



The vaginal microbiome

And how to treat vaginal complaints



Microbiome Center: evidenzbasierte, personalisierte Präzisionsmedizin

Medical topics



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Moderator



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Scientific topics



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*Director for science and
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Microbiome Center

- Founded in **2018**
- Microbiome Center **support practitioners** with the treatment of chronic patients through **personalised, evidence base microbiome treatments** advice based on a patient's complaints, medical background and stool analyses.
- Network of **more than 600** Medical Doctors and practitioners in The Netherlands, Germany, Austria, Switzerland, Italy, etc.
- **Partnerships**
 - BIOVIS Diagnostik
 - Blumenau Apotheke München
 - Scientist, Universities and patientgroups



**Microbiome
Center**

600+

Practitioners

>10

Countries

10.000+

Patients

72 %

Positive
effect

Today's topics

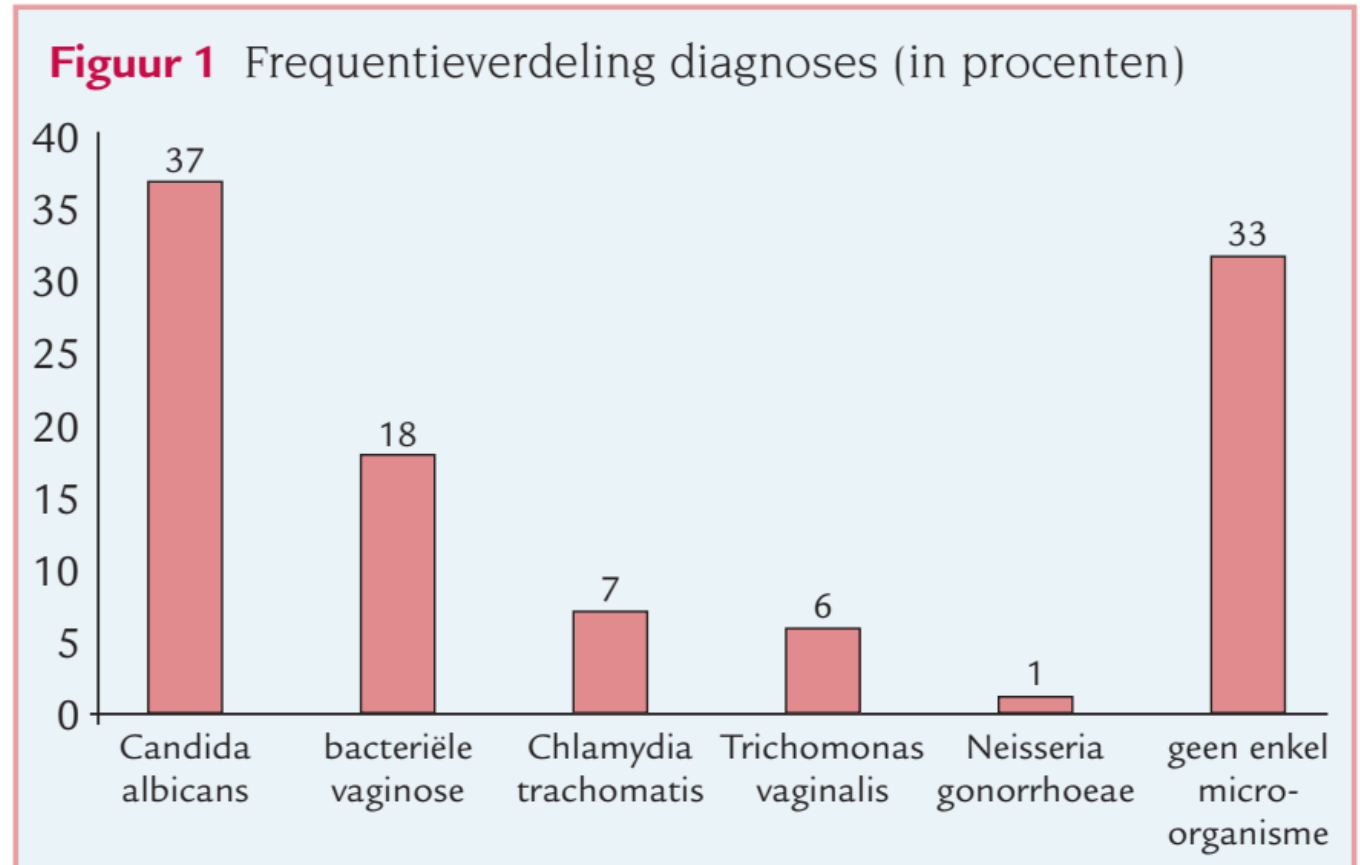


1. Introduction: vaginal complaints
2. The vaginal microbiome
3. Various causes of vaginitis
 - Bacterial vaginosis
 - Vulvovaginal candidiasis
4. Fertility
5. Urinary tract infections
6. Treatment with probiotics
7. Vaginal suppositories
8. Conclusions

Introduction: vaginal complaints

Vaginal complaints

- According to the Dutch Family Doctor Association (NHG), the incidence of vaginal complaints is¹:
 - 15.1 per 1000 per year for fluor vaginalis
 - Double for younger women
 - 24.6 per 1000 per year for urogenital candidiasis
- An older Dutch study among 682 women who visited the family doctor shows similar numbers (figure)².
- International figures of BV prevalence vary substantially (5–60%), with Finland and the US being most representative (5% and 30%)³



1. Fluor vaginalis | NHG-Richtlijnen, accessed 14-2-2023

2. Boeke, A. et al. Huisarts & Wetenschap 11, 616–620 (2002)

3. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)

Vaginal complaints

- Not only are vaginal complaint common, mental wellbeing is affected too¹:
 - Fear (e.g. for severe disease or STD)
 - Shame
 - Problems in sexual relationship
- Bacterial vaginosis (BV) is most common cause of abnormal vaginal discharge²:
 - Symptoms: malodor, increased pH, itching
 - 50% asymptomatic
 - Risk factors: sexual history, intravaginal practices, contraceptive use, antibiotic use.



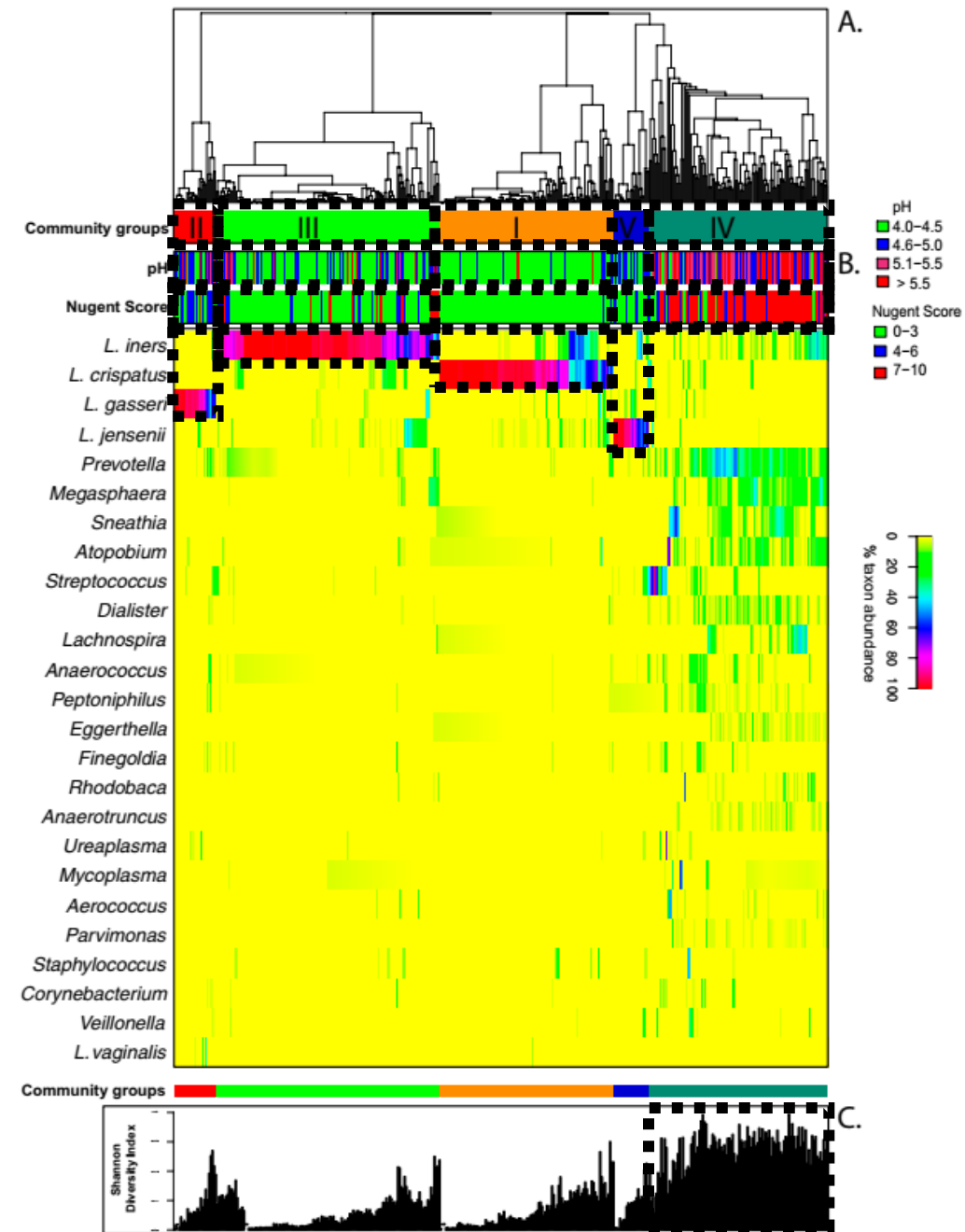
1. Boeke, A. et al. Huisarts & Wetenschap 11, 616–620 (2002)

2. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)

The vaginal microbiome

Vaginal microbiome

- The most cited study on the vaginal microbiome was published in 2011¹.
 - 5 groups were identified
 - In 4 of these the vaginal microbiome is dominated by a single Lactobacillus species: *L. iners*, *L. crispatus*, *L. gasseri*, *L. jensenii*.
 - The 5th group was characterized by high diversity. This 5th group has been regrouped into two subgroups^{2,3}.
 - One with high Nugent score.
- Although this grouping has been debated, conclusion remains that most women have a vaginal microbiome characterized by:
 - Single dominating Lactobacillus species (1 of 4)².
 - pH below 4.5



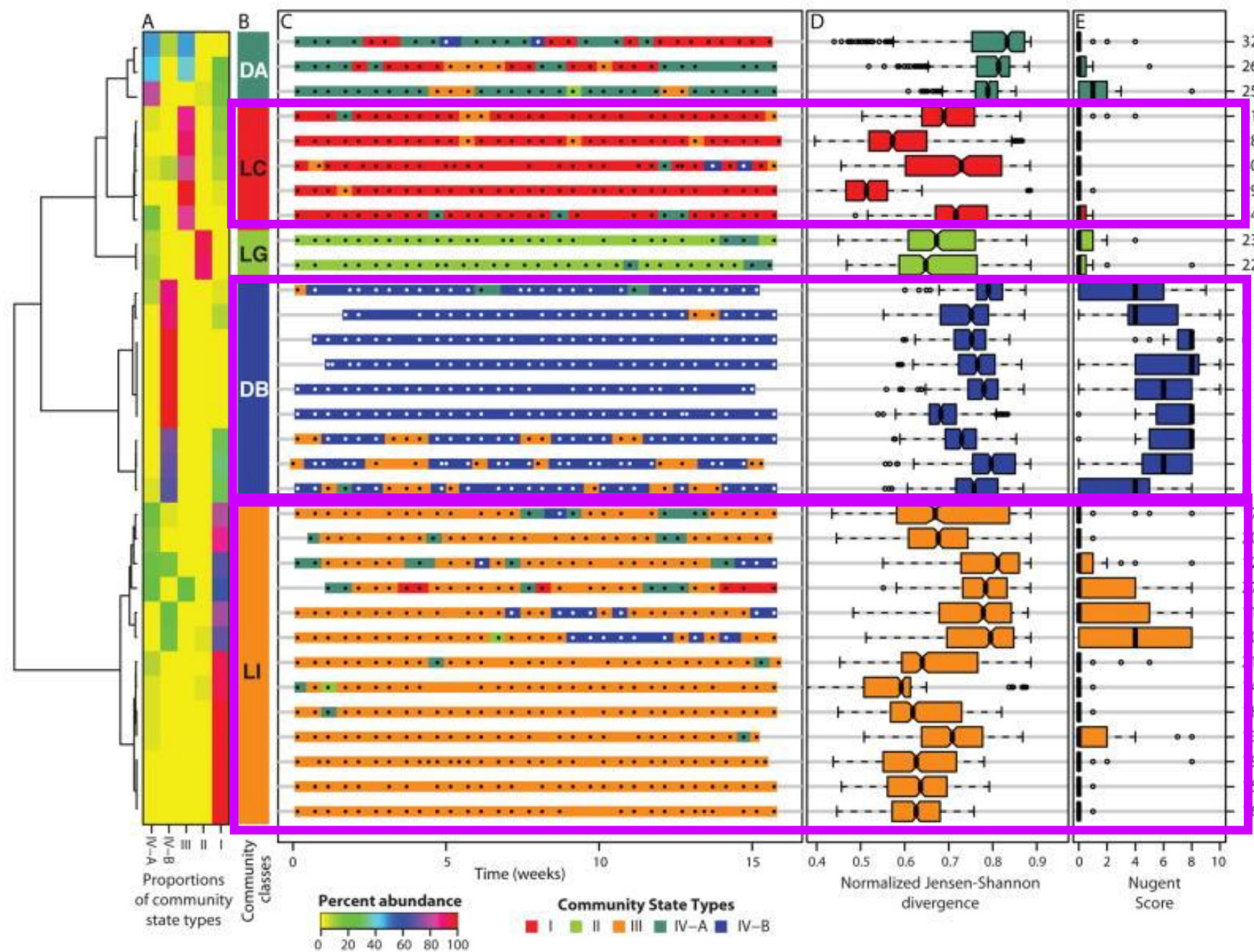
1. Ravel, J. et al. Proc Natl Acad Sci U S A 108, 4680–4687 (2011)

2. Verstraelen, H. et al. Journal of Lower Genital Tract Disease 26, 73 (2022)

3. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)

Vaginal microbiome

- Longitudinal research shows variation of the vaginal microbiome over time¹.
 - One of the high diversity subgroups was characterized by high Nugent scores (i.e. BV).
 - The *L. iners*-dominated vaginal microbiome is most prevalent in this population. The association of *L. iners* with health remains unclear, occurring in both dysbiotic and healthy states².
 - The *L. crispatus*-dominated and *L. gasseri*-dominated microbiomes are most stable and associated with lowest Nugent scores.

