

Impact of periodic presumptive treatment for bacterial vaginosis on the vaginal microbiome among women participating in the Preventing Vaginal Infections trial

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Background

- Bacterial vaginosis (BV) is the most common vaginal infection globally, affecting hundreds of millions of women each year
- BV frequently recurs following successful treatment and is associated with HIV-1 and STI acquisition, and adverse reproductive health outcomes¹
- Emerging evidence suggests specific vaginal bacteria that are associated with BV may increase risk of adverse health outcomes in women²
- Innovative treatment and prevention approaches are needed to reduce the burden of disease caused by BV and its associated complications
 - Critical to assess the effect of such interventions on detection and quantity of key vaginal bacterial species

¹Brotman *et al.* *JID* (2010); Allsworth *et al.* *AJOG* (2011); Martin *et al.* *JID* (1999); Wiesenfeld *et al.* *CID* (2003) ; Balkus *et al.* *STD* (2014); Atashili *et al.* *AIDS* (2008)

²McClelland *et al.* *International AIDS Meeting* (2016); Nelson *et al.* *Matern Child Health J* (2015); Gorgos *et al.* *STD* (2015)

Preventing Vaginal Infections (PVI) trial

Randomized Trial of Periodic Presumptive Treatment With High-Dose Intravaginal Metronidazole and Miconazole to Prevent Vaginal Infections in HIV-negative Women

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Table 2. Number and Proportion of Visits With Vaginal Infections by Study Arm in the Intent-to-treat Population

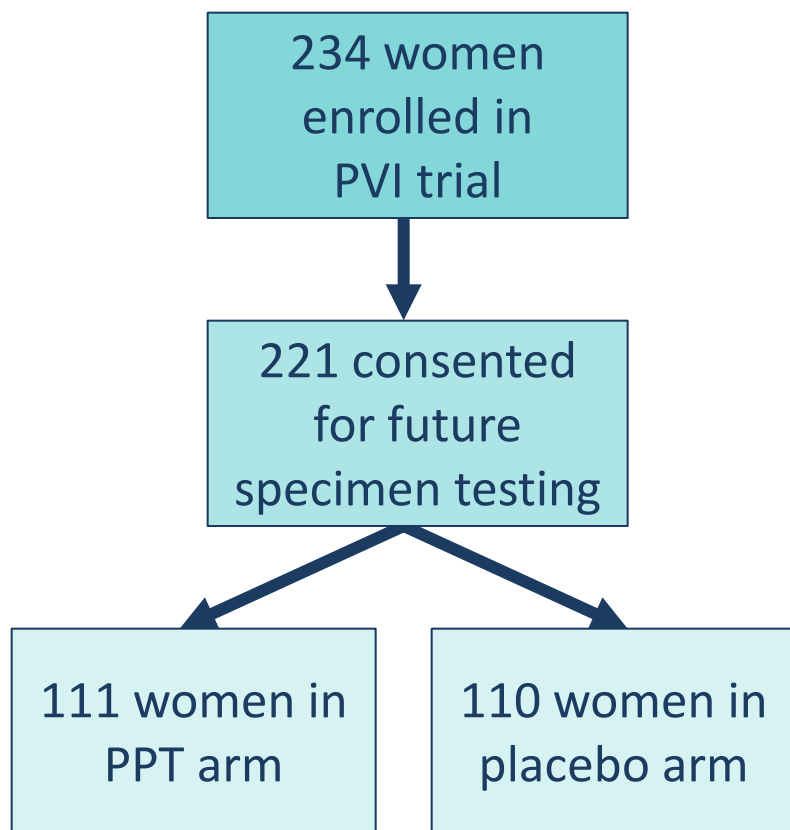
Outcome	Intervention Arm ^a 645 Visits Number (%)	Placebo Arm ^b 665 Visits Number (%)	Relative Risk Intervention/ Placebo (95% CI)	P Value ^c
Bacterial vaginosis (Nugent)	137 (21.2)	216 (32.5)	0.65 (.48, .87)	.005
Vulvovaginal candidiasis	67 (10.4)	75 (11.3)	0.92 (.62, 1.37)	.7
<i>Trichomonas vaginalis</i> ^d	35 (5.4)	57 (8.6)	0.63 (.30, 1.32)	.3
Any vaginal infection ^e	210 (32.6)	309 (46.5)	0.70 (.57, .86)	.001
Bacterial vaginosis (Amsel)	93 (14.4)	121 (18.2)	0.81 (.55, 1.19)	.2
Abnormal vaginal microbiota ^d	227 (35.2)	325 (48.9)	0.72 (.58, .90)	.004



