalium	not detected ☐	

1 The main assessed parameters: SIC (number of epithelial cells with microbial biofilm) and TBM (total bacterial load of biotope).

2 Relative values (proportion) of normal flora (lactobacilli) against TBM: severe dysbiosis ■, moderate dysbiosis ■, normal state ■

3 Relative values of facultative anaerobic microorganisms (they are often called aerobes based on their cultivation method, because they preserve viability in aerobic and anaerobic conditions): outside the normal range ■, increased value ■, norm variant ☐

4 Relative values (proportion) of obligate anaerobic microorganisms (they are often called strict anaerobes, because oxygen kills them): outside the normal range ■, increased value ■, norm variant ☐

5 Mycoplasmas (comparison with clinically significant value 10^4 GE/sample): more than 10^4 GE/sample ■, less than 10^4 GE/sample ■, norm variant ☐

6 Yeast-like fungi Candida spp. (absolute value): more than 10^4 GE/sample ■, less than 10^4 GE/sample ■, norm variant ☐

7 Quantitative value of Mycoplasma genetalium: detected ■, not detected ☐

8 Conclusion: state of microbiome composition. If the main assessment parameters are outside the range, there will be recommendations on preanalytical phase (to repeat the specimen collection/to repeat the study).

9 Histogram: another way of results presentation.

TEST FEMOFLOR®

Description of the results form

The test is performed by real time-PCR. The test is designed to diagnose infectious diseases of the reproductive system of women. In the same sample from the patient simultaneously are tested:

- presence/absence Mycoplasma genitalium;
- the amount of human genomic DNA (SIC — sample intake control), total number of all bacteria (total bactenal mass — TBM), amount of lactobacilli, commensals, opportunistic bacteria;
- amount of Candida spp.

The absolute number of microorganisms is shown in genome equivalents (GE), the number of which is proportional to the microbial contamination of the biotope from which the biomaterial sample was obtained. Absolute values of GE are shown in the column of the form «Result. Quantitative".

Relative values are shown in the column of the form "Result. Relative» in two versions: as the difference between the absolute values of each indicator and TBM (Lg10) and in percentage (%) of TBM.

Percent are the traditional format of quantitative data, so they are shown as readily available information, but they are not used in the conclusion formation algorithm, therefore it is not correct to sum percentages (%).

The number of Candida and opportunistic mycoplasmas (Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis) are shown only in absolute values.

To facilitate the perception of test results*, a color marking is used in the table with test results. Depending on the parameter being measured, the color markers indicate the following:

Verification indicators (sample intake control, total bacterial mass):

☐ – meet the criteria,

■ – do not meet the criteria.

Lactobacillus spp.:

■ – compliance with the criteria of norm — normocenosis,

■ – moderate deviation from the criteria of norm — moderate dvsbiosis,

■ – serious deviation from the criteria of norm — severe dvsbiosis.

Opportunistic bacteria and Candida spp.:

☐ – compliance with the criteria of norm,

■ – moderate deviation from the criteria of norm,

■ – erious deviation from the criteria of norm.

Sexually Transmitted Infections:

☐ – not dected

■ – detected.

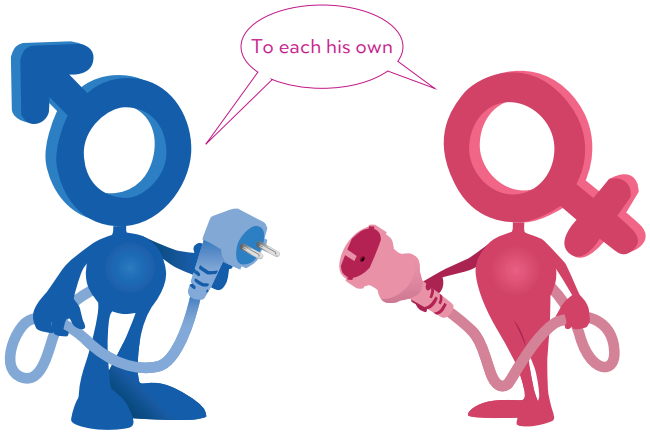
Additionally, in order to facilitate the perception, the results of the study are presented on a histogram in percentage / log formats.