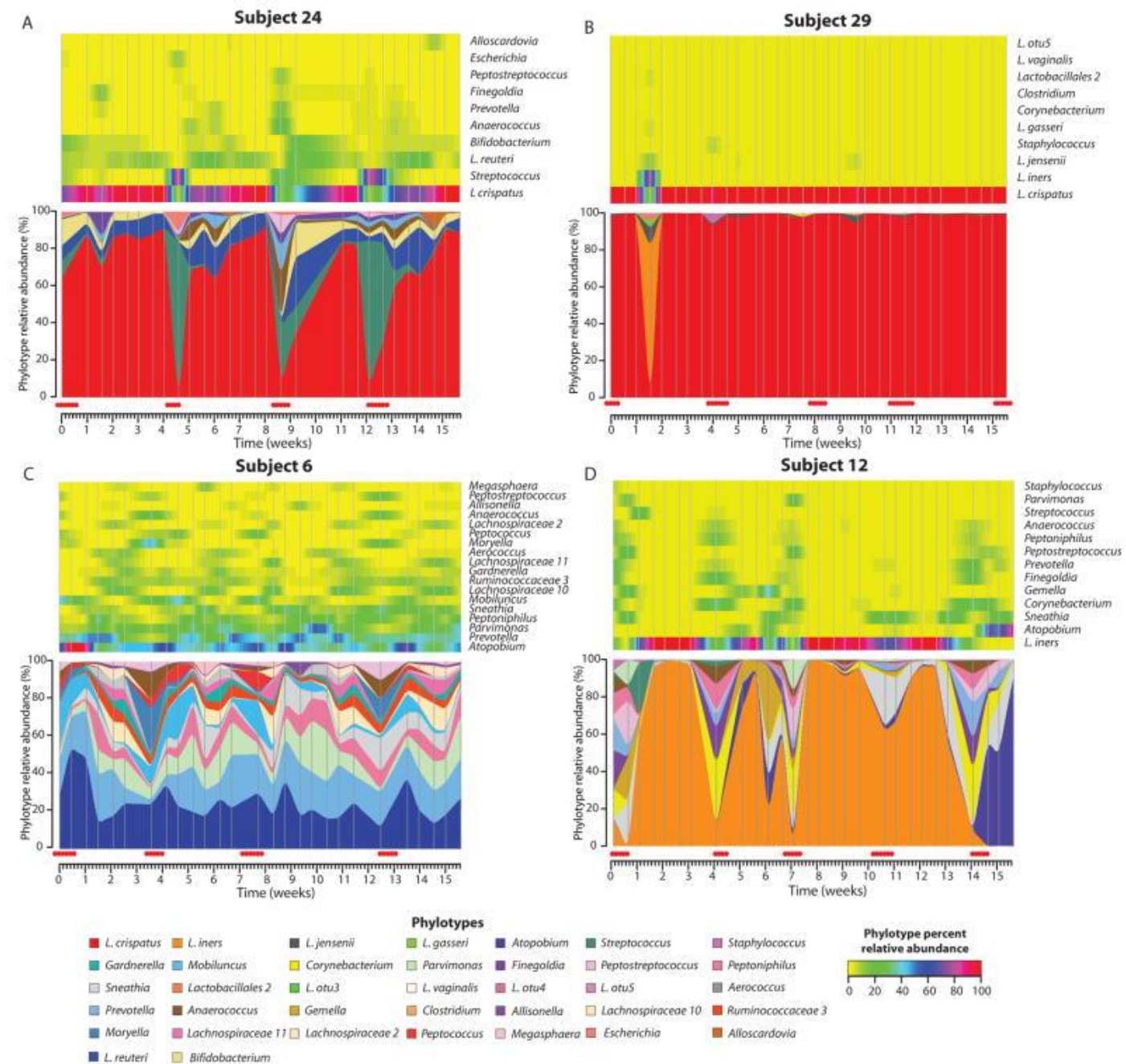


1. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)

2. Verstraalen, H. et al. Journal of Lower Genital Tract Disease 26, 73 (2022)

Vaginal microbiome

- Menses is major disturbing factor during the menstrual cycle^{1,2}:
 - *L. crispatus* abundance declines during menses
 - *L. iners* concentrations increase along with *Gardnerella vaginalis* and is the dominant species during menses.
 - There is certain “dynamic stability”: most women retain their typical state or alternate between states.
 - Alternation is aligned with menses.

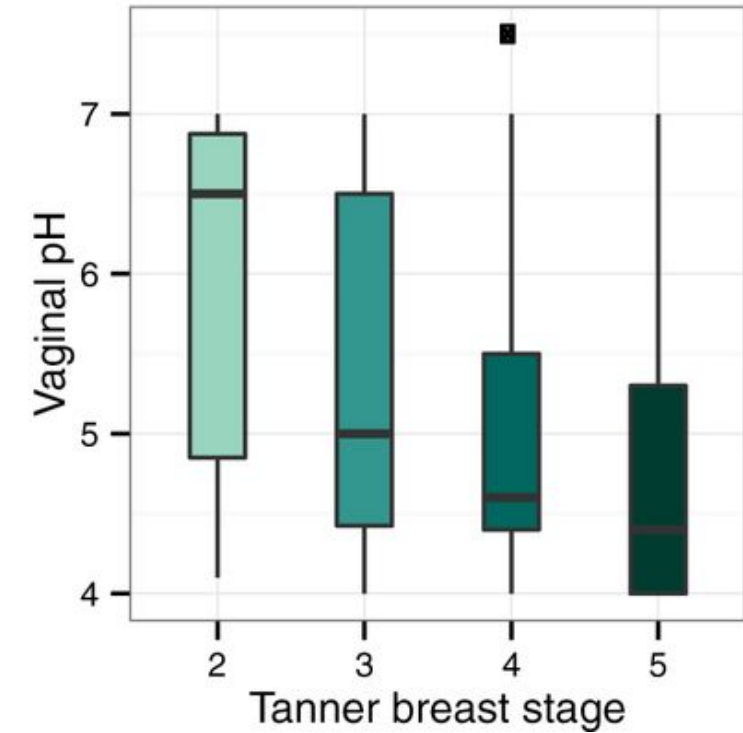
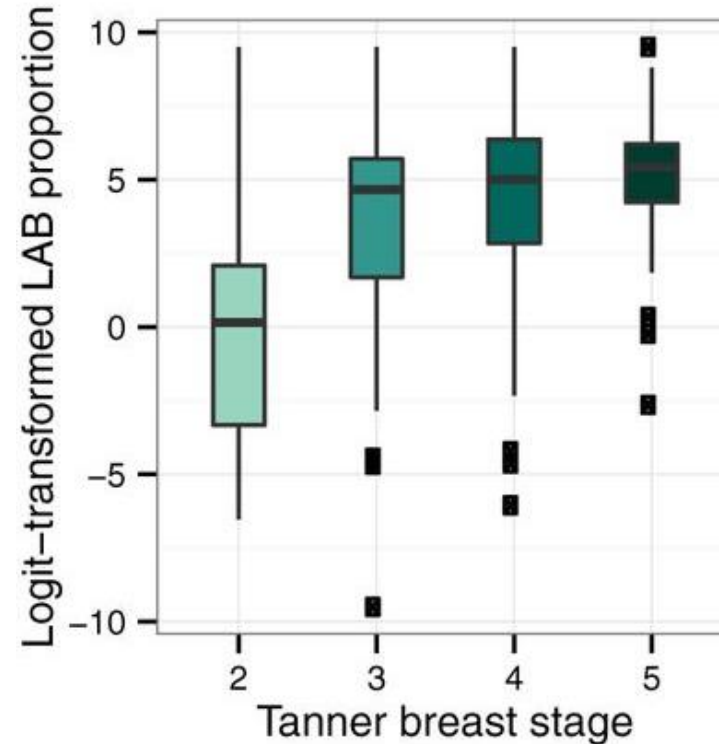


1. Gajer, P. et al. Sci Transl Med 4, 132ra52 (2012)

2. Verstraalen, H. et al. Journal of Lower Genital Tract Disease 26, 73 (2022)

Vaginal microbiome: prepuberty/premenarche

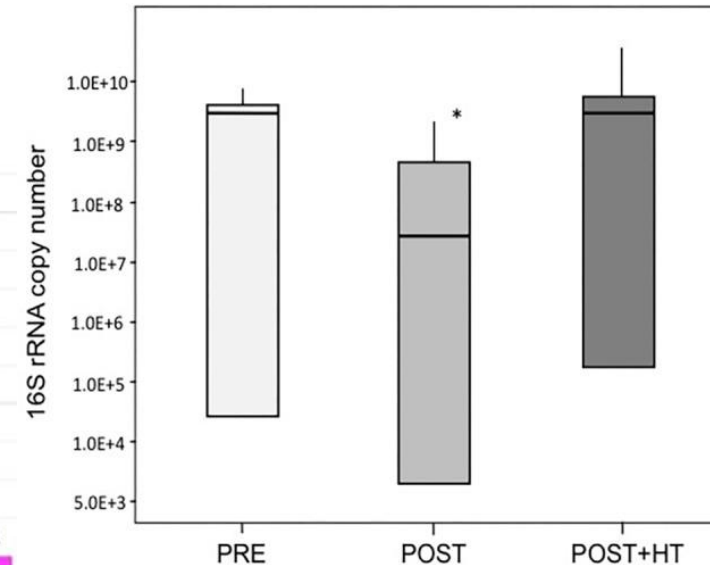
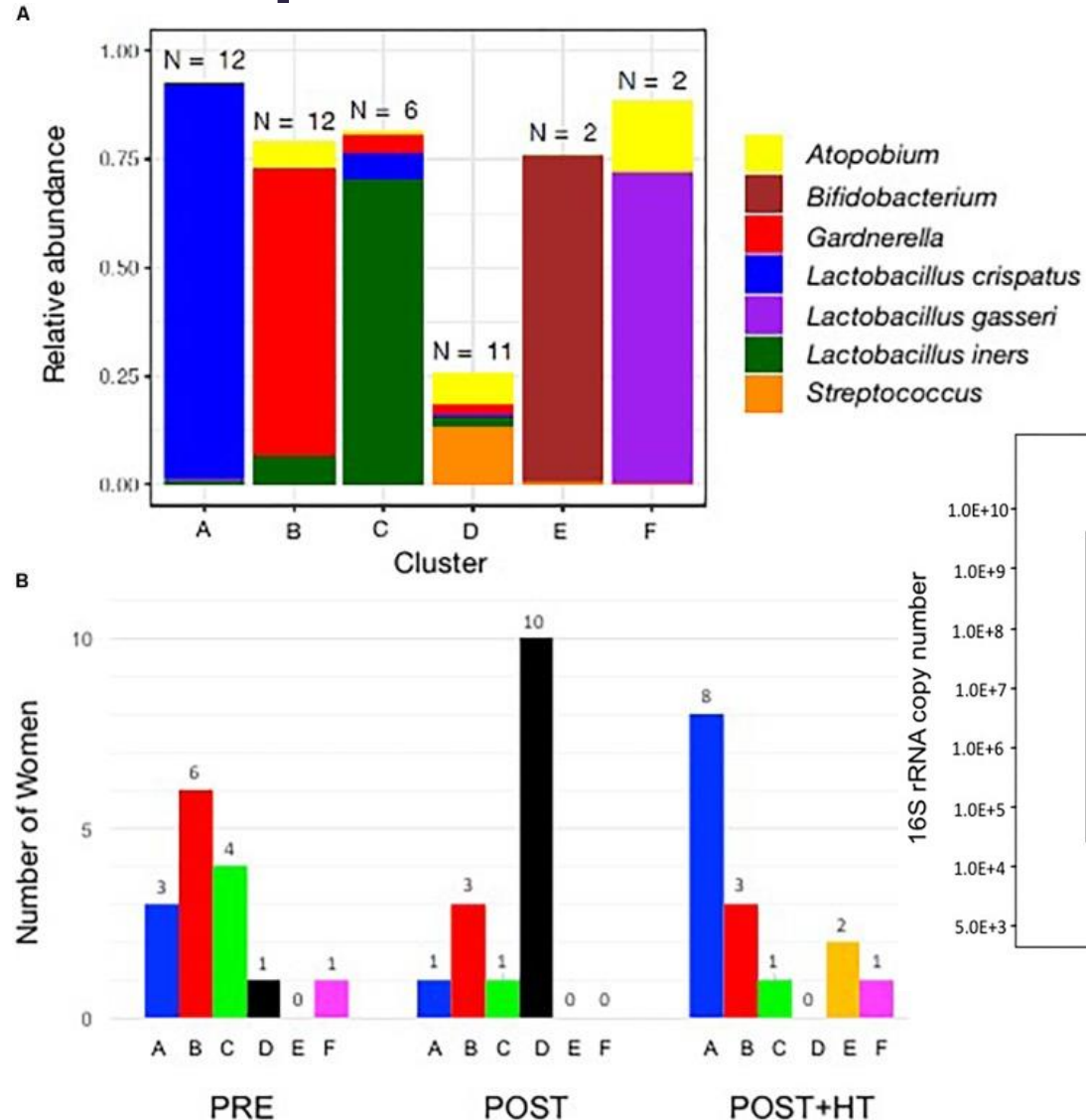
- Longitudinal research shows transition of vaginal microbiome from premenarche (~11y/o) to post-menarche¹.
- BV-like microbiome gradually shifts toward an adult-like *Lactobacillus*-dominated microbiome¹:
 - In almost all girls the microbiome changed to *Lactobacillus*-dominant after menarche. In some girls already at baseline.
 - Transition occurred before or shortly after menarche
 - Various trajectories occur, e.g. *L. iners*-dominated to *L. crispatus*-dominated.



