

MEDICAL REPORT: ANALYSIS OF THE VAGINAL MICROBIOME USING NEXT-GENERATION SEQUENCING

Sender information

Patient information

Sender: Musterarzt Frauenarzt Street: Musterstrasse 1 Postcode/city: 12345 Musterhausen Phone number: 1234/4567890	Name of patient: MUSTERFRAU, ERIKA Date of birth: 01.01.1950 Sample ID: 1234567890 Request date: 15.06.2023 Report date: 20.06.2023
--	---

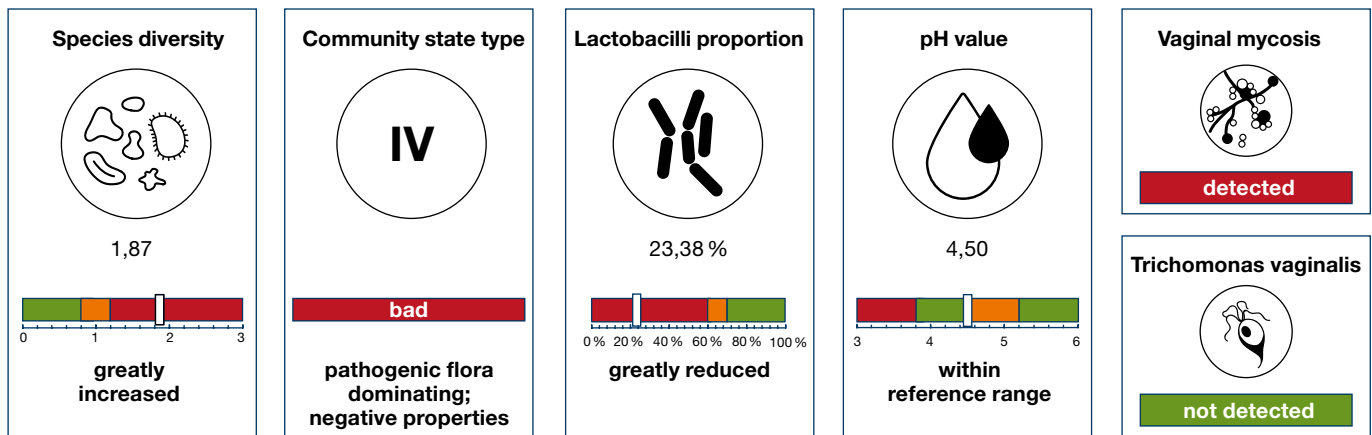
Diagnosis

Specimen type

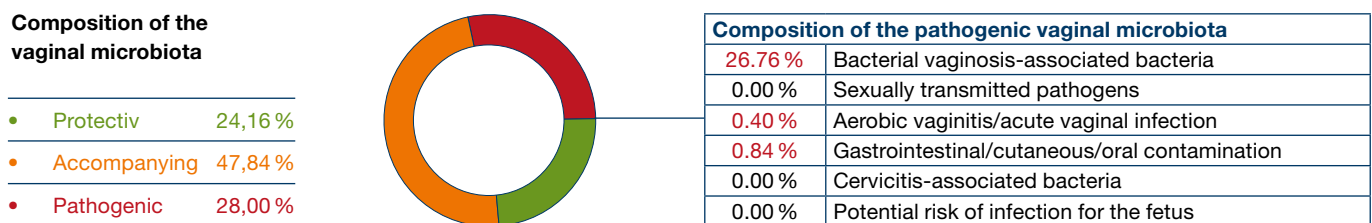
Pregnancy

Genital pruritus
Vaginal swab
None
WG: -

Results overview



The vaginal microbiome composition



Medical findings

Pathological findings with reduction of the protective vaginal flora dominated by lactobacilli. The Shannon index (species diversity) is greatly increased and the proportion of lactobacilli correspondingly greatly reduced. *Lactobacillus iners* is dominant among the lactobacilli. This species is considered to have a less positive protective effect than other lactobacilli. Significant increase of vaginosis-associated bacilli (in particular *Gardnerella vaginalis*). Positive detection of *Candida albicans* in the sense of vaginal mycosis. No trichomonads were detected. The pH value is within the physiological range.