09

IS IT POSSIBLE TO USE FEMOFLOR® FOR MEN?

No. Femoflor® and Androflor® differ not only in the list of microorganisms to be tested, but also in the algorithm of results interpretation. In case of a man, it is extremely difficult for the expert to evaluate the result of Femoflor®.

Automated interpretation of the test results («traffic light» system in the table and colored bars on the histogram) and the conclusion based on the Femoflor® results have been developed for women and does not apply to men.



10

IS IT RECOMMENDED TO EXAMINE A COUPLE (SEXUAL PARTNERS), IF ONE OF THEM HAS A CHRONIC INFLAMMATORY PROCESS?

Yes, in these cases it is recommended to examine both partners. You can use Femoflor® for women and Androflor® for men. Both tests are based on the same real-time PCR technology, so a correct comparison and result analysis of the couple is possible.

Therefore, it is important to examine a couple, and not to prescribe an empirical treatment, based on the results of one partner, because it is often ineffective. In the case of chronic forms of diseases, for example, bacterial vaginosis, the fact of microflora exchange during unprotected sexual intercourse can become a trigger of exacerbation. In this case, individual diagnostics can be useful for adjusting treatment regimens for each partner and reducing the risk of disease recurrence.

WHY DO THE RESULTS OF MICROBIAL CULTURE AND FEMOFLOR® NOT MATCH?

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Differing results are due to the fundamental difference between the two technologies. It most visibly reflects in dominance of obligate anaerobes in the microbiome composition and presence of viral-bacterial associations.

Microbial culture is the cultivation, identification and assessment of antibiotic resistance of microorganisms that are isolated during bacteriological analysis. The results of bacteriological analysis directly depend on the preservation of microbial viability during specimen collection, transportation and the ability of microbes to multiply on culture media. Bottlenecks of culture methods are strict requirements for the preanalytical phase (temperature, duration of transportation to the laboratory, usage of special transport and culture media) and cultivation in a specific bacteriological laboratory (with special equipment and highly qualified employees), difficulties in isolating obligate anaerobes (CO_2 incubators and other equipment for creating anaerobic conditions for the cultivation of bacteria). There are very fastidious or unculturable microorganisms, such as Chlamydia, Mycoplasma Genitalium; they require special cultivation conditions. The «no growth» statement does not necessarily correspond to the absence of bacteria.

Femoflor® allows a direct detection and quantification of microorganisms by their DNA that got into the tube during sample collection — regardless of their sensitivity to oxygen, viability and the possibility of cultivation. In case of PCR-diagnostics using Femoflor®, it is not required to preserve the viability of microorganisms. However, the recommendations for specimen collection in the manual must be strictly observed.

The PCR method does not allow detecting microorganisms outside the list included in the test. Therefore, Femoflor® tests include all clinically significant groups of microorganisms. There are no “extra” indicators in the test.
The coincidence of the bacterial culture and Femoflor® has been demonstrated for culturable microorganisms (aerobes and facultative anaerobes), mycoplasmas (they are detected in a standard culture, the specimen must be taken into a special transport medium for a separate microbiological test for opportunistic mycoplasmas), yeast-like fungi of Candida spp.

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WHY DO WE USE FEMOFLOR® FOR THE DIAGNOSIS OF BACTERIAL VAGINOSIS?

Firstly, the following methods are not recommended for confirming the diagnosis of bacterial vaginosis (BV):

- microbial culture due to low specificity;
- non-quantitative study of G. vaginalis, A. vaginae and / or Mobiluncus spp. by the method of molecular genetic analysis (PCR) due to the need for quantitative assessment of opportunistic bacteria.

Secondly, prescription for Femoflor® is advisable in cases where it is difficult to make a diagnosis based on the Amsel criteria. For example, if it is impossible to perform a microscopic examination of the native (wet) mount, amine test or pH measurement. Another difficulty with Nugent-scoring and Ison-Hay criteria might be the availability of a highly qualified doctor performing microscopy.