1. Hickey, R. J. et al. mBio (2015)

Vaginal microbiome: postmenopausal

- Estrogen levels strongly affect the vaginal microbiota^{1,2}.
- Research on the postmenopausal vaginal microbiome is scarce. One study found marked differences between¹:
 - Premenopausal
 - Postmenopausal
 - Postmenopausal with hormone replacement therapy
- Postmenopausal women had:
 - Much more diverse microbiome without a single dominant species and few lactobacilli
 - 10x lower total abundance



1. Gliniewicz, K. et al. *Frontiers in Microbiology* 10, (2019)

2. Verstraalen, H. et al. *Journal of Lower Genital Tract Disease* 26, 73 (2022)

Vaginitis: various causes

Distinctions between causes of vaginitis

Signs and Symptoms of Vaginitis

Diagnosis	Etiology	Symptoms	Signs	Other risks
Bacterial vaginosis	Anaerobic bacteria (<i>Prevotella</i> , <i>Mobiluncus</i> , <i>Gardnerella vaginalis</i> , <i>Ureaplasma</i> , <i>Mycoplasma</i>)	Fishy odor; thin, homogenous discharge that may worsen after intercourse; pelvic discomfort may be present	No inflammation	Increased risk of HIV, gonorrhea, chlamydia, and herpes infections
Vulvovaginal candidiasis	<i>Candida albicans</i> , can have other <i>Candida</i> species	White, thick, cheesy, or curdy discharge; vulvar itching or burning; no odor	Vulvar erythema and edema	—
Trichomoniasis	<i>Trichomonas vaginalis</i>	Green or yellow, frothy discharge; foul odor; vaginal pain or soreness	Inflammation; strawberry cervix	Increased risk of HIV infection Increased risk of preterm labor Should be screened for other sexually transmitted infections
Atrophic vaginitis	Estrogen deficiency	Thin, clear discharge; vaginal dryness; dyspareunia; itching	Inflammation; thin, friable vaginal mucosa	—
Irritant/allergic vaginitis	Contact irritation or allergic reaction	Burning, soreness	Vulvar erythema	—
Inflammatory vaginitis	Possibly autoimmune	Purulent vaginal discharge, burning, dyspareunia	Vaginal atrophy and inflammation	Associated with low estrogen levels

HIV = human immunodeficiency virus.

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Poll 1



- How often do you see patients with vaginal complaints in your practice?
 1. Every day
 2. Every week
 3. Every month
 4. Never

Poll 2



- Do you ask female patients about vaginal complaints?
 1. Always
 2. Sometimes (e.g. if I suspect a problem)
 3. Only if she reports vaginal problems
 4. Never

Bacterial vaginosis

Diagnosis of bacterial vaginosis

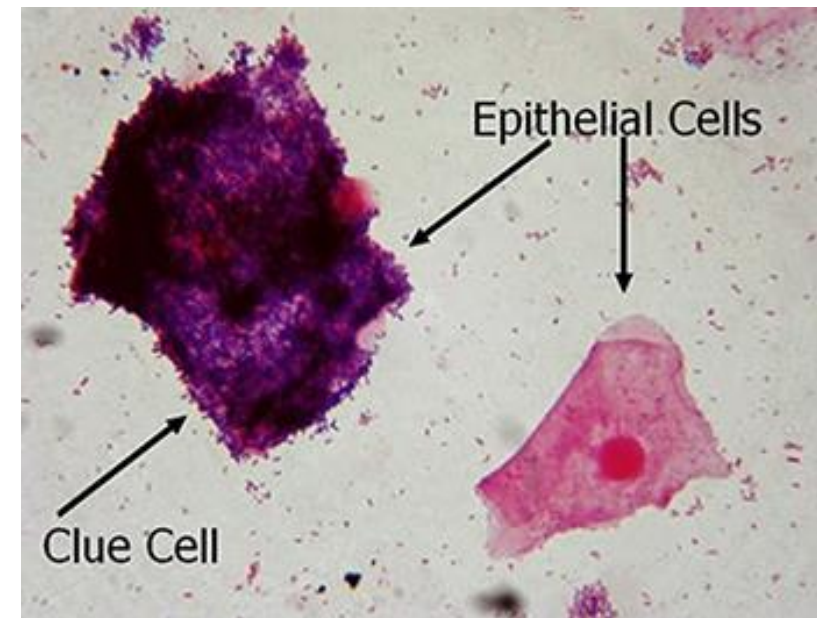
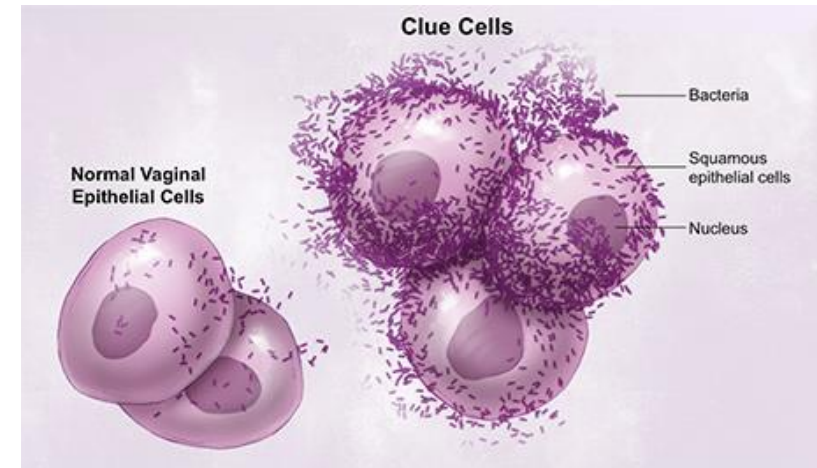
- “Vaginosis” is vaginal form of dysbiosis but is not characterized by typical inflammatory changes generally implied by the suffix ‘-itis’¹.
- Diagnosis of BV can be done using various criteria²:
 - **Nugent score.** Vaginal smears plated. Minimum of 10 high power fields examined for three bacteria morphotypes: *Lactobacillus*, *Gardnerella*, and curved gram rods. Each category receives score for cell count. Scores added together and:
 - 0–3: negative for BV
 - 4–6: intermediate
 - 7+: positive for BV

Method is cumbersome.

- **Amsel criteria.** There are four parameters used to determine the presence or absence of BV. These are:
 - Thin, white, yellow, homogeneous discharge
 - Clue cells on wet mount microscopy
 - a vaginal fluid pH of over 4.5 when placing the discharge on litmus paper
 - Release fishy odor after adding 10% potassium hydroxide (KOH) solution to wet mount - also known as “whiff test.”

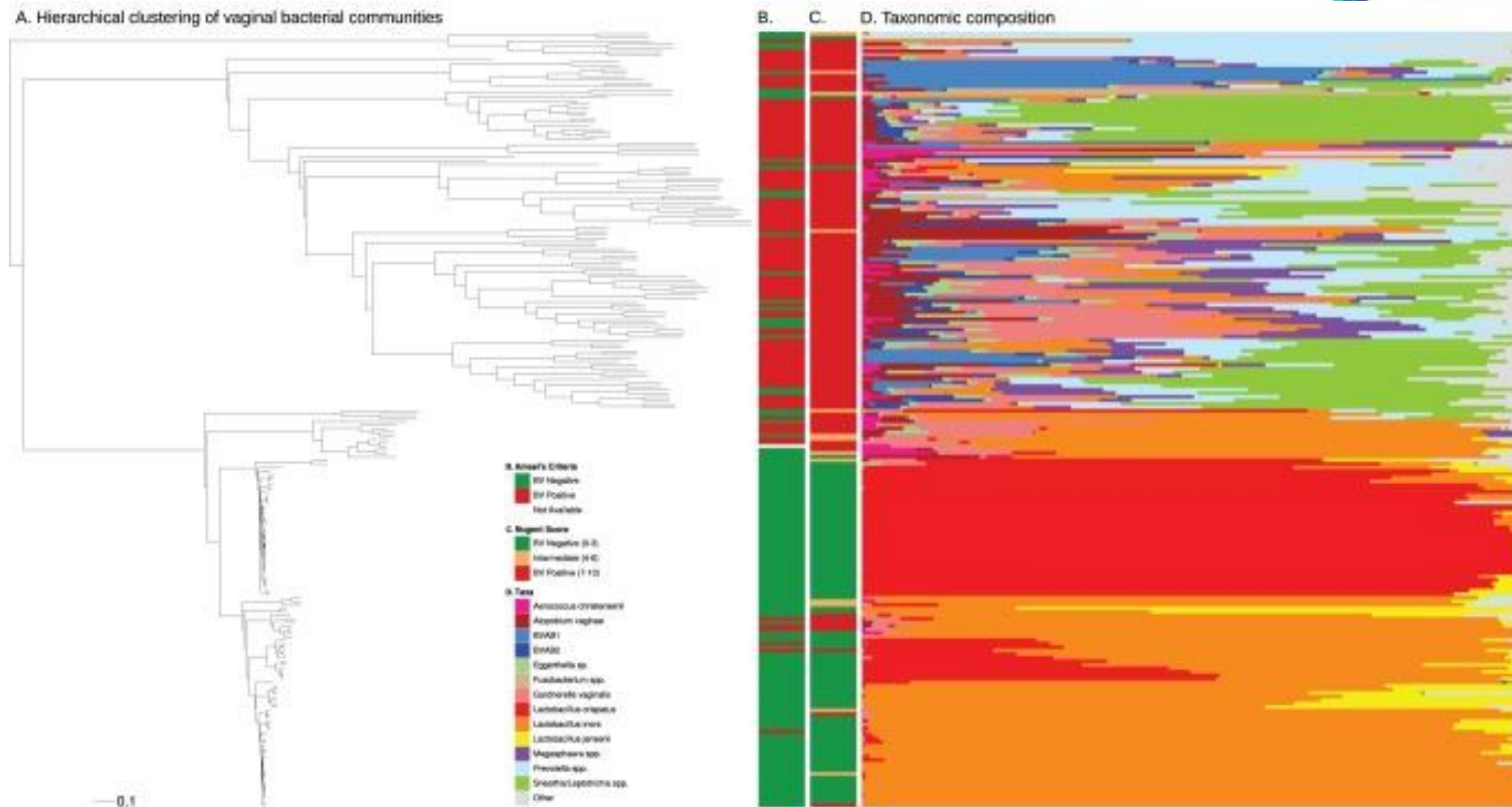
More practical when there is limited diagnostician's microscopic experience, availability of microscope tools, or time. Meeting three (or two) criteria is considered positive for BV.

- Some women do not report symptoms even though diagnostic criteria are met. Many women with BV may consider their discharge to be normal.



1. Schellenberg, J. J. et al. *Research in Microbiology* 168, 837–844 (2017)
2. Colonna, C. et al. in *StatPearls* (StatPearls Publishing, 2022)
3. Lev-Sagie, A. et al. *Journal of Lower Genital Tract Disease* 26, 79 (2022)

Vaginal microbiome: healthy vs bacterial vaginosis



1. Srinivasan, S. et al. PLoS ONE 7, (2012)

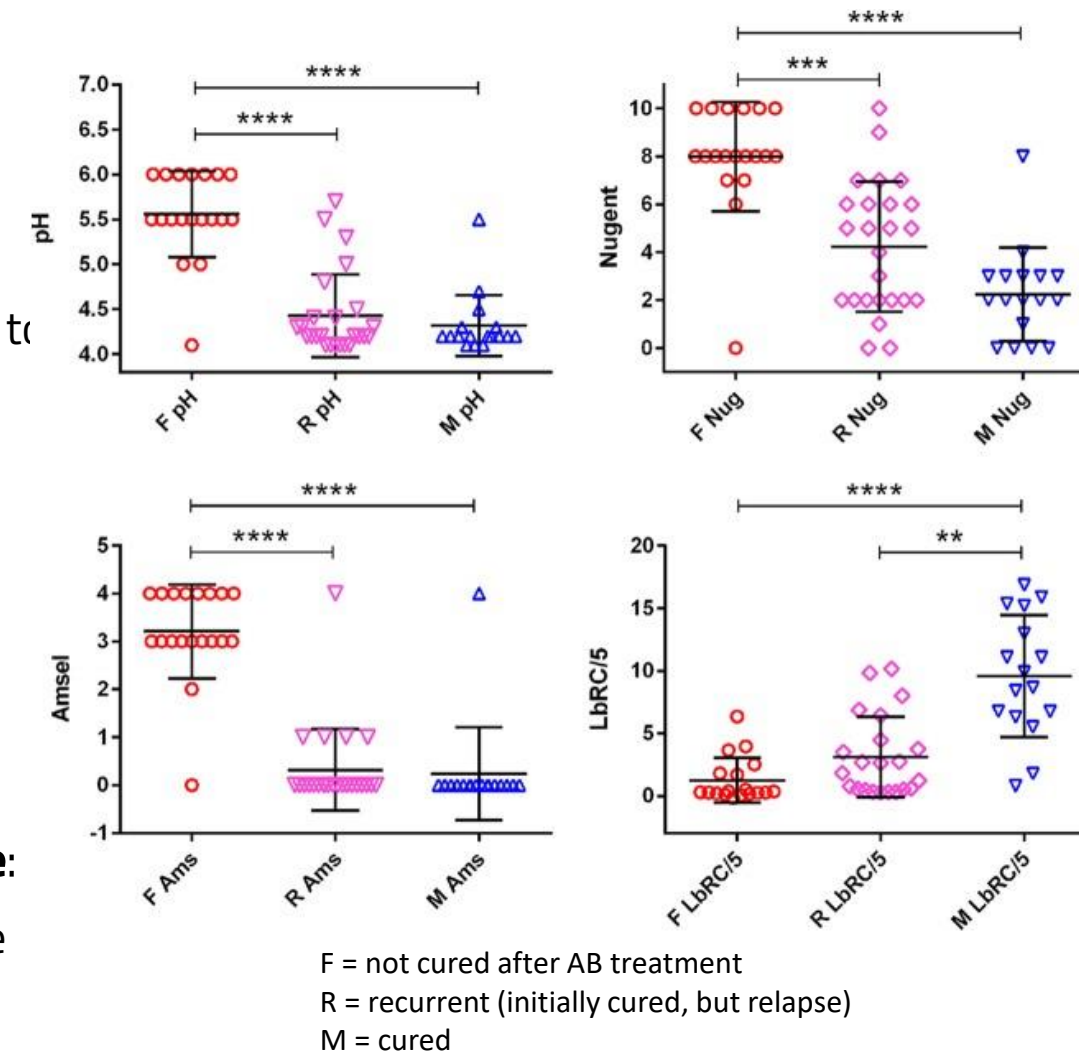
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Poll 3