Objective

To determine the impact of the PVI intervention on detection of bacteria associated with the presence or absence of vaginal health using highly sensitive species-specific quantitative PCR assays

PVI trial design & analysis population

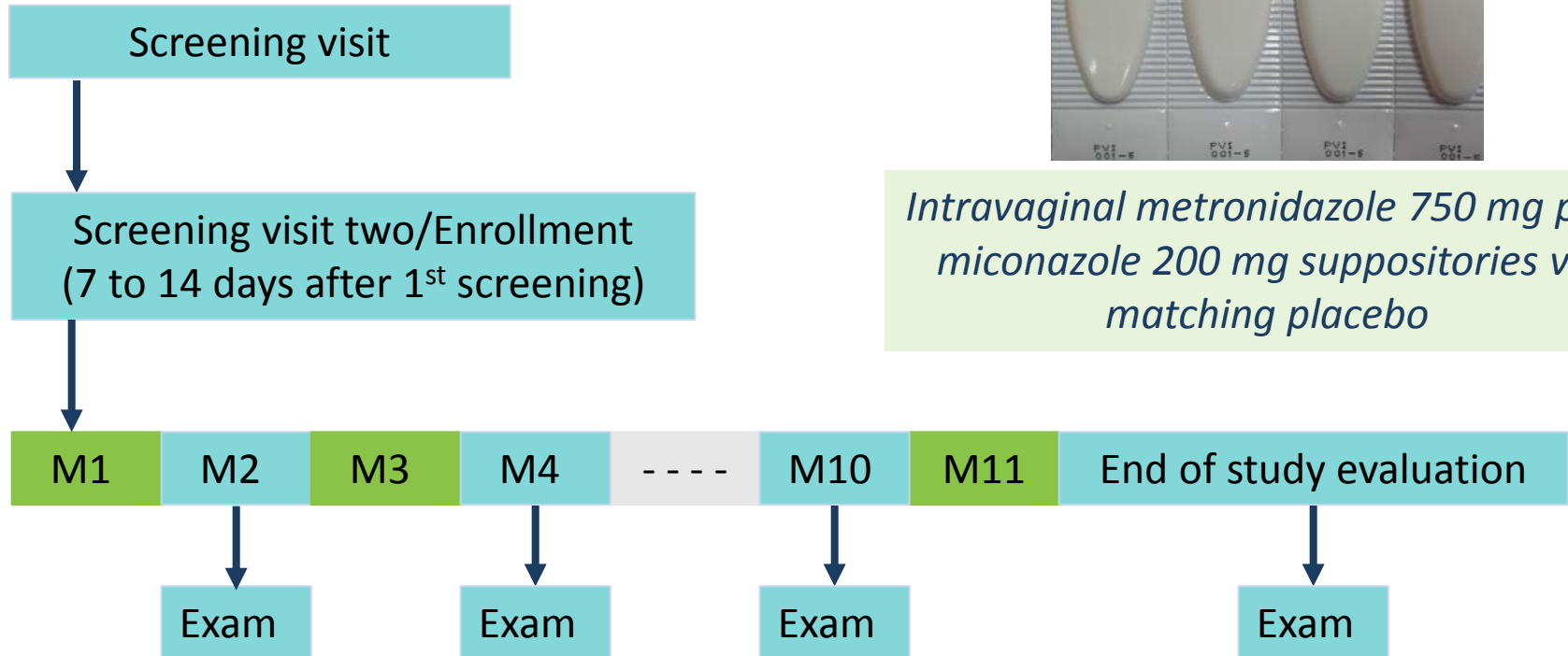


- HIV-negative, non-pregnant women enrolled at 4 sites between 2011-2012:
 - Nairobi, Kenya (two sites)
 - Mombasa, Kenya
 - Birmingham, USA
- Eligible participants had a vaginal infection detected at screening:
 - Bacterial vaginosis
 - Vulvovaginal candidiasis
 - *Trichomonas vaginalis*

PVI study schedule

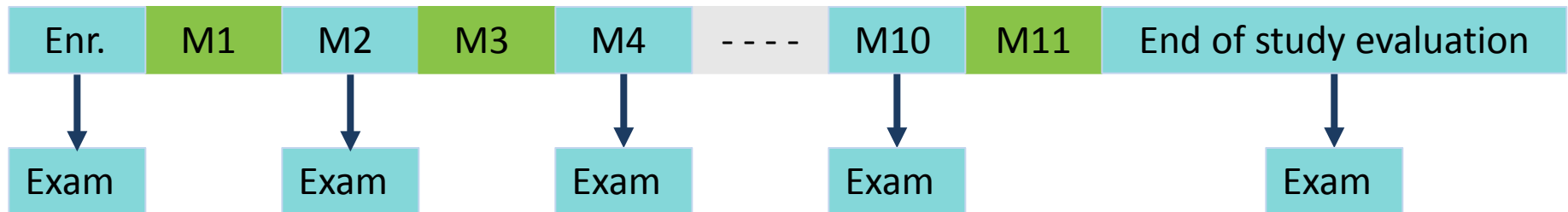


Intravaginal metronidazole 750 mg plus miconazole 200 mg suppositories vs. matching placebo



- Study product dispensed at monthly treatment visits
- Women with self-reported vaginal discharge or odor received open label treatment with oral metronidazole and fluconazole plus study product

Assessment of vaginal bacteria using qPCR



- Vaginal fluid collected using Dacron swabs and stored at -80C
- A MoBio BiOstic Bacteremia DNA Isolation kit was used to extract and purify DNA
- Species-specific quantitative PCR assays targeting the 16S rRNA gene were performed for:

<i>Lactobacillus crispatus</i>	BVAB1	<i>Atopobium vaginae</i>
<i>Lactobacillus jensenii</i>	BVAB2	<i>Leptotrichia/Sneathia</i>
<i>Lactobacillus iners</i>	<i>Mageeibacillus indolicus</i> (BVAB3)	<i>Megasphaera</i> species
		<i>Gardnerella vaginalis</i>

Analytic methods

- **Primary analysis:**

- For each bacterium, the proportion of follow-up visits with detectable quantities (i.e. greater than the lower limit of detection [LLD]) was calculated by arm
 - 94 copies per swab ($\log_{10} 1.97$)
- Relative risks [RR] were generated using generalized estimating equations with a log link and exchangeable correlation structure to separately assess the effect of the intervention on species detection
- Analyses were repeated using a species-specific bacterial concentration that was predictive of BV by Nugent score (ROC cut-off)
 - Cut-off range = $\log_{10} 2.15$ to 8.84

- **Secondary analysis:**

- Evidence suggests that the vaginal microbiota may differ by geographic region
- Repeated primary analyses for each country separately in order to assess potential differences in intervention effect