Overall findings indicate a bacterial vaginosis and vaginal mycosis.

Therapy recommendations

Therapy options in case of BV requiring treatment (e.g. symptomatic pat/planned gynaecological or invasive procedures): Amoxicillin/clavulanic acid (oral) OR metronidazole (2 × 500 mg p.o. OR vaginal gel 1 × 0.75 % intravaginally) for 5–7 days OR clindamycin (2 × 300 mg p.o. OR vaginal cream 1 × 1 × 2 % intravaginally) for 7 days. Subsequent treatment of the vaginal mycosis with antifungal drugs (clotrimazole (topically), fluconazole or itraconazole (orally)).

The vaginal microbiome composition

Protective vaginal microbiota

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
Bifidobacterium	spp.	0.77 %	<i>Lactobacillus</i>	<i>helveticus</i>	0.00 %
Lactobacillus	spp.	23.38 %	<i>Lactobacillus</i>	<i>iners</i>	20.55 %
<i>Lactobacillus</i>	<i>acidophilus</i>	0.00 %	<i>Lactobacillus</i>	<i>jensenii</i>	0.00 %
<i>Lactobacillus</i>	<i>casei</i>	0.00 %	<i>Lactobacillus</i>	<i>paracasei</i>	0.00 %
<i>Lactobacillus</i>	<i>crispatus</i>	0.00 %	<i>Lactobacillus</i>	<i>plantarum</i>	0.00 %
<i>Lactobacillus</i>	<i>delbrueckii</i>	0.00 %	<i>Lactobacillus</i>	<i>reuteri</i>	0.00 %
<i>Lactobacillus</i>	<i>fermentum</i>	0.00 %	<i>Lactobacillus</i>	<i>rhamnosus</i>	0.00 %
<i>Lactobacillus</i>	<i>gasseri</i>	0.00 %	<i>Lactobacillus</i>	<i>salivarius</i>	0.00 %

proportion of the total vaginal microbiota **24.16 %**

Bacterial vaginosis-associated bacteria (BVAB)

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
Actinomyces	spp.	0.00 %	<i>Leptotrichia</i>	<i>amnionii</i>	0.00 %
Anaerococcus	spp.	0.00 %	<i>Mageeibacillus</i>	<i>indolicus</i>	0.00 %
<i>Atopobium</i>	spp.	2.11 %	Megasphaera	spp.	1.17 %
<i>Bacteroides</i>	<i>fragilis</i>	0.00 %	Mobiluncus	spp.	0.00 %
<i>Bacteroides</i>	spp.	0.00 %	<i>Parvimonas</i>	<i>micra</i>	0.00 %
Clostridium	spp.	1.19 %	Peptoniphilus	spp.	0.18 %
Dialister	spp.	0.00 %	Porphyromonas	spp.	0.00 %
Eggerthella	spp.	0.00 %	Prevotella	spp.	0.00 %
<i>Gardnerella</i>	<i>vaginalis</i>	19.01 %	<i>Sneathia</i>	<i>sanguinegens</i>	0.39 %
Lachnospira	spp.	0.00 %	<i>Sneathia</i>	spp.	3.69 %

proportion of the total vaginal microbiota **26.76 %**

Sexually transmitted pathogens

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
<i>Chlamydia</i>	<i>trachomatis</i>	0.00 %	<i>Neisseria</i>	<i>meningitidis</i>	0.00 %
<i>Haemophilus</i>	<i>ducreyi</i>	0.00 %	<i>Treponema</i>	<i>pallidum</i>	0.00 %
<i>Klebsiella</i>	<i>granulomatis</i>	0.00 %	<i>Trichomonas</i>	<i>vaginalis</i>	negative (PCR)
<i>Mycoplasma</i>	<i>genitalium</i>	0.00 %	<i>Ureaplasma</i>	<i>parvum</i>	0.00 %
<i>Mycoplasma</i>	<i>hominis</i>	0.00 %	<i>Ureaplasma</i>	<i>urealyticum</i>	0.00 %
<i>Neisseria</i>	<i>gonorrhoeae</i>	0.00 %			