- Would you conduct a vaginal microbiome analysis, or pragmatically start treatment?
 1. The test does not influence the treatment, so I always immediately start with treatment.
 2. For vaginal complaints I pragmatically start with treatment, but for e.g. fertility problems I prescribe a vaginal microbiome analysis first.
 3. I want to know if there is a dysbiosis before starting any treatment, so I always prescribe vaginal analysis first.
 4. Other

Traditional treatment of BV and recurrence

- According to guidelines BV is usually treated with antibiotics¹.
 - The reported initial cure rate in studies is high (80-90%)
 - The recurrence rate is high, ranging from 15-30% after treatment to 60-80% after 9 months
- One hypothesis for recurrence is reinfection with associated microbes via sexual partners¹:
 - But: treatment of partners does not reduce recurrence rates.
 - Recurrence can also be due to relapse or ineffective treatment.
- **This is indirect evidence that susceptibility may be a more important factor. A disturbed vaginal microbiome plays a role:**
 - Indeed, the *Lactobacillus* relative composition is prognostic of cure rate and recurrence rate².

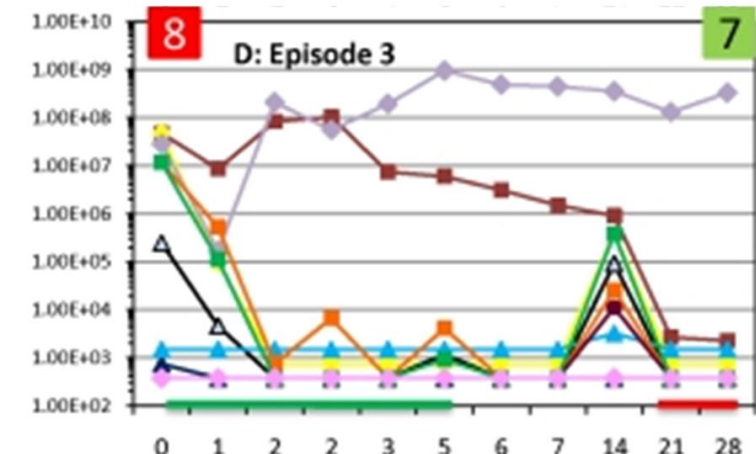
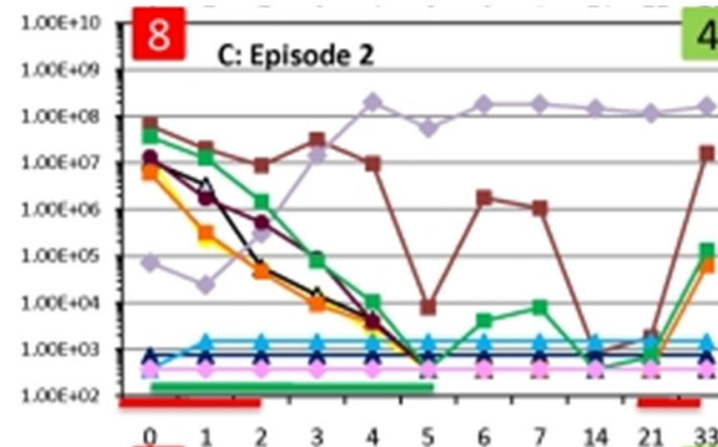
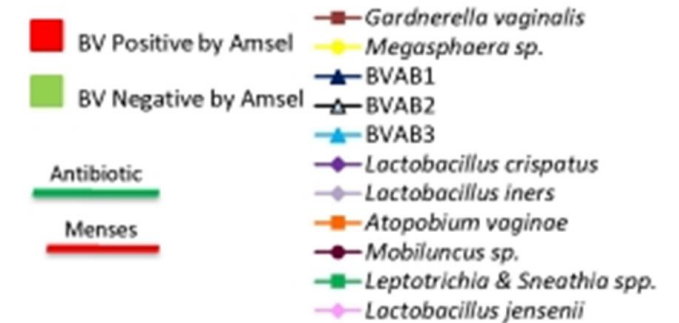
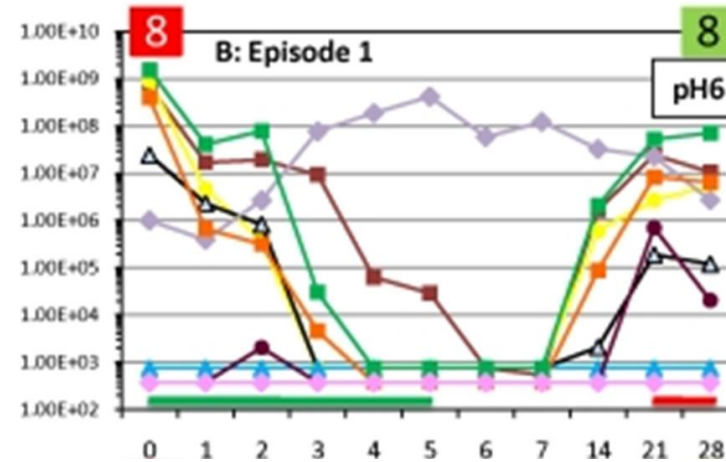


1. Coudray, M. S. et al. Eur J Obstet Gynecol Reprod Biol 245, 143–148 (2020)

2. Sobel, J. D. et al. J Clin Microbiol 57, e00227-19 (2019)

Vaginal microbiome: healthy vs bacterial vaginosis

- In women without BV¹:
 - Vaginal microbiome is relatively stable
 - Disturbance occurs during menses
 - BV-associated *Gardnerella vaginalis* can be present and increase during menses
- In women with BV¹:
 - Typically BV-associated species dominate (undefined species from *Clostridiales* order) and/or composition is diverse.



1. Srinivasan, S. et al. PLoS One 5, e10197 (2010)

Case report 1: BV



Woman, 75 years old

- Recurrent UTIs: complaints of dry vagina, slight burning sensation vagina (not when urinating), dyspareunia, urine stick nitrite pos
- Bowel: flatulence, diarrhea, histamine symptoms (headache, fuzzy, tired).
- Treatment: vitC, cranberry, D-mannose, solidago.
- Gut microbiome: MyOwnBlend after which bowels improved (headache and fatigue were gone) but vaginal complaints not yet.
- Vaginal microbiome analysis requested.
- Awaiting results: Vagifem (estrogens), probiofem Bonusan inserted vaginally.
- Results analysis.

Test	Uitslag	Eenheid	Normbereik	Vorig onderzoek
Vaginaal diagnostiek				
Vaginaal microbloom				
Kenmerken van vaginale afscheiding				
pH-waarde	7,0		4,0 - 4,4	AB NA) MGSEQ
Diversiteit				
Diversiteit	4,55		< 0,90	AB NA) MGSEQ

De diversiteit in de vaginale microbiota kan van mens tot mens verschillen. Een lage diversiteit s is een teken van een gezonde fysiologische vaginale microbiota. Een hoge diversiteit kan op mycoses, pathogene bacteriën of seksueel overdraagbare aandoeningen wijzen.

Grad

4,5

Vagina type / Community state type (CST)

De samenstelling van de vaginale microbiota kan in verschillende groepen verdeeld worden, de zogenaamde vagitypen. De vijf vagitypes I, II, III, IV en V worden onderscheiden door de dominerende lactobacillus species.

Vaginaltyp

IV

Bacteriële vaginose-score

Aan de hand van de BV-score wordt de vaginale microbiota beoordeeld op een bacteriële vaginose (BV). Voor deze beoordeling wort gekeken naar de fysiologische lactobacillen flora, de anaerobe begeleidende flora en de BV-geassocieerde flora.

BV Score

< 5 normaal
5 - 8 intermediair
> 8 positief

8

H2O2-opbouw

H2O2 synthese index	1,00		> 4	AB NA) MGSEQ
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Lactobacillus (meest voorkomende spp.)

Lactobacillus crispatus	0,00	%		AB NA) MGSEQ
Lactobacillus gasseri	0,00	%		AB NA) MGSEQ
Lactobacillus jensenii	0,00	%		AB NA) MGSEQ
Lactobacillus iners	0,00	%		AB NA) MGSEQ
Lactobacillus (totaal)	0,0	%	> 70,0	AB NA) MGSEQ

Bacteriën geassocieerd met bacteriële vaginose

Test	Uitslag	Eenheid	Normbereik	Vorig onderzoek
Atopobium vaginae	0,00	%	< 0,01	AB NA) MGSEQ
BVAB*-1	0,00	%	< 0,01	AB NA) MGSEQ
BVAB*-2	0,00	%	< 0,01	AB NA) MGSEQ
BVAB*-3	0,00	%	< 0,01	AB NA) MGSEQ
Bacteroides fragilis	0,00	%	< 0,01	AB NA) MGSEQ
Gardnerella vaginalis	0,00	%	< 0,01	AB NA) MGSEQ
Megasphaera spp.	0,00	%	< 0,01	AB NA) MGSEQ
Eggerthella spp.	0,00	%	< 0,01	AB NA) MGSEQ
Aerococcus christensenii	0,00	%	< 0,01	AB NA) MGSEQ
Dialister microaerophilus	0,55	%	< 0,01	AB NA) MGSEQ
Prevotella spp.	23,03	%	< 0,01	AB NA) MGSEQ
Dialister invisus	0,55	%	< 0,01	AB NA) MGSEQ
Mobiluncus spp.	0,00	%	< 0,01	AB NA) MGSEQ

Anaërobe bacteriën

Anaerococcus spp.	14,17	%	< 0,01	AB NA) MGSEQ
Bacteroides spp.	12,10	%	< 0,01	AB NA) MGSEQ
Corynebacterium spp.	4,68	%	< 0,01	AB NA) MGSEQ
Escherichia spp.	12,02	%	< 0,01	AB NA) MGSEQ
Fingoldia spp.	2,09	%	< 0,01	AB NA) MGSEQ
Gemella spp.	0,00	%	< 0,01	AB NA) MGSEQ
Lachnospiraceae	0,60	%	< 0,01	AB NA) MGSEQ
Mycoplasma spp.	0,00	%	< 0,01	AB NA) MGSEQ
Parvimonas spp.	0,00	%	< 0,01	AB NA) MGSEQ
Sneathia spp.	0,00	%	< 0,01	AB NA) MGSEQ
Streptococcus spp.	0,00	%	< 0,01	AB NA) MGSEQ
Ureaplasma spp.	0,00	%	< 0,01	AB NA) MGSEQ
Veillonella spp.	0,00	%	< 0,01	AB NA) MGSEQ
Overig	30,16	%		AB NA) MGSEQ

Candidiasis

Candida albicans	negatief	negatief		AB NA) QPCR
Candida dubliniensis	negatief	negatief		AB NA) QPCR
Candida glabrata	negatief	negatief		AB NA) QPCR
Candida krusei	negatief	negatief		AB NA) QPCR
Candida lusitanae	negatief	negatief		AB NA) QPCR
Candida parapsilosis	negatief	negatief		AB NA) QPCR
Candida tropicalis	negatief	negatief		AB NA) QPCR

Case report 1: BV



- Started ovules.
- Feels almost normal now, almost no more complaints vaginally.
- Personally feels ovules have done a lot.
- Repeat measurement

Test	Uitslag	Eenheid	Normbereik	Vorig onderzoek
Vaginaal diagnostiek				
Vaginaal microbiom				
Kenmerken van vaginale afscheiding				
pH-waarde	4,4		4,0 - 4,4	7,0
Diversiteit				

Diversiteit	0,15		< 0,90	4,5
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De diversiteit in de vaginale microbiota kan van mens tot mens verschillen. Een lage diversiteit s is een teken van een gezonde fysiologische vaginale microbiota. Een hoge diversiteit kan op mycoses, pathogene bacteriën of seksueel overdraagbare aandoeningen wijzen.

Grad

0,1

Vagina type / Community state type (CST)

De samenstelling van de vaginale microbiota kan in verschillende groepen verdeeld worden, de zogenaamde vagitypen. De vijf vagitypes I, II, III, IV en V worden onderscheiden door de dominerende lactobacillus species.

Vaginaltyp

I

Bacteriële vaginose-score

Aan de hand van de BV-score wordt de vaginale microbiota beoordeeld op een bacteriële vaginose (BV). Voor deze beoordeling wordt gekeken naar de fysiologische lactobacillen flora, de anaerobe begeleidende flora en de BV-geassocieerde flora.