Enrollment characteristics by study arm and country

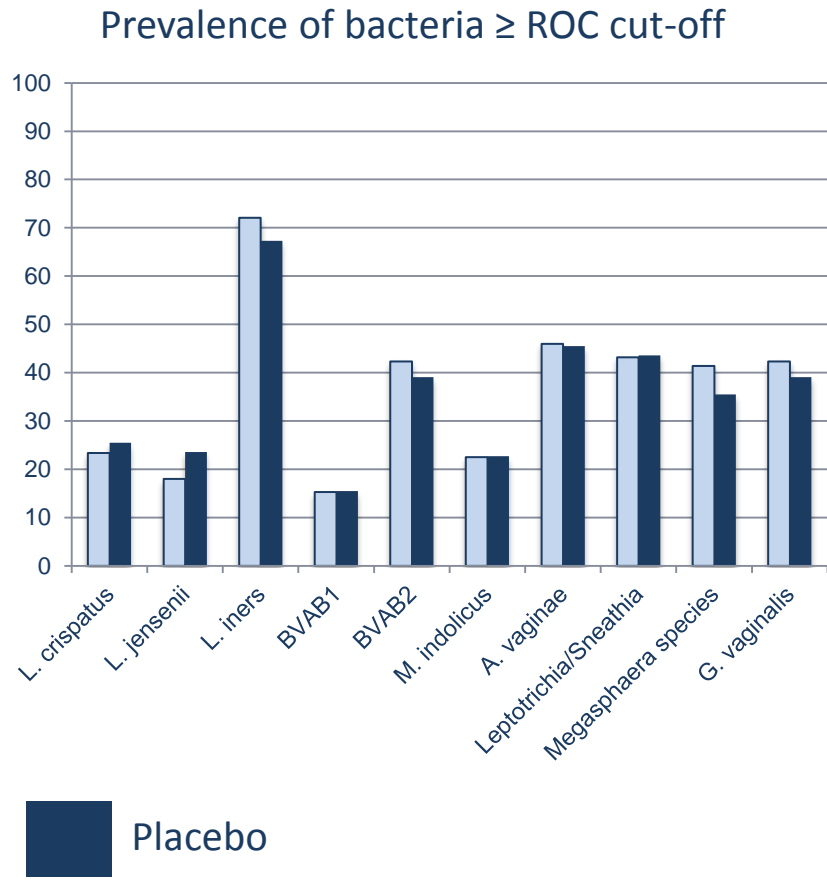
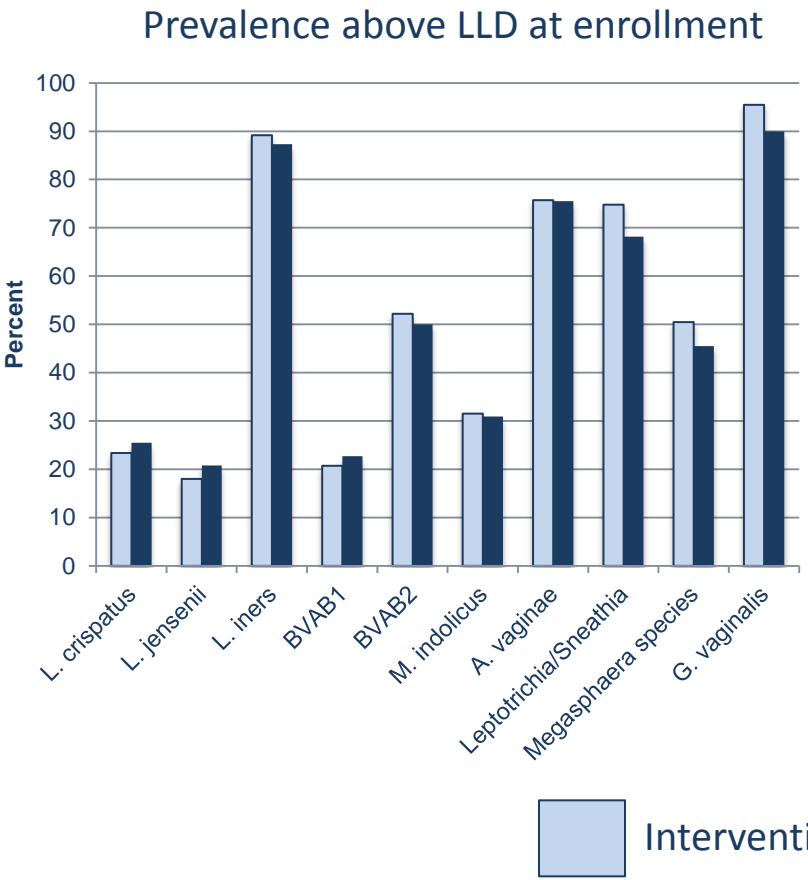
	Placebo N=110	Intervention N=111	p- value ¹	US N=53	Kenya N=168	p- value ¹
Age (years)	29 (23-34)	29 (24-34)	0.47	30 (23-36)	29 (24-34)	0.99
Education (years)	11 (8-12)	10 (8-13)	0.61	13 (12-15)	9 (8-12)	<0.001
Black race ²	106 (96)	111 (100)	0.06	49 (92)	168 (100)	0.003
Number of live births	2 (1-3)	2 (1-3)	0.97	1 (0-2)	2 (1-3)	<0.001
Vaginal washing in the past month	55 (50)	56 (50)	0.95	20 (38)	91 (54)	0.04
Ever had sex in exchange for goods/money/services ³	60 (55)	59 (53)	0.84	1 (2)	118 (70)	<0.001
Sexual behaviors in the past week						
# of partners	1 (1-2)	1 (1-2)	0.25	1 (1-1)	1 (1-3)	<0.001
New partner	23 (21)	22 (20)	0.84	1 (2)	44 (26)	<0.001
History of anal sex	13 (12)	12 (11)	0.84	21 (40)	4 (2)	<0.001
Nugent score						
BV (7-10)	40 (36)	41 (37)	0.76	25 (47)	56 (33)	0.02
Intermediate (4-6)	20 (18)	24 (22)		4 (8)	40 (24)	
Normal (0-3)	50 (45)	46 (41)		24 (45)	74 (43)	