proportion of the total vaginal microbiota **0.00 %**

Aerobic vaginitis/acute vaginal infection

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
Enterococcus	spp.	0.00 %	<i>Streptococcus</i>	<i>agalactiae</i>	0.20 %
<i>Escherichia</i>	spp.	0.18 %	<i>Streptococcus</i>	<i>anginosus</i>	0.00 %
<i>Staphylococcus</i>	<i>aureus</i>	0.12 %	<i>Streptococcus</i>	<i>dysgalactiae</i>	0.00 %
<i>Staphylococcus</i>	<i>epidermidis</i>	0.00 %	<i>Streptococcus</i>	<i>pyogenes</i>	0.00 %
<i>Staphylococcus</i>	<i>lugdunensis</i>	0.00 %			

proportion of the total vaginal microbiota **0.40 %**

The vaginal microbiome composition

Gastrointestinal/cutaneous/oral contamination

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
<i>Acinetobacter</i>	<i>johnsonii</i>	0.00 %	<i>Kluyvera</i>	spp.	0.00 %
<i>Acinetobacter</i>	<i>lwoffii</i>	0.00 %	<i>Pseudomonas</i>	spp.	0.00 %
<i>Acinetobacter</i>	<i>ursingii</i>	0.00 %	<i>Ruminococcus</i>	spp.	0.00 %
<i>Clostridium</i>	spp.	1.19 %	<i>Staphylococcus</i>	<i>capllis</i>	0.00 %
<i>Enterococcus</i>	<i>avium</i>	0.00 %	<i>Staphylococcus</i>	<i>caprae</i>	0.00 %
<i>Enterococcus</i>	<i>faecalis</i>	0.00 %	<i>Staphylococcus</i>	<i>epidermidis</i>	0.00 %
<i>Enterococcus</i>	<i>faecium</i>	0.00 %	<i>Staphylococcus</i>	<i>hominis</i>	0.15 %
<i>Escherichia</i>	spp.	1.18 %	<i>Streptococcus</i>	<i>pneumoniae</i>	0.00 %
<i>Haemophilus</i>	spp.	0.00 %	<i>Veillonella</i>	<i>parvula</i>	0.00 %
<i>Klebsiella</i>	spp.	0.00 %			

proportion of the total vaginal microbiota **0.84 %**

Cervicitis-associated bacteria

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
<i>Actinomyces</i>	spp.	0.00 %	<i>Streptococcus</i>	<i>pyogenes</i>	0.00 %
<i>Chlamydia</i>	<i>trachomatis</i>	0.00 %	<i>Trichomonas</i>	<i>vaginalis</i>	negative (PCR)
<i>Neisseria</i>	<i>gonorrhoeae</i>	0.00 %			

proportion of the total vaginal microbiota **0.00 %**

Potential risk of infection for the fetus

Genus	Species	Rel. abundance	Genus	Species	Rel. abundance
<i>Chlamydia</i>	<i>trachomatis</i>	0.00 %	<i>Neisseria</i>	<i>gonorrhoeae</i>	0.00 %
<i>Haemophilus</i>	<i>influenzae</i>	0.00 %	<i>Treponema</i>	<i>pallidum</i>	0.00 %
<i>Listeria</i>	<i>monocytogenes</i>	0.00 %	<i>Ureaplasma</i>	spp.	0.00 %
<i>Mycoplasma</i>	spp.	0.00 %			

proportion of the total vaginal microbiota **0.00 %**

Vaginal mycosis

Aerobic vaginitis/acute vaginal infection

Genus	Species	Result	Genus	Species	Result
<i>Candida</i>	<i>albicans</i>	+	<i>Candida</i>	<i>parapsilosis</i>	-
<i>Candida</i>	<i>dubliniensis</i>	-	<i>Candida</i>	<i>tropicalis</i>	-
<i>Candida</i>	<i>glabrata</i>	-			
<i>Candida</i>	<i>krusei</i>	-			
<i>Candida</i>	<i>lusitaniae</i>	-			

Additional fungal pathogens:

Genus	Species	Result
<i>Candida</i>	spp.	+

Legend

- not detected
- (+) slightly positive
- +
- ++ clearly positive
- +++ strongly positive

General information about the NGS microbiome analytics

Next-generation sequencing (NGS)

NGS technology enables the analysis of microbial compositions of complex samples using various sequencing strategies approved by the government.