BV Score

4

< 5 normaal
5 - 8 intermediair
> 8 positief

H2O2-opbouw

H2O2 synthese index	6,00		> 4	1,00
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Lactobacillus (meest voorkomende spp.)

Lactobacillus crispatus	98,92	%		0,00
Lactobacillus gasseri	0,00	%		0,00
Lactobacillus jensenii	0,00	%		0,00
Lactobacillus iners	0,00	%		0,00
Lactobacillus (totaal)	98,9	%	> 70,0	0,0

Bacteriën geassocieerd met bacteriële vaginose				
Atopobium vaginae	0,00	%	< 0,01	0,00
BVAB*-1	0,00	%	< 0,01	0,00
BVAB*-2	0,00	%	< 0,01	0,00
BVAB*-3	0,00	%	< 0,01	0,00
Bacteroides fragilis	0,00	%	< 0,01	0,00
Gardnerella vaginalis	0,00	%	< 0,01	0,00
Megasphaera spp.	0,00	%	< 0,01	0,00
Eggerthella spp.	0,00	%	< 0,01	0,00
Aerococcus christensenii	0,00	%	< 0,01	0,00
Dialister microaerophilus	0,01	%	< 0,01	0,55
Prevotella spp.	0,04	%	< 0,01	23,03
Dialister invisus	0,01	%	< 0,01	0,55
Mobiluncus spp.	0,00	%	< 0,01	0,00

Anaërobe bacteriën

Anaerococcus spp.	0,20	%	< 0,01	14,17
Bacteroides spp.	0,00	%	< 0,01	12,10
Corynebacterium spp.	0,00	%	< 0,01	4,68
Escherichia spp.	0,00	%	< 0,01	12,02
Finegoldia spp.	0,02	%	< 0,01	2,09
Gemella spp.	0,00	%	< 0,01	0,00
Lachnospiraceae	0,00	%	< 0,01	0,60
Mycoplasma spp.	0,00	%	< 0,01	0,00
Parvimonas spp.	0,00	%	< 0,01	0,00
Sneathia spp.	0,00	%	< 0,01	0,00
Streptococcus spp.	0,00	%	< 0,01	0,00
Ureaplasma spp.	0,00	%	< 0,01	0,00
Veillonella spp.	0,00	%	< 0,01	0,00
Overig	0,81	%		30,16

Candidiasis

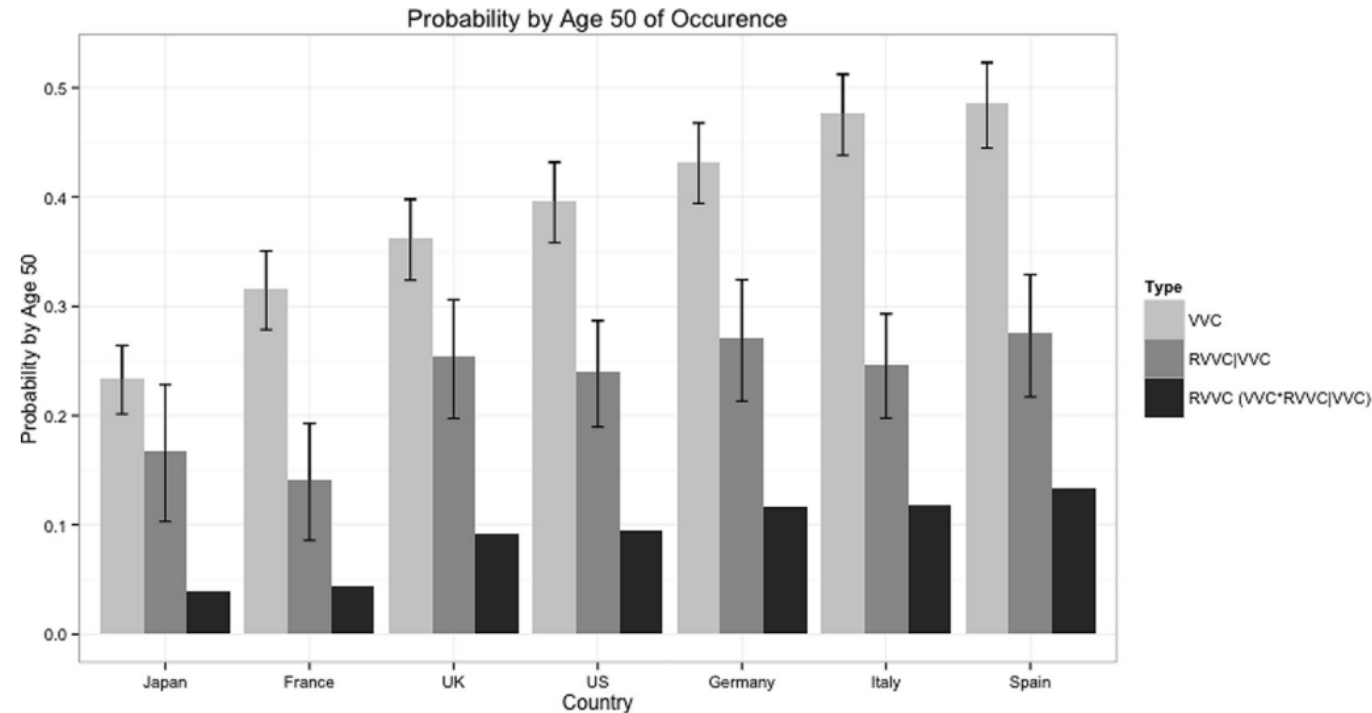
Candida albicans	negatief		negatief	negativ
Candida dubliniensis	negatief		negatief	negativ
Candida glabrata	negatief		negatief	negativ
Candida krusei	negatief		negatief	negativ
Candida lusitanae	negatief		negatief	negativ
Candida parapsilosis	negatief		negatief	negativ
Candida tropicalis	negatief		negatief	negativ

Vulvovaginal candidiasis

Vulvovaginal candidiasis

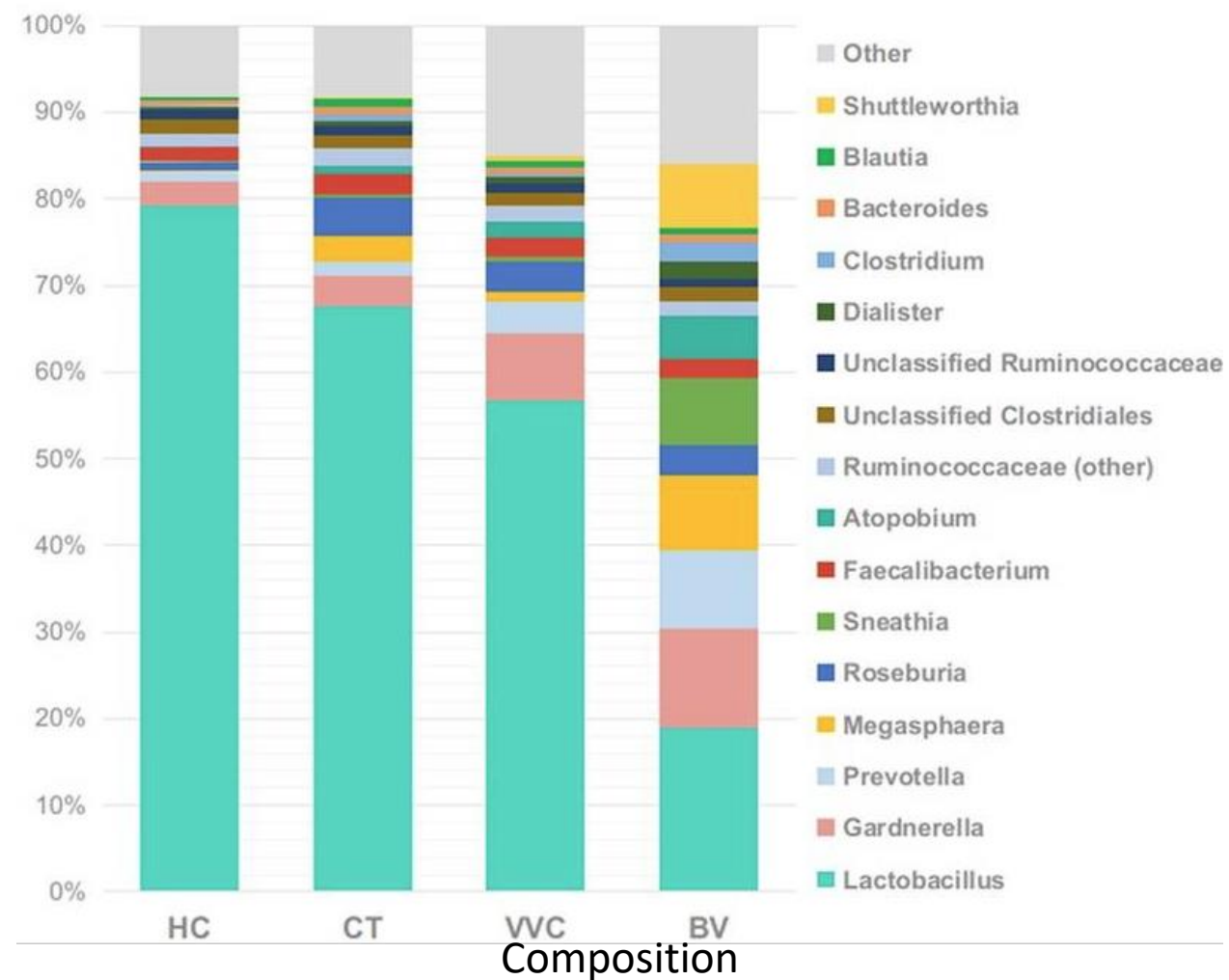
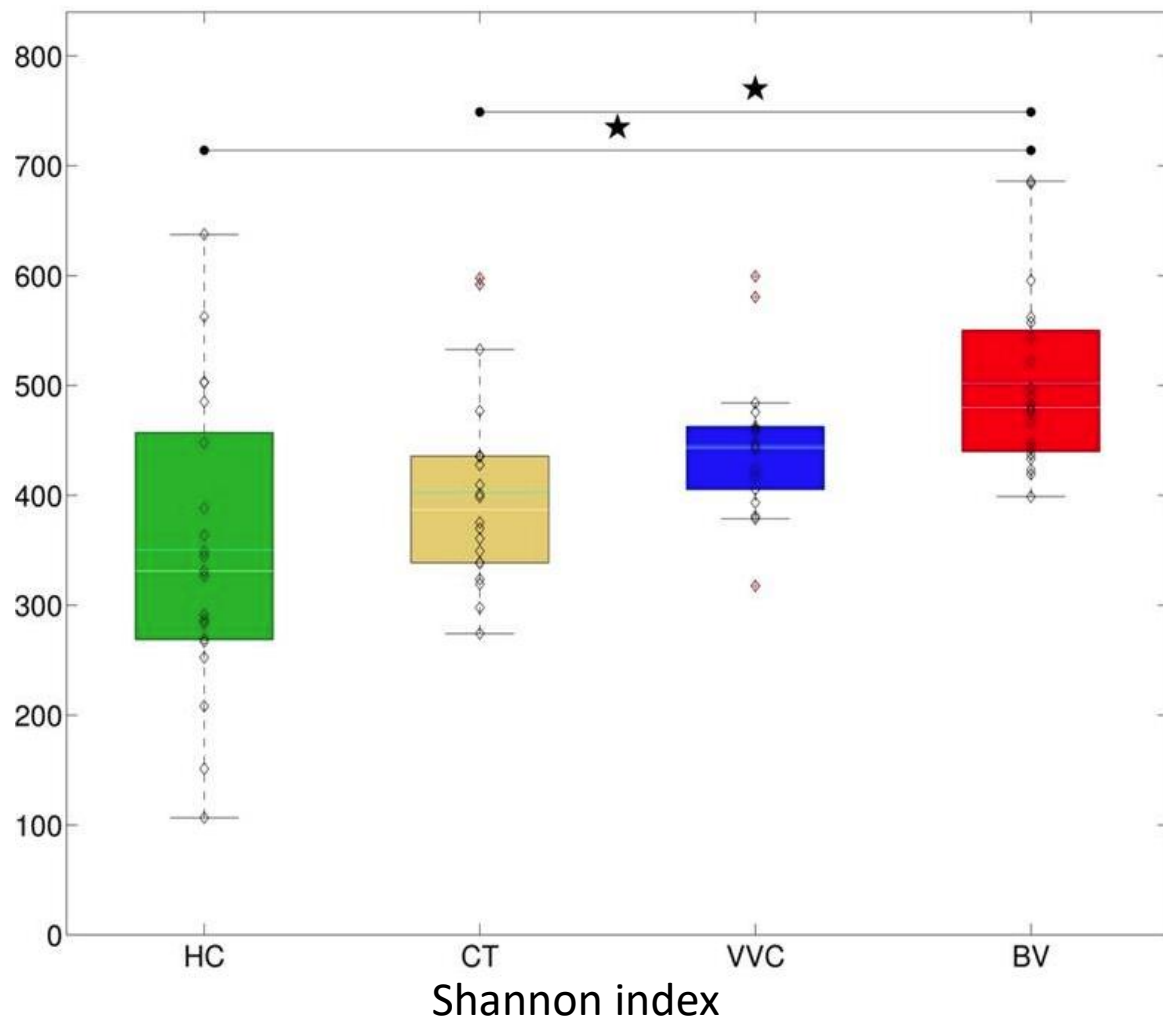
Vulvovaginal candidiasis¹:

- Is most often caused by *C. albicans* (85-90%), sometimes by other candida species.
- Is characterized by vulvar erythema, excoriation, pruritus, and an abnormal “cheese-like” or watery vaginal discharge.
- Diagnosis is not straightforward: defined by combination of nonspecific vaginal symptoms and presence of yeast that is commensal.
 - Is often diagnosed and treated empirically, with much self-diagnosis and treatment.
- Has high cumulative incidence: 23-49% at age of 50.
- Is typically treated with vaginal azoles.
- Has often recurrence (50-60% within 1-2 years)