¹Data presented as N(%) or median(interquartile range). Mantel-Haenzel test stratified by site Chi-squared test or Fisher's exact test used for categorical factors and Wilcoxon rank sum test used for continuous factors.

²All participants enrolled in Kenya reported Black race. Of those enrolled at the US site 49 of 53 women reported identifying as Black or African-American race.

³Two of the sites in Kenya recruited participants from longitudinal cohort studies following women who reported a history of engaging in transactional sex.

Prevalence of species at enrollment – by arm



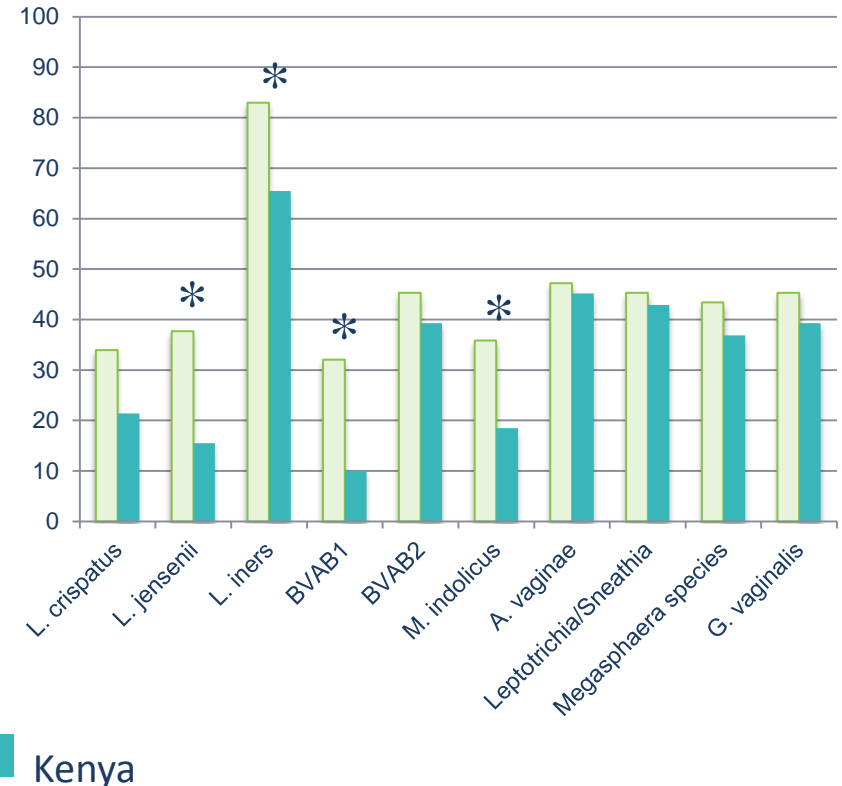
Similar frequency of bacteria detection at both cut-offs by study arm at enrollment