SYNLAB NGS technology performs amplification and subsequent sequencing of the conserved ribosomal 16S rRNA gene. The sequencing data is processed using a bioinformatic analysis algorithm and phylogenetically profiled through database comparison.

Result interpretation

The results of the NGS data analysis are quantified using relative abundance. DNA fragments corresponding to certain bacteria genus/species are compared to the entirety of all detected DNA fragments and depicted in percentage. The detection limit of our NGS test has been set to 0.1 % relative abundance.

Analysis of the vaginal microbiome by NGS

Under the influence of oestrogens, the vaginal mucosa is physiologically dominated by lactobacilli (> 70 %), which metabolise glucose to lactate, thus reducing the pH value. In addition to the lactobacilli, numerous other species (up to approx. 180 in total) can be found, many of them in low bacterial counts, which belong to the normal vaginal microbiota. Equilibrium shifts within the normal vaginal microbiota are central to the microbial pathogenicity of the vagina. Various clinical conditions (bacterial vaginosis (BV)/aerobic vaginitis, potentially pathogenic bacteria/sexually transmitted diseases (STD)/cervicitis/vaginal mycosis or gastrointestinal/cutaneous contamination) can be associated with increased numbers of pathogenic bacteria. In contrast to the classic microbiological cultivation of bacteria supplemented by singular amplification methods (PCR), the molecular genetic method NGS (next-generation sequencing) records all apathogenic and potentially pathogenic bacteria within the vaginal microbiome. The analytics is supplemented by a specific molecular-biological detection of trichomonads, which are often underdiagnosed with conventional methods. On the other hand, viruses – such as herpes simplex virus 1/2 (HSV 1/2) and human papilloma viruses (HPV) – are not recorded by this NGS technology. The molecularly and biologically measurable variety of the vaginal microbiota increases with pathologies being present as part of the shift in equilibrium (species diversity). This shift is measured using the Shannon index.

Shannon index

The Shannon index is a mathematical dimension for recording the biodiversity (species diversity) in a community. The healthy, physiological vaginal microbiome is characterised by low biodiversity due to the dominance of lactobacilli (index threshold: < 0.8). An increased index is always a sign of increased biodiversity or a shift in balance and therefore a disturbed vaginal bacterial community.

Community state type (CST)

To simplify the interpretation of the vaginal microbiome, a classification based on typical constellations was introduced by Ravel et al. along with the dominant bacteria, all other bacteria are also taken into account in terms of type and prevalence. CST (community state type) I, II and V define favourable constellations with dominance of *Lactobacillus crispatus*, *Lactobacillus gasseri* and *Lactobacillus jensenii*, respectively. *Lactobacillus iners* is dominant in CST III and has less favourable properties. CST IV describes constellations of a bacterial vaginosis with a reduction of lactobacilli and increased abundance of vaginosis-associated bacteria. Meanwhile, further CSTs have been introduced in order to record other pathologies as well.

Lactobacilli

More than 120 lactobacillus species can be differentiated with the new NGS technology. Lactobacilli are the main bacteria species in the physiological vaginal microbiome. They represent a physiological protective microbiota against other bacteria in the microbiome and even against STDs. By metabolising sugars to D/L-lactate, they lower the pH value. In addition, many lactobacilli release hydrogen peroxide and other bactericides. These and other factors, in particular the so-called surface-active molecules (SAMs), create a microbial protective film, which prevents infections caused by pathogens that would otherwise result in a shift in equilibrium and the formation of a pathological biofilm. However, not all lactobacilli are equally protective. For example, *Lactobacillus iners* (CST III) does not form D-lactate but predominantly L-lactate, which has no protective effect. Thus, the exact knowledge of the lactobacilli species – which is challenging to achieve with classic cultivation methods – is decisive for the evaluation of the vaginal microbiome.