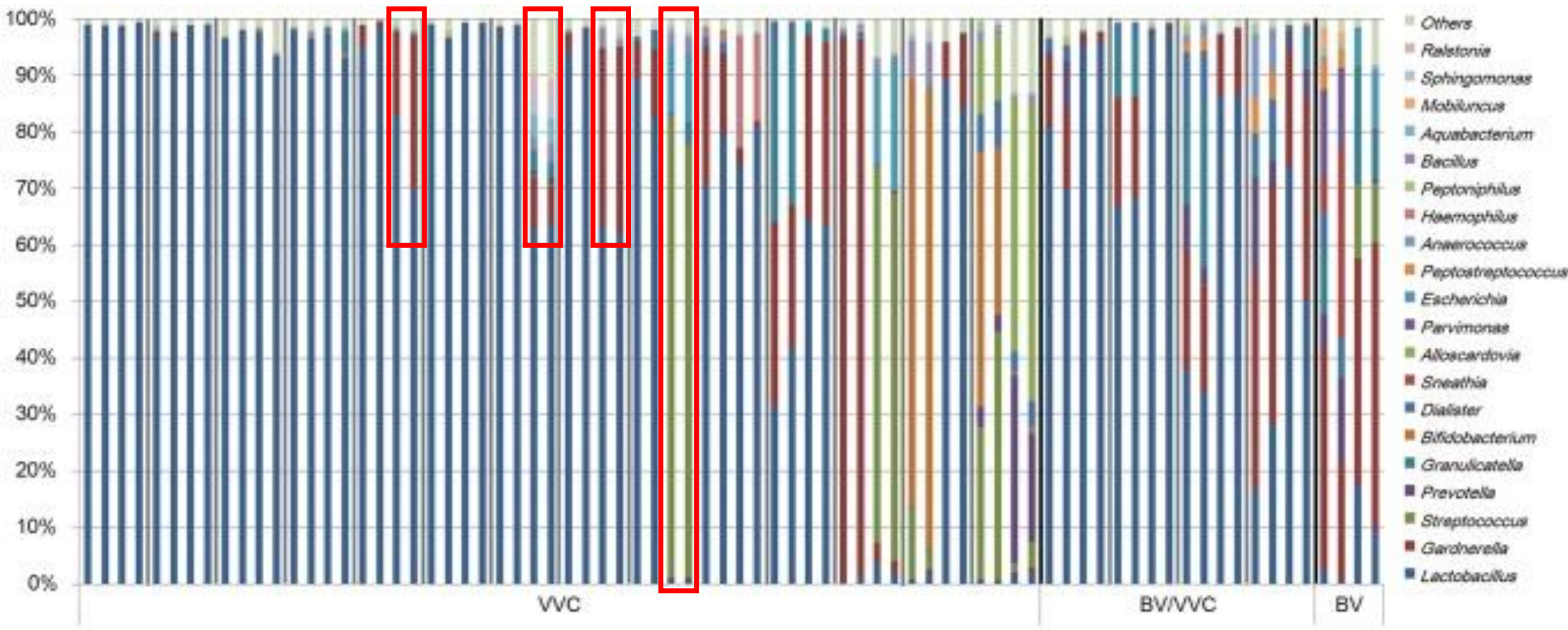


1. Blostein, F. et al. *Annals of Epidemiology* 27, 575-582.e3 (2017)

Vaginal microbiome: healthy vs VVC



VVC: treatment may lead to less healthy microbiome



1. Liu, M.-B. et al. PLoS One 8, e79812 (2013)

Case report 2: VVC + BV



- Woman 22 years old
- Alternating vaginal complaints due to candida or due to bacterial vaginosis
- Was prescribed vaginal suppositories
- Were they easy to use?
 - *“There seems to be some kind of coating over them that makes it easy. It doesn't hurt either.”*
- Did you experience any side effects?
 - *“I did not experience any side effects.”*
- Have they had the desired effect?
 - *“The suppositories often had the desired effect, especially with vaginal yeast infections. With bacterial infections, it seems to come back more quickly.”*

Case report 3: VVC



- Women 40 years, doctor
- Recurring yeast infections, for which she preferred not to use medication.
- Prescribed vaginal suppositories to herself

- Her findings:

“The symptoms of itching and crumbly discharge were over very quickly. I used the ovules for over a week, and then I got my period and didn't use them. Now I have used 1 more ovule twice on the day after my period, and that has ensured that to date both the Candida and an unpleasant odor stay away.

So, I would definitely recommend the ovules”

Fertility

Role in fertility and pregnancy

- A canonical (culture-based) study on fertility found higher prevalence of vaginal dysbiosis in infertile women compared to healthy controls without genealogical problems (figure)^{1,2}.
 - Only 3.5% of infertile women had *Lactobacillus* species.
- Similar results were found in study with IVF patients³.
 - Notably, women with abnormal vaginal microbiome had much less successful pregnancy (figure).
 - *L. crispatus* was found in 59% of women with normal microbiome vs. 12% in intermediate and 0% in BV.
- Some studies do not find clear associations¹.

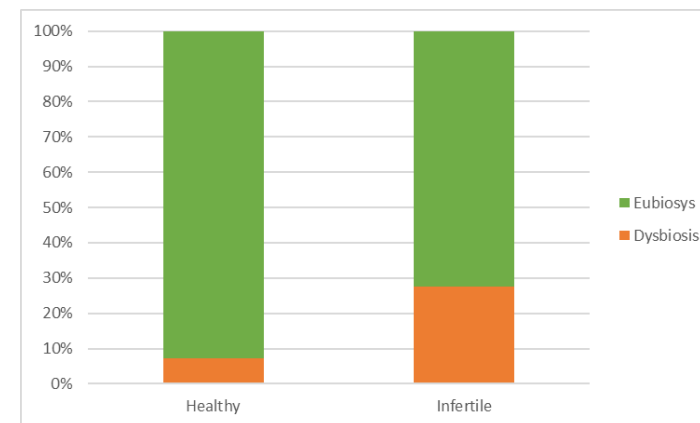


Table IV qPCR classification of vaginal microbiota (VM) and reproductive outcome of IVF patients.

	Biochemical pregnancy	Clinical pregnancy
Normal VM (N = 62)	32 (52)	27 (44)
Abnormal VM (N = 22)	6 (27)	2 (9)

Data are n (percent of patients per row).

Table V Nugent score, reproductive outcome of IVF patients.

	Biochemical pregnancy	Clinical pregnancy
Normal flora (N = 60)	30 (50)	24 (40)
Intermediate (N = 12)	6 (50)	4 (33)
BV (N = 12)	2 (17)	1 (8)

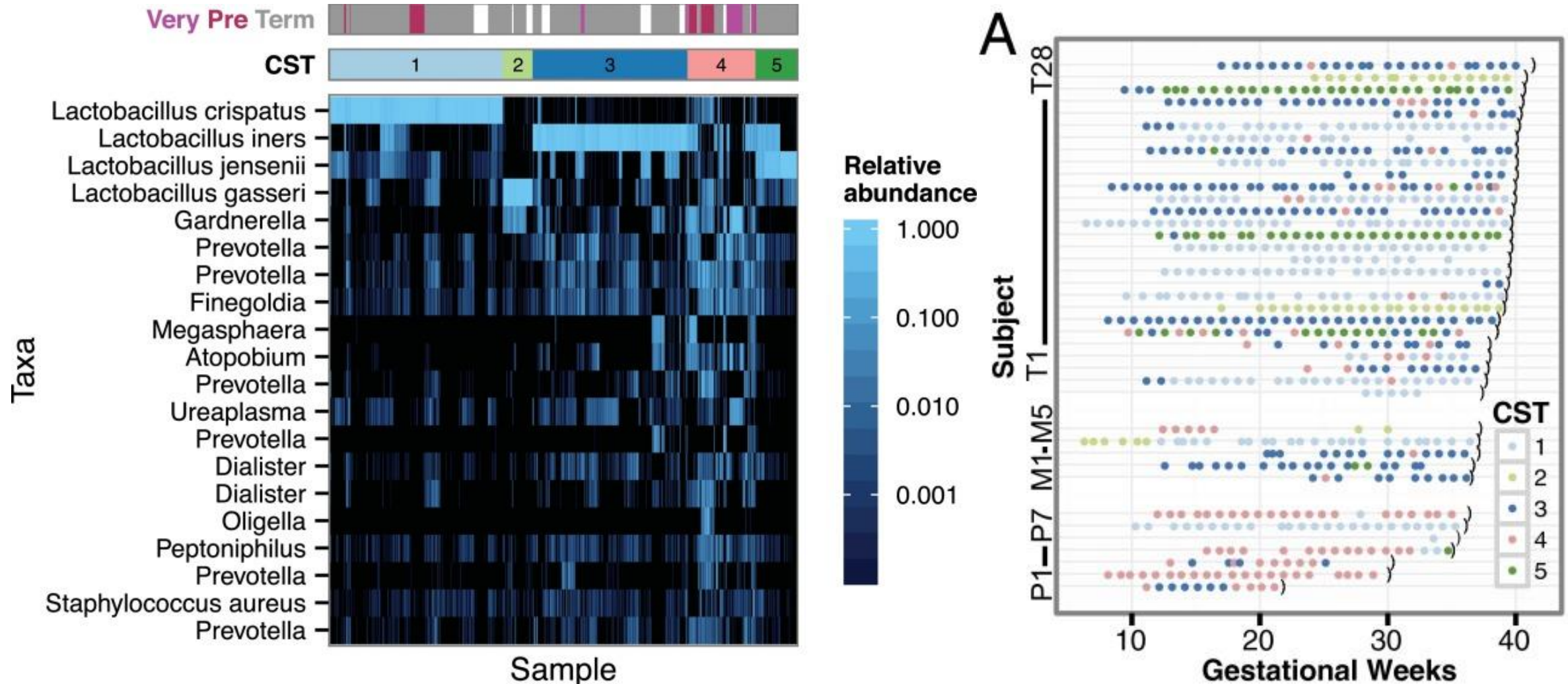
Data are n (percent of patients per row).

1. Hong, X. et al. Arch Gynecol Obstet 302, 569–578 (2020)

2. Babu, G. et al. J Clin Diagn Res 11, DC18–DC22 (2017)

3. Haahr, T. et al. Hum. Reprod. 31, 795–803 (2016)

Preterm delivery is associated with highly diverse vaginal microbiome¹



1. DiGiulio, D. B. et al. *Proc Natl Acad Sci U S A* 112, 11060–11065 (2015)

Case report 4: IVF

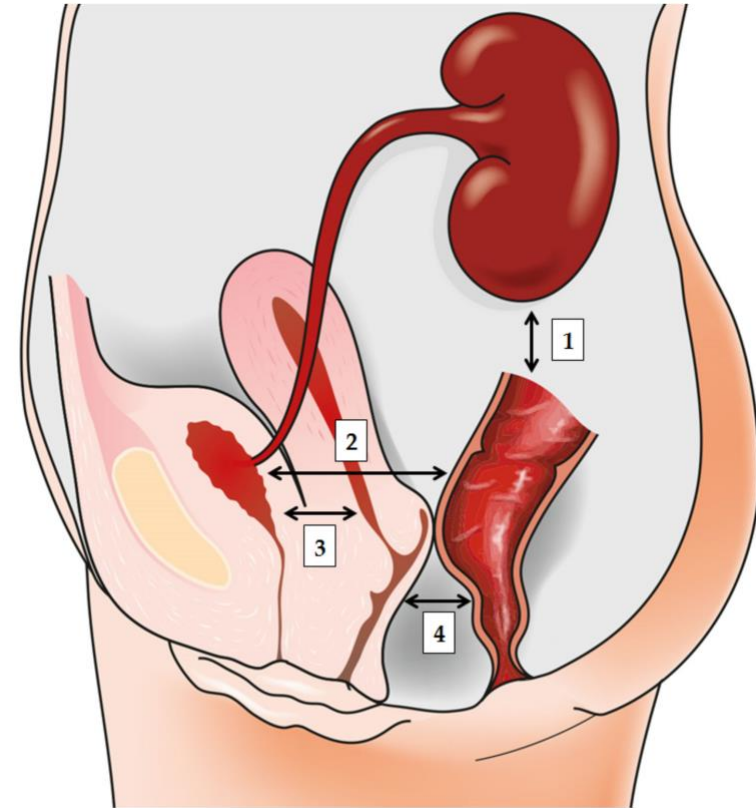


- Woman 35 years old
- Since 2015 stress-related disturbance of cycle, endometriosis, failed intrauterine insemination and two IVF attempts (low yield). Over 4 years of unfulfilled pregnancy wish.
- Estrogen breakdown problem, methylation only 3%.
- After low-dose naltrexone (LDN) with exorphin-free diet, recovery of stress and immune system. Improvement of methylation with B vitamins and NAC.
- IVF yield suddenly very good (17 oocytes), 6 months after setting LDN pregnant. Labor after 38 weeks with cesarean section, son in 2020.
- For second pregnancy again setting up LDN from April till June 2020 with methylation support.
- From June also used vaginal suppositories. Also around embryo transfer.
- **Immediately pregnant!** Calculated to give birth in April 2023.

Urinary tract infections

Role in urinary tract infections

- Historically, role of gut microbes in UTIs has been recognized:
 - Via contamination of the periurethral space by uropathogens residing in the gut¹. In most cases UTI is caused by pathogenic *E. coli* strains.
- More recently, the role of the vaginal microbiome has been identified too.
 - In fact, various axes are identified¹.
- Lactobacilli (most notably *Lactobacillus crispatus*) of the vaginal microbiome can inhibit *E. coli* growth¹.
 - In women with UTIs, there is often no lactobacilli-dominance and/or a dysbiotic vaginal microbiome².
- Antibiotic use can increase risk of UTI².