The research carried out at the «Research Institute of Obstetrics, Gynecology and Reproductology named after D.O. Ott» (St. Petersburg), proved that a low concentration of lactobacilli (<10% of TBM) in combination with an increased number of obligate anaerobes, detected by Femoflor®-16, make it possible to diagnose bacterial vaginosis with a sensitivity of 99% and specificity of 93%. The conclusion «severe anaerobic dysbiosis» usually corresponds to the diagnosis of «bacterial vaginosis».

In the «Clinical guidelines for the diagnosis and treatment of diseases with pathological discharge from the genital tract of women (2nd edition, revised and supplemented)», approved by ROAG (Russian Association of Obstetricians and Gynecologists) in 2019, it is indicated that the Femoflor® test have a high diagnostic value by detection of high concentrations of BV-associated microorganisms.

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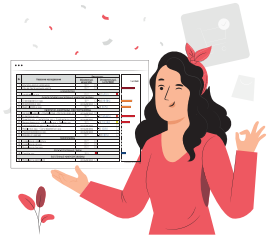
IS IT POSSIBLE TO TEST WITH FEMOFLOR®-16 AND SCREEN FOR MAJOR PATHOGENS AND HPV STARTING OUT FROM ONE SAMPLE TUBE? TO WHOM AND WHEN IS IT RECOMMENDED?

Yes, it is advisable to prescribe an extended comprehensive assessment of vaginal or cervical samples for pathogenic microorganisms and HPV when preparing a patient for pregnancy, ART procedures, before planned gynecological surgeries and in case of relapses of infection and ineffective treatment of infectious and inflammatory diseases. It is also advisable to evaluate the microbiome composition of patients with cervical intraepithelial neoplasia (CIN) and HPV infection. An extended PCR analysis analysis of the micriobiome composition of the vaginal flora can help to make a more comprehensive diagnosis. Correction of dysbiosis based on Femoflor results can reduce the risk of CIN progression.

The real-time PCR method technically allows to combine Femoflor®-16 testing with other molecular tests e.g. for microbiological pathogens and HPV. All can be done starting out from one single tube with specimen collected from the vagina and cervical canal. In practice, some laboratories nevertheless ask to collect specimens for Femoflor®-16 and / or monoplex PCR tests in separate tubes. So it is advisable to clarify the preanalytical questions before prescribing studies.

14

WHY DO I RECEIVE A REPORT NOT IN THE FORM OF A TABLE WITH COLORED MARKERS AND A HISTOGRAM, BUT IN THE FORM OF DIGITAL VALUES? HOW TO INTERPRET SUCH RESULT?



Sometimes laboratories cannot immediately transfer data from the real-time PCR instrument on which Femoflor® tests were performed to the laboratory information system (LIS) through which the results are issued. In these cases, doctor can get the result in the form of a table; sometimes it contains only the absolute values, without relative values and color markers, which makes clinical interpretation impossible. In this situation, you need to contact the laboratory with a request to issue Femoflor®-16 result from the device software.

The color-coding of indicators based on the traffic light system was created for the convenience and speed of interpretation of results, therefore we recommend to use a colored printed form, if it is impossible — to view the colored result on a PC or smartphone.

IS IT RECOMMENDED TO PRESCRIBE SEVERAL METHODS OF TESTING AT THE SAME TIME FOR DIAGNOSING INFECTIONS?

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What is necessary for examining a particular patient is always the choice of the attending physician. The simultaneous use of several direct methods is advisable for the differential diagnosis of inflammatory and non-inflammatory diseases of female reproductive tract (for example, vaginosis-vaginitis) and for correction of the treatment of patients with relapses associated with resistant strains of bacteria. Whether initially microbiological or molecular methods (e.g. using Femoflor) have been employed, it is always necessary to evaluate the state of the vaginal microbiocenosis also by microscopy. It is also important to determine the quantity and quality of epithelium, the ratio of leukocytes and epithelial cells, phagocytosis, lactobacilli, «clue» cells, pseudo «clue» cells and other parameters to get a complete picture. This is especially important if a moderate dysbiosis was diagnosed with Femoflor®. This holds true only if the laboratory is proficient in such microscopy techniques. If a microscopic conclusion is based on the presence of the absolute value of leukocytes and the identification of bacterial morphotypes in the vaginal biotope, then it is better not to carry out a microscopic examination at all and examine the patient with Femoflor® only.