Prevalence of species at enrollment – by country

Prevalence above LLD at enrollment

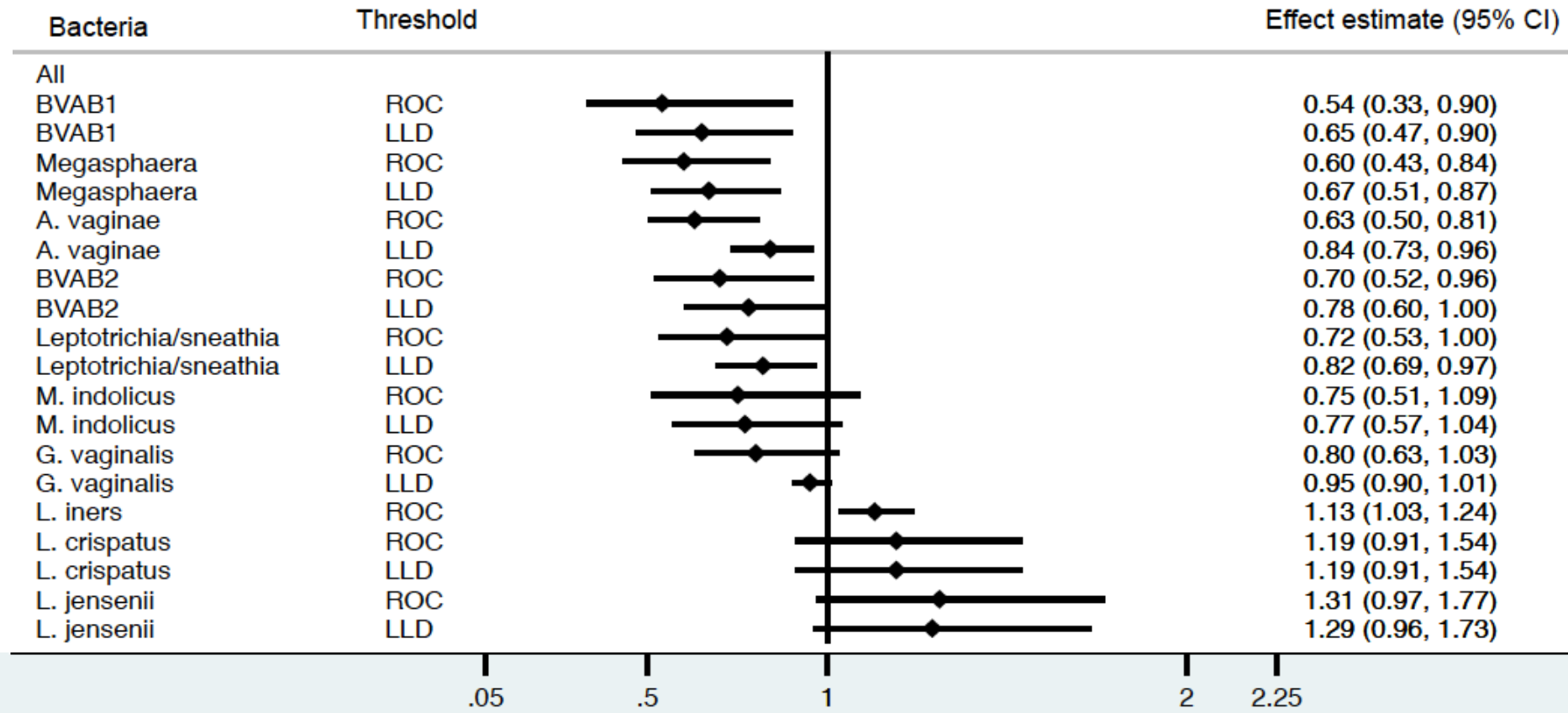


Prevalence of bacteria \geq ROC cut-off



**At both cut-offs, L. jensenii, L. iners, BVAB1, and M. indolicus detected more often in US women than Kenyan women*

Effect of the PVI intervention on detection of select bacterial species – all participants



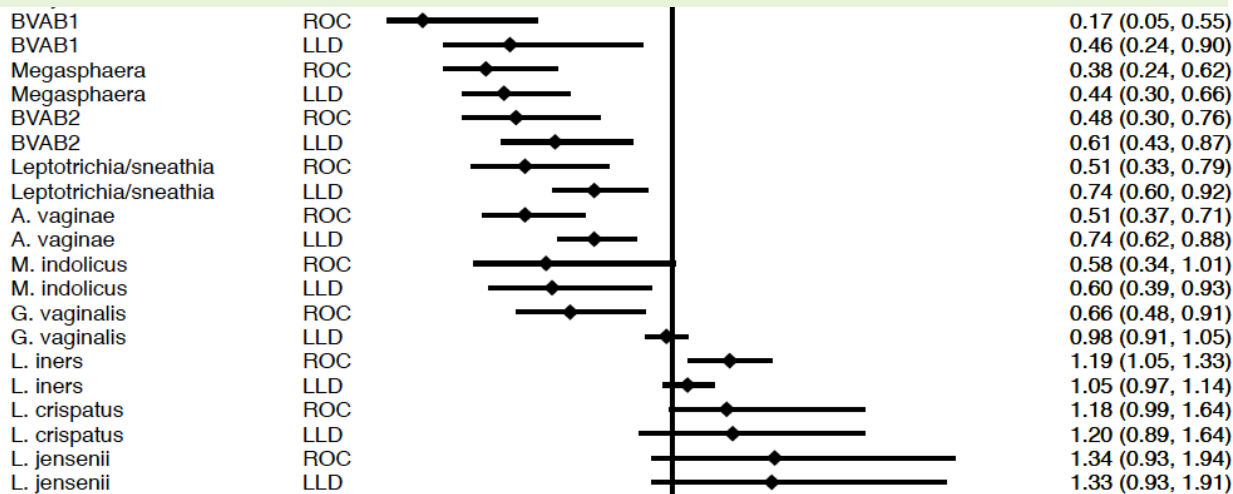
LLD = bacterial quantity above the lower limit of detection