1. Meštrović, T. et al. *Diagnostics (Basel)* 11, (2020)

2. Stapleton, A. E. *Microbiol Spectr* 4, (2016)

Case report 5: UTI



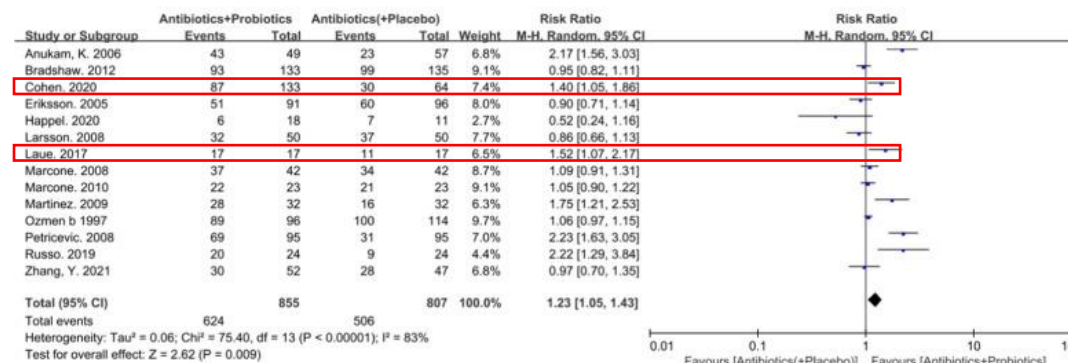
- 70-year-old woman
- Recurrent urinary tract infections; 2022 5 x AB, 2023 3 x AB
- Has been using vaginal estrogens for a while.
- No intestinal issues, therefore, no consideration of microbiome analysis or treatment.
- Treatment with vaginal suppositories for one month, starting in March 2023.
- August 2023: 19 week free of UTI's

Treatment with probiotics

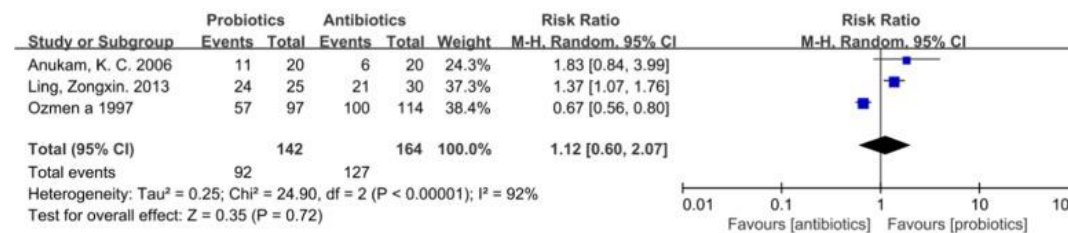
Probiotics for bacterial vaginosis

- Several meta-analyses on probiotics for treatment of bacterial vaginosis are published.
- One meta-analysis from 2022 includes 14 studies with 2093 participants¹:
 - Antibiotics + probiotics are somewhat better than antibiotics alone (RR: 1,23)
 - Probiotics are much better than placebo (RR 15,2)
 - Half of studies used oral administration
 - Only 2 studies use a *L. crispatus* strain.

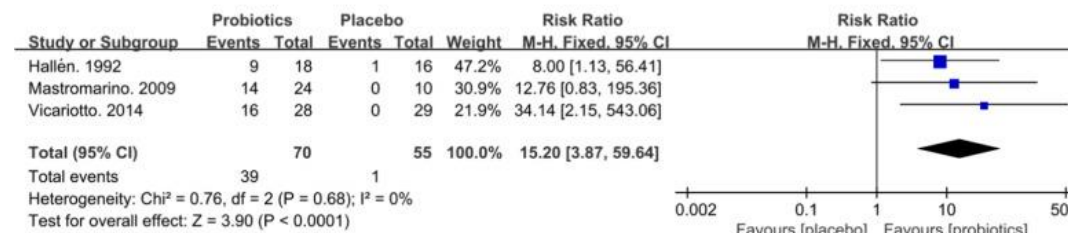
A. Antibiotics + Probiotics/Antibiotics (+Placebo)



B. Probiotics/Antibiotics.



C. Probiotics/Placebo.



1. Chen, R. et al. Reprod Health 19, 137 (2022)

Probiotics for vulvovaginal candidiasis

- A review from 2019 includes 14 studies using probiotics against VVC¹:
 - The results are in general positive, although they are strain-specific.
- Two open label studies with vaginal product containing *L. acidophilus* La02 showing^{2,3}:
 - La02 inhibits growth of various candida species
 - Administered 1dd for one week, then 1x per 3 days for three weeks, and finally once a week during second month.
 - Vaginal product cured vulvovaginal candidiasis in >86% of the women after 1 month and in >76% after two months².
 - Prevents recurrence of VVC in 72% of women with history of rVVC³.

4.1 | Patients with acute VVC

Multiple studies have demonstrated that probiotics may be beneficial to patients with acute VVC treated with standard antifungals by improving vaginal symptoms. Kovachev et al. compared women with

TABLE 1. Clinical Outcome (Efficacy Analysis) of Study Population

Time	n/N (%)	
	Cure	Recurrence
Prophylactic phase		
I phase—10 d	58/57 (98.3)	58/1 (1.7)
II phase—10 wk	57/49 (86.0)	57/8 (14.0)
Observation phase (mo)		
1	49/45 (91.8)	49/4 (8.2)
3	45/43 (95.6)	45/2 (4.4)
7	43/42 (97.7)	43/1 (2.3)
Cumulative rate	58/42 (72.4)	58/16 (27.6)

TABLE 3. Overview of Data and Statistical Analysis

Parameters	Time 0	Time 28	p (T28 vs. T0)	Time 56	p (T56 vs. T0)	p (T56 vs. T28)
Total women with infection	30	4	< 0.001	7	< 0.001	—
Total women without infection	0	26	—	23	—	—
Percentage of healing	—	86.67%	—	76.67%	—	—
Total women with recurrences	—	0	—	3	—	0.083
Percentage of recurrences	—	—	—	11.54%	—	—

1. Shenoy, A. et al. *Dermatologic Therapy* e12970 (2019)

2. Vicariotto, F. et al. *Journal of Clinical Gastroenterology* 46, S73–S80 (2012)

3. Murina, F. et al. *Journal of Clinical Gastroenterology* 48, S102–S105 (2014)

Probiotics for urinary tract infections

- A study with a *L. crispatus* strain shows reduced risk for recurring UTI after 10w, compared to placebo¹.
- Women who had high levels of *L. crispatus* colonization, had substantial lower risk of recurrent UTI than women with low levels of colonization.

Table 2. Urinary Tract Infection Rates by Intervention and *Lactobacillus crispatus* Colonization Pattern

Intervention	No. (%) of participants developing recurrent UTI	Relative risk (95% CI)
Lactin-V (<i>n</i> = 48)	7 (15)	.5 (.2–1.2)
Placebo (<i>n</i> = 48)	13 (27)	...
Intervention, <i>L. crispatus</i> colonization pattern		
Lactin-V, high level (<i>n</i> = 41)	2 (5)	.07 (.02–.3)
Lactin-V, low level (<i>n</i> = 7)	5 (71)	...
Placebo, high level (<i>n</i> = 32)		
Placebo, high level (<i>n</i> = 32)	9 (28)	1.1 (.4–3.1)
Placebo, low level (<i>n</i> = 16)	4 (25)	...

1. Stapleton, A. E. et al. *Clinical Infectious Diseases* 52, 1212–1217 (2011)

Vaginal suppositories

Vaginal suppositories

- Microbiome Center together with the pharmacist and small group of doctors developed vaginal suppositories.
 - Meant to replace over-the-counter options because:
 - Medical devices (i.e. vaginal applications) no longer allowed to contain live microorganisms¹
 - Most commercial products do not contain *L. crispatus*
 - Often fecal strains being repurposed
 - The only medicine with a *L. crispatus* strain (CTV-05) is still under development and contains only a single strain².

Variable	Lactin-V (N = 152) no. (%)	Placebo (N = 76) no. (%)	Risk Ratio (95% CI)*	P Value
Status of recurrence by wk 12				
Recurrence†	46 (30)	34 (45)	0.66 (0.44–0.87)	0.01
No recurrence	87 (57)	30 (39)		
Unknown	19 (12)	12 (16)		
Status of recurrence by wk 24				
Recurrence	59 (39)	41 (54)	0.73 (0.54–0.92)	
No recurrence	63 (41)	21 (28)		
Unknown	30 (20)	14 (18)		

	Pre-clinical	Phase I	Phase II	Phase III
LACTIN-V				
	Reduce the rate of recurrence of bacterial vaginosis			
	Reduce pre-term birth in women at high risk of PTB			
	Enhance success rates of <i>In vitro</i> fertilization			

6. This Regulation does not apply to:

- (h) products, other than those referred to in points (d), (f) and (g), that contain or consist of viable biological material or viable organisms, including living micro-organisms, bacteria, fungi or viruses in order to achieve or support the intended purpose of the product;

1. European Union. Official Journal of the European union L 117, (2017)

2. Cohen, C. R. et al. N Engl J Med 382, 1906–1915 (2020)

Vaginal suppositories

- Vaginal suppositories:
 - Contains three active strains:
 - *Lactobacillus crispatus* SP28 (2.5×10^9 cfu/ovule)
 - *Lactobacillus crispatus* LCR01 (2.5×10^9 cfu/ovule)
 - *Lactobacillus acidophilus* La02 (2.5×10^9 cfu/ovule)
 - Matrix from cacao butter with coconut butter.
- Available as 30 vaginal suppositories for EUR 88,-
- Includes leaflet with clear instructions



Vaginal suppositories

Constipation	3	Does the patient experience constipation? 0 = no; 1 = yes, may skip a day; 2 = yes, defecation every 2-3 days; 3 = yes, defecation every 4-5 days; 4 = yes, defecation every 6 days or longer;
Diarrhea	0	How often does the patient experience diarrhea? 0 = never; 1 = once every two weeks; 2 = once every week; 3 = two-three times per week; 4 = four-six times per week; 5 = daily.
Bloating / flatulence	0	How often does the patient experience bloating/flatulence? 0 = never; 1 = once every two weeks; 2 = once every week; 3 = two-three times per week; 4 = four-six times per week; 5 = daily.
Abdominal pain (gut related)	0	How often does the patient have abdominal pain? 0 = never; 1 = a few times per week; 3 = daily, but not throughout the day; 4 = daily throughout the day.
Cognition	0	Does the patient experience cognitive problems? 0 = no; 1 = somewhat; 2 = moderate; 3 = severe.
Susceptibility to sad mood	5	Does the patient experience sad mood? 0 = never; 1 = sometimes; 2 = less than half of the time; 4 = most of the time; 5 = always.
Increased stress level	1	Does the patient experience an increased level of stress? 0 = never; 1 = sometimes; 2 = less than half of the time; 3 = more than half of the time; 4 = most of the time; 5 = always.
Anxiety	0	Does the patient experience an increased level of anxiety? 0 = never; 1 = sometimes; 2 = less than half of the time; 3 = more than half of the time; 4 = most of the time; 5 = always.
Fatigue	4	How severe was the fatigue/exhaustion during the last six months? 0 = none; 1 = mild; 2 = moderate; 3 = severe; 4 = very severe.
Bacterial vaginosis (smelling gray-white discharge, itching, burning)	4	Does the patient have complaints that are indicative for bacterial vaginosis (smelling gray-white discharge, itching, burning)? 0 = N/A, never; 1 = sometimes; 2 = regularly; 3 = often; 4 = all the time.
Osteoporosis	0	Does the patient have osteoporosis? 0 = no, 1 = yes.
Autoimmune diseases	0	Does the patient have an autoimmune disease? 0 = no, 1 = yes.
Urinary tract infections	4	How often does the patient have urinary tract infections? 0 = never; 1 = once every two months; 2 = once every month; 3 = once every two weeks; 4 = monthly; 5 = almost continuously.
Kidney stones	0	Does the patient have had calcareous kidney stones? 0 = no, 1 = yes.
Lactose intolerance	0	Is the patient lactose intolerant? 0 = no; 1 = yes.
Allergic conditions	0	How much does the patient suffer from allergic conditions? 0 = none (negative); 1 = minor complaints (weak positive); 2 = moderate; 3 = severe.