It is important to notice that the prescription for bacteriological study in patients with severe anaerobic dysbiosis is inappropriate, because this is most likely a bacterial vaginosis. You can immediately prescribe antibacterial drugs in accordance with Russian and international guidelines for the treatment of BV. If, according to Femoflor®-16 results, the conclusion «severe aerobic dysbiosis» is obtained, then in most cases, it is an aerobic vaginitis (AV) and high-quality microscopic examination is required. If necessary, you can also perform a bacteriological test to determine the sensitivity of microorganisms to antibacterial drugs, although the documents also contain recommendations for choice of drugs for AV treatment.

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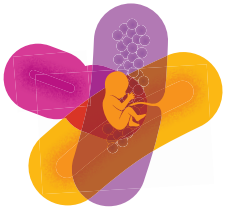
DOES FEMOFLOR® MEET MODERN TRENDS?

Yes, over the past few years, there has been a global tendency to use high-tech methods for evaluation of the microbiome composition of different biotopes. In many scientific articles of international peer-reviewed journals, there are more and more reports on the potential advantages of using molecular genetic tests for the diagnosis of infectious and inflammatory processes of the reproductive tract. PCR was included in the CDC and IUSTI clinical guidelines, showing its main advantages over traditional methods — high sensitivity and specificity.



WORLD OF
MICROBIOME
PREGNANCY, BIRTH
& INFANCY

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HOW LONG DO TEST RESULTS TAKE?

The test itself takes about 3-4 hours. The total lead time is 1-2 days, it is influenced by the speed of samples transportation to the laboratory, the schedule of test runs and reruns (rarely required).

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WHY DO YOU NEED FEMOFLOR® IF THE MOST MODERN BROAD-SPECTRUM DRUGS ACT AGAINST «EVERYTHING»?

The main reason is if there is a correct diagnosis, there is a correct treatment. Therefore, diagnostics is in the first place, and then treatment. The empiric therapy is unacceptable.

It is not sufficient to start treatment solely based on complaints and clinical symptoms. Complaints can be subjective. If there are complaints of vaginal discharge, only 30% of women may have a disease, and 70% have a physiological condition. Therefore, diagnosis based on highly sensitive methods such as Femoflor® is very important.

The principle of action of broad-spectrum drugs can be compared with «carpet bombing», after which not only microorganisms that should be «neutralized» can die, but also useful lactobacilli. What is the right approach to sustainably restore a normal state of microbiome composition and thereby minimize the risk of relapse? The answer is individual for each case.

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CAN WE PRESCRIBE FEMOFLOR® FOR PREGNANT WOMEN? ARE THERE DIFFERENCES IN THE INTERPRETATION OF THE RESULT?

The results of the study carried out at UGMU (Yekaterinburg, Russia) showed that vaginal microbiome composition in healthy pregnant women without complaints and symptoms of vaginal pathology meets the criteria for normal state. Thus, the interpretation of Femoflor® results in pregnant women can be carried out according to standard criteria.

In principle it is not necessary to prescribe microbiome test for women with normal pregnancy. However, the dominance of lactobacilli in the first trimester of pregnancy is a significant prognostic sign of the preservation of physiological microbiocenosis throughout pregnancy. Therefore, Femoflor® can be prescribed in preparation for pregnancy, which is due to the influence of infectious and inflammatory diseases on reproductive function, the development of obstetric and neonatal complications. At the same time, Femoflor® Screen can be recommended in case of complaints of vaginal discomfort, itching, burning or pathological discharge for evaluation of the vaginal microbiome state of pregnant women and ahead of prescribing an etiotropic treatment.

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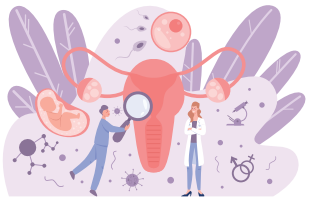
IS IT ADVISABLE TO USE FEMOFLOR®-16 AND FEMOFLOR® SCREEN FOR EXAMINATION OF PATIENTS WITH MISCARRIAGE, INFERTILITY AND OTHER REPRODUCTIVE PROBLEMS?