ROC = bacterial quantity \geq the ROC cut-point

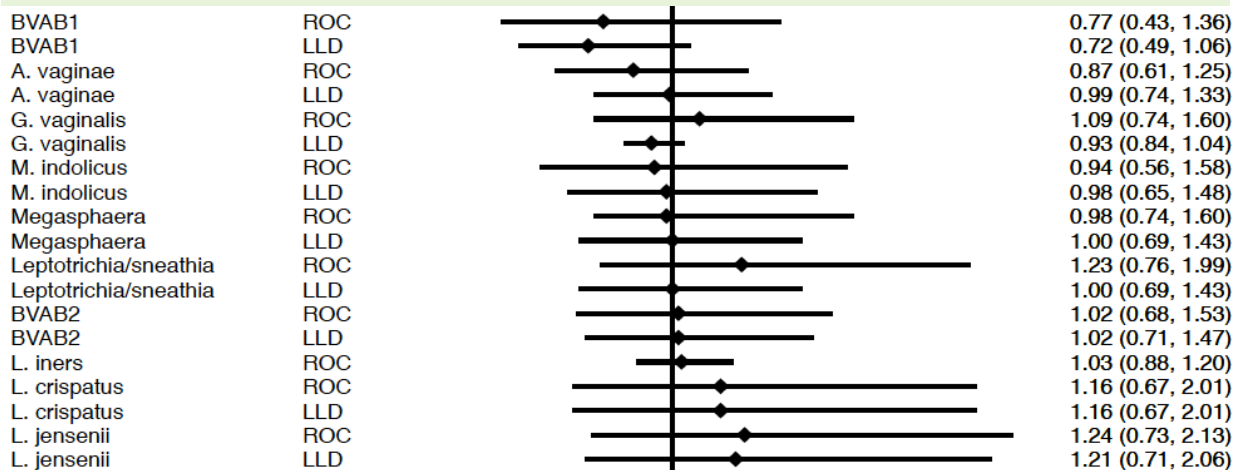
Effect estimate = Relative risks [RR] generated using generalized estimating equations with a log link and exchangeable correlation structure and adjusted for site

Effect of the PVI intervention on detection of select bacterial species – by country

Kenyan participants



US participants



Self-reported use of all 5 doses of study product at each visit where product was dispensed:

99% of Kenyan participants

88% of US participants (p<0.001)

0.05 0.5 1 2 2.25

Key findings (1)

- Women in the PPT arm were less likely to have BVAB1, BVAB2, *A. vaginae*, *Leptotrichia/Sneathia*, and *Megasphaera* species detected over the course of the trial (> LLD)
- Similarly, women in the PPT arm were less likely to have BVAB1, BVAB2, *A. vaginae*, *Leptotrichia/Sneathia*, and *Megasphaera* species detected at high concentrations
- Reductions observed species associated with:
 - Recurrent BV - BVAB2, *A. vaginae*, *Leptotrichia/Sneathia*, and *Megasphaera* species
 - HIV acquisition - *Leptotrichia/Sneathia*, *Megasphaera* species

Key findings (2)

- Broad-range vaginal microbiome results are pending → Will allow for further assessment of the intervention impact on detection of other bacteria potentially associated with HIV acquisition
 - *Prevotella bivia*, *Gemella asaccharolytica*
- Differences in bacteria prevalence by country were noted at enrollment, highlighting the importance of geographic location in assessments of the vaginal microbiome

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Clinical characteristics at enrollment

	Placebo N=110		Intervention N=111		p- value ¹	US N=53		Kenya N=168		p- value ¹
Other infections										
Gonorrhea	0	(0)	3	(3)	0.35	0	(0)	3	(3)	1.00
Chlamydia	8	(7)	8	(7)	0.99	2	(4)	14	(8)	0.37
HSV-2 ¹	68	(62)	71	(64)	0.74	29	(55)	110	(65)	0.16
<i>Trichomonas vaginalis</i> ²	6	(5)	10	(9)	0.33	3	(6)	13	(8)	0.77
Vulvovaginal candidiasis	24	(22)	28	(25)	0.55	15	(28)	37	(22)	0.35

¹Kenyan participants with an optical density (OD) value >2.1 were considered HSV-2 positive.

²One participant from Mombasa site missing baseline TV result. TV by NAAT.

Effect of PVI intervention on species detection (>LLD)