Ok

Vaginal suppositories

✓

Complaints

✓

Medical background

✓

Laboratory results

4

Show suggestion

Treatment Suggestion

☐ Standard Product

☒ Magistral product

☐ Magistral mixture (MyOwnBlend)

Possible Magistral products

☒ Vaginal suppositories (30 pcs)

i

Based on these patient-scores

- Bacterial vaginosis (smelling gray-white discharge, itching, burning) 📄 🔥
- Urinary tract infections 📄 🔥

Ok, Create Prescription

Vaginal suppositories

Anamnesis-aid & Advice-aid

Analyses

New

Search Existing

Search

Clear

Prescription

New

Search Existing

Search

Clear

Client *

Dennis Zeilstra (23/01/1979)

☐ Standard Product

☒ Magistral product

☐ Magistral mixture (MyOwnBlend)

Ingredients *

Vaginal superpositories (30 pcs) 1 Stuks € 88.00

How To Use

Total pieces

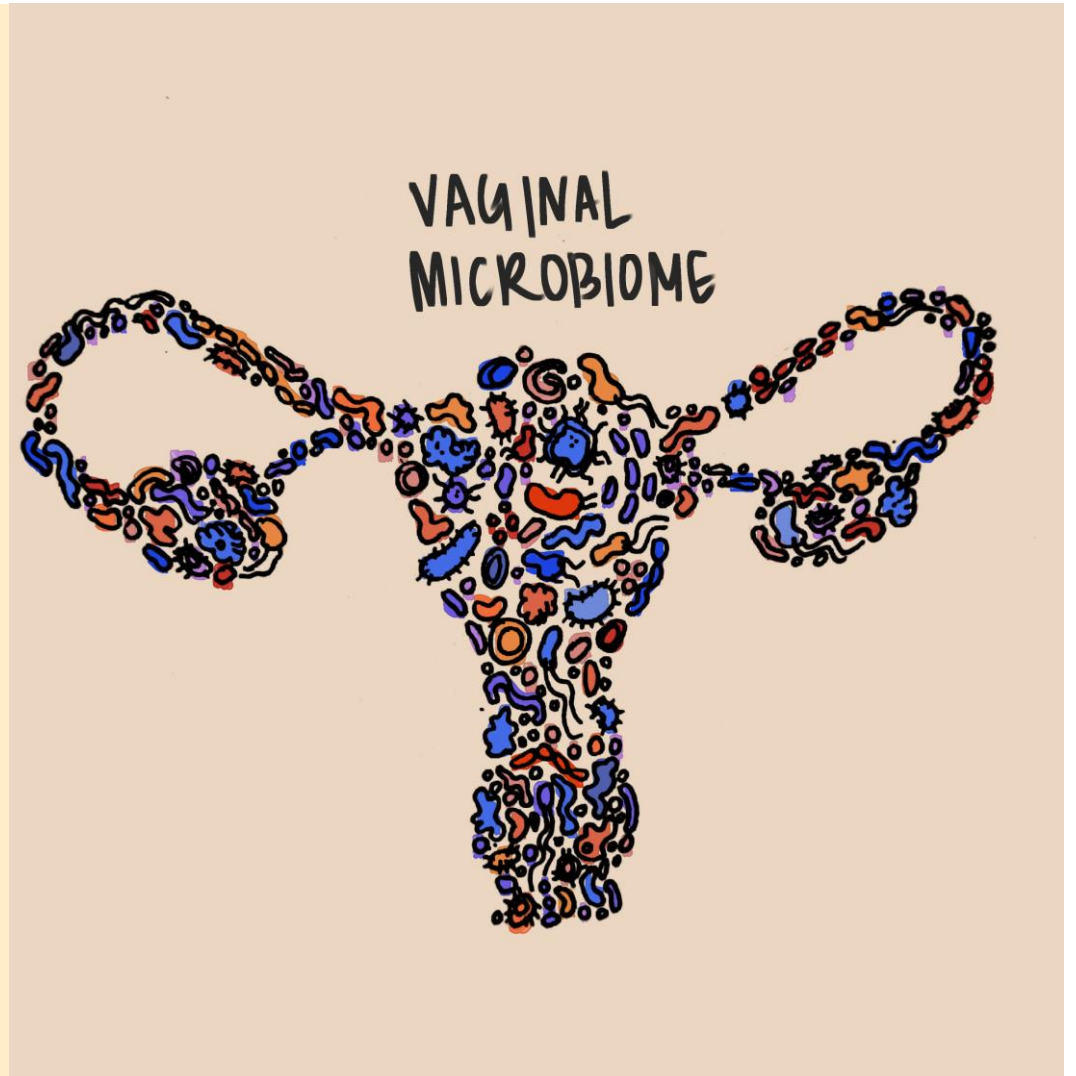
1.00

Product	pieces	Amount
Vaginal superpositories (30 pcs)	1	88

Conclusions and outlook

Conclusions

- Vaginal complaints are very prevalent
- A healthy vaginal microbiome:
 - Is dominated by *Lactobacillus* species
 - Has pH below 4,5
 - Has very low diversity
- Four dominating *Lactobacillus* species are found, with *L. crispatus* most consistently being associated with good health.
- Bacterial vaginosis, vulvovaginal candidiasis, and infertility are characterized by high diversity and elevated pH.
- Probiotics can be effective against BV, VVC and UTIs, depending on strains and route of administration.
- New vaginal suppositories fill gap in market due to regulatory changes, lack of products with *L. crispatus*, and slow developments of pharmaceutical products.
- Use shows good effects in most women, minor side effects (primarily inconvenience).



1. <https://sqonline.ucsd.edu/2021/02/what-i-wish-i-learned-in-sex-ed/>

Advice Aid: continuously improved & fast



Automatic processing of Biovis results

The screenshot shows the 'Laboratory results' tab selected. Below the tab, there are two buttons: 'Show available Biovis results' (highlighted with a red circle) and 'Log in to Biovis for (more) results'. Below these buttons, there is a section for 'Diversity' with a dropdown menu set to '0' and a description: 'The value of the diversity in the lab report.'

The screenshot shows the 'Laboratory results' tab with several dropdown menus and buttons. The 'Hide available Biovis results' button is highlighted with a red circle. Below the buttons, there is a section for 'Diversity' with a dropdown menu set to '6' and a description: 'The value of the diversity in the lab report.' Other dropdown menus include 'Dysbiosis index' (set to '0'), 'Low levels of butyrate' (set to '1'), '(Potential) pathogenic bacteria overgrowth' (set to '0'), and 'Oxalate degrading capacity' (set to '0').

Option to limit non-probiotics

The screenshot shows the 'Limit non-probiotics' dropdown menu. The dropdown is open, showing options from 0 to 4. Option '3' is highlighted with a blue bar. The description for this option is: 'Limit the amount of non-probiotic ingredients such as prebiotics or glutamine. This can be useful if the patient reacts strongly to e.g. fibers. 0 = no restriction (typically maximum 7g/day in total of non-probiotic ingredients); 1 = limit to 5g/day; 2 = limit to 3g/day; 3 = limit to 1g/day; 4 = entirely exclude.'

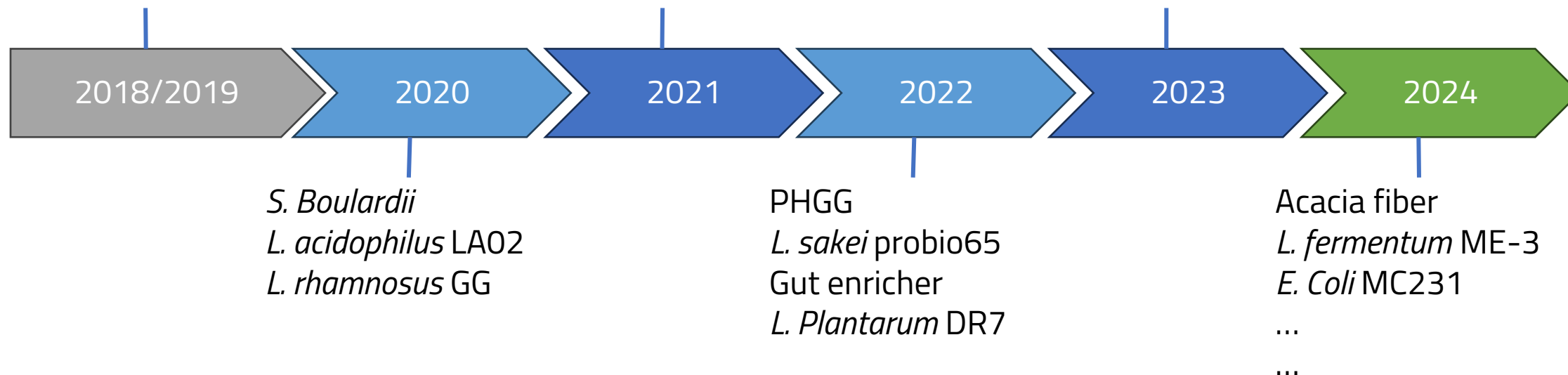
The screenshot shows a list of probiotics and their associated health conditions. The 'PHGG' probiotic is highlighted with a red circle. The list includes: 'S. Boulardii', 'L. rhamnosus GG', 'PHGG', and 'Lactiplantibacillus plantarum DR7'. Each probiotic is associated with a list of health conditions, such as '(Potential) pathogenic bacteria overgrowth', 'Intestinal permeability', 'Inflammation', 'Constipation', 'Sad mood', 'General diarrhea', and 'Stress'.

Ingredients are continuously expanded

Barrier
Bifido booster
DJ repair
IL-10 Immune balancer
TH1/TH2 Immune balancer
Pathogene reduction
Yeast reduction
Vitamin K2 booster
Butyrate generator
Microbiota booster

Bacillus clausii UBBC-07
Bacillus coagulans Unique IS-2
Enterococcus faecium Rosell-26 +
Bacillus subtilis Rosell-179
2'-Fucosyllactose
L. plantarum P8

L. rhamnosus SP1
Akkermansia
Bifidobacterium lactis HN019
Vaginal suppositories



New ingredient

Added acacia fiber

Acacia fiber



Product information:

This prebiotic is derived from the gum of Acacia Senegal trees and consists of over 90% fibers (also known as gum arabic). Research on acacia fibers has been conducted for decades. In MyOwnBlend, a realistically achievable dosage of approximately 5g/day is assumed, but in most clinical studies, a daily dosage of 25g/day or higher has been used. Therefore, the evidence score for many indications is somewhat lower. With this caveat, there is reasonable evidence from an RCT for bloating (1). There is also evidence from several clinical, non-blinded studies for a beneficial effect on constipation (2-4, 18). One of these studies shows a trend of improvement in IBS-C, which can be seen as some degree of evidence for IBS (18). A number of RCTs show an effect on metabolic dysfunction/insulin resistance (including 5-7). Additionally, there is some evidence for an effect in ulcerative colitis (8), inconsistent evidence for an effect on diarrhea (9-11), and some evidence for an effect on periodontitis (12). Furthermore, there is some clinical and in vitro evidence that acacia fibers can increase butyrate production (13, 14). Finally, various open-label studies demonstrate anti-inflammatory effects (including 15-17).

Active components: Organic Acacia Senegal Fiber

Ingredients: Organic Acacia Senegal Fiber

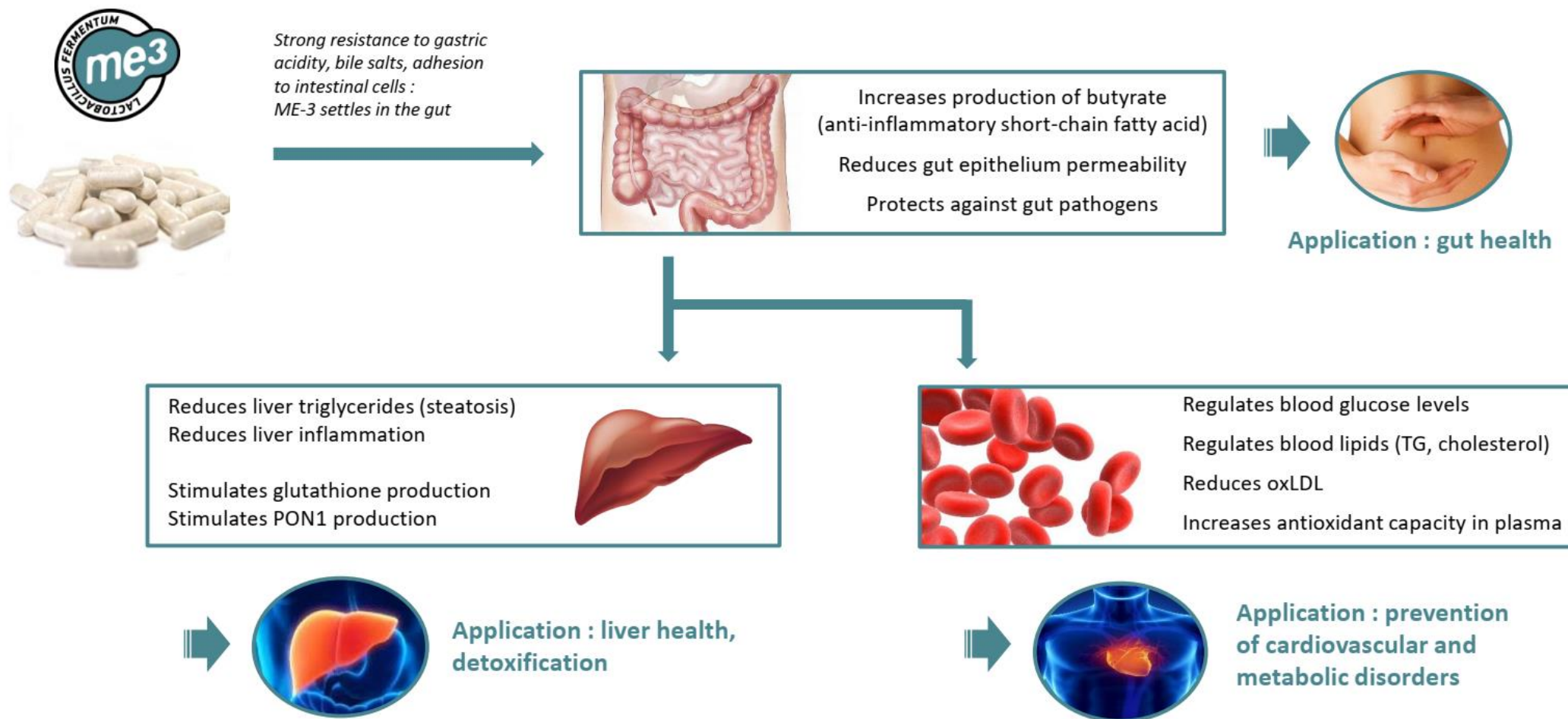
Min:3 g/d, **Max:**20 g/d

References:

(1) The Effect of Gum Arabic (Acacia senegal) on Cardiovascular Risk Factors and Gastrointestinal Symptoms in Adults at Risk of Metabolic Syndrome: A Randomized Clinical

New ingredient: launched this week

In the process of adding *L. fermentum* ME-3



Calendar



Thematic webinars

- Tuesday September 10th : SIBO part 1 with Frau Anja Pietzsch (DE)
- Monday September 23rd : SIBO part 2 (NL)
- Thursday September 26th : SIBO part 2 (ENG)
- Wednesday October 23rd : Depression (NL)
- Monday October 28th : Depression (ENG)

Thank you for your attention!

For more information, contact:



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