STDs are associated with infertility and increased risk of spontaneous abortion in ART-programs, which allows us to recommend Femoflor® Screen for testing.

Abnormal state of the vaginal microbiota is associated with infertility and increased risk of spontaneous miscarriage. In order to clarify the cause of infertility and establish the role of the infectious factor, it is possible to prescribe a comprehensive microbiome composition analysis (guidelines «Female infertility»).

The research carried out at Research Institute of Obstetrics and Gynecology named after D.O. Ott (Saint Petersburg, Russia) showed that dysbalance of vaginal microbiota is more common in pregnant women with a history of miscarriage. In this context, it is necessary to follow the diagnostic protocols for the correction and prevention of reproductively significant infections.

The research at National Medical Research Center of Obstetrics, Gynecology and Perinatal Medicine named after V.I. Kulakov (Moscow, Russia) showed that vaginal dysbiosis approximately doubles the risk of premature birth.

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IS IT POSSIBLE TO USE FEMOFLOR® FOR WOMEN TAKING MHT? HOW TO EVALUATE THE RESULT?

Yes, the vaginal microbiome composition of women in menopause and postmenopause taking MHT does not differ significantly from reproductive-age women. The results are evaluated in a standard way, taking into account the recommendations above. Therefore, Femoflor® is an excellent tool to monitor the success of MHT treatment.

The interpretation of Femoflor® results in women in menopause and postmenopause who do not take MHT is significantly different. In these cases, we recommend to pay attention to absolute values. Both cases of dominance of lactobacilli and obligate anaerobes can be an individual normal state.

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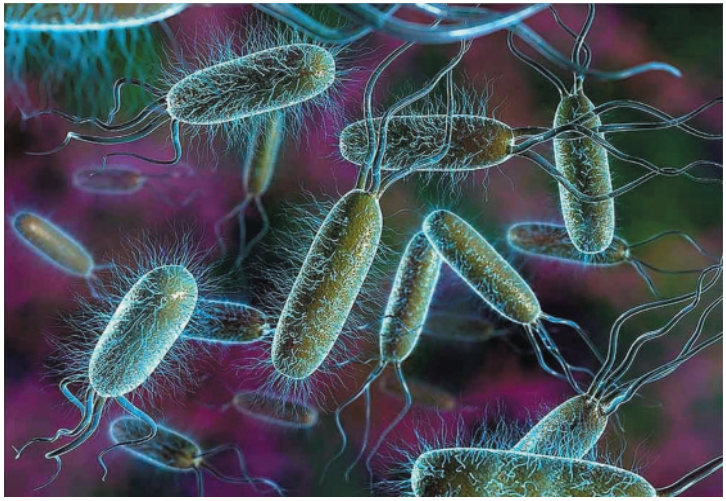
WHY IS E.COLI NOT A PART OF THE TESTED PARAMETERS?

This microorganism is included in the test. *Escherichia coli* is detected as part of the *Enterobacteriaceae* family with other related bacteria.



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DO AEROBES ALWAYS DOMINATE IN THE MICROBIOME COMPOSITION OF WOMEN WITH A CONFIRMED DIAGNOSIS OF «AEROBIC VAGINITIS»? CAN THERE BE EXCEPTIONS?

An unexpected answer to this question was obtained in the research carried out at Ural State Medical University (Yekaterinburg, Russia) in 2019: patients (n = 333) with microscopic evidence of aerobic vaginitis (AV) according to G. Donders criteria had aerobic dysbiosis (according to Femoflor®) only in 9%. In most cases, there was a dysbiosis with a predominance of obligate anaerobes. In these cases, not only a study according to Donders or Nugent criteria is needed, but also a microscopic examination with assessment of vaginal microbiome composition.

If AV is suspected, it is important to analyze the quantity and quality of epithelium, the ratio of leukocytes and epithelial cells, phagocytosis, lactobacilli, “clue” cells, pseudo “clue” cells and other microscopic parameters.

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DNA-TECHNOLOGY

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