Vaginosis-associated bacteria

The prevalence of bacterial vaginosis (BV) in Europe is around 23 %. The main causative pathogens are *Gardnerella vaginalis* followed by *Atopobium vaginae*. In addition, there are numerous other potentially pathogenic bacteria, which largely elude the classic cultural detection methods but can clearly be detected with the new NGS technology. Since there are different pathogenic subtypes (A–D) of *Gardnerella vaginalis*, its detection does not automatically correlate with bacterial vaginosis. Therefore, knowing the entire spectrum of pathogens in a case of bacterial vaginosis enables the application of targeted antibiotics and probiotics, if necessary.

General information about the NGS microbiome analytics

Potentially pathogenic microbiota

Aerobic vaginitis

Aerobic vaginitis (according to Donders/Belgium) is a condition where women experience an unpleasant fluorine and in which pH values of five to six are reported. The numerous leucocytes, which can be seen in the native compound with coccoid microbiota, are difficult to treat. A clear inflammatory reaction is the main difference to bacterial vaginosis. The cause is often a mixed microbiota of haemolytic streptococci, mostly *Streptococcus agalactiae* (*B-streptococci*) but also of groups A, C or G, or of the *Streptococcus anginosus* group, *Staphylococcus aureus*, Enterobacteriaceae, enterococci and lastly *Trichomonas vaginalis*. This acute inflammation is difficult to treat. Therapy attempts with vaginal clindamycin cream for one to two weeks are possible, but often not sufficient. Additionally, hydrocortisone in a vaginal cream seems to improve the results. Substitution with lactobacilli or acidification for weeks to months is always advisable. This is especially recommended during pregnancy, as prematurity is also significantly increased by aerobic vaginitis-associated bacteria. Furthermore, acute infections can occur very rarely, some of which massively affect the patient. These are caused by *Staphylococcus aureus* and streptococci of groups A, C or G, some of which used to be called 'childbed fever', up to their most threatening form, Fournier's gangrene.

Gastrointestinal/cutaneous contamination

If the vaginal microbiome is not sufficiently colonised with lactobacilli, *E. coli* and other faecal bacteria can be translocated from the intestine into the vagina and urinary tract and cause infections. Therefore, particular attention should be paid to proper toilet hygiene.

Sexually transmitted diseases (STDs)

NGS is used to reliably detect sexually transmitted pathogens. Trichomonads are protozoa and cannot be detected by 16S-RNA sequencing. Therefore, a singular PCR analysis for the detection of these often underdiagnosed pathogens is performed. If one of these pathogens is detected, diagnostics of the partner should be carried out. Corresponding therapy recommendations can be found in our medical report.

The most common symptoms of STDs are urethral or vaginal discharge, painful or painless genital ulceration, inguinal swelling and lower abdominal pain. As STDs can occur without pain or symptoms, they are often undiagnosed and untreated.

Cervicitis-associated bacteria

In this group, we have summarised typical pathogens associated with the clinical condition of cervicitis. Please refer to our medical report for corresponding therapy recommendations.

Vaginal mycosis

Vaginal mycosis is widely spread among women. Statistically, three out of four women have a vaginal fungal infection at least once in their lifetime. In about 3–4 % of these women, the disease recurs.

The most common pathogens of vaginal mycosis are yeasts of the *Candida* type. *Candida* species in small numbers belong to the saprophytic inhabitants of the vaginal flora and are usually harmless. Hormonal influences, general or local immune deficiencies, changes in the pH value and/or disturbances of the normal microbial colonisation of the vagina favour the increase of yeasts and can subsequently lead to the emergence of disease. The most common type of fungal pathogen is *Candida albicans*, with an incidence of over 80 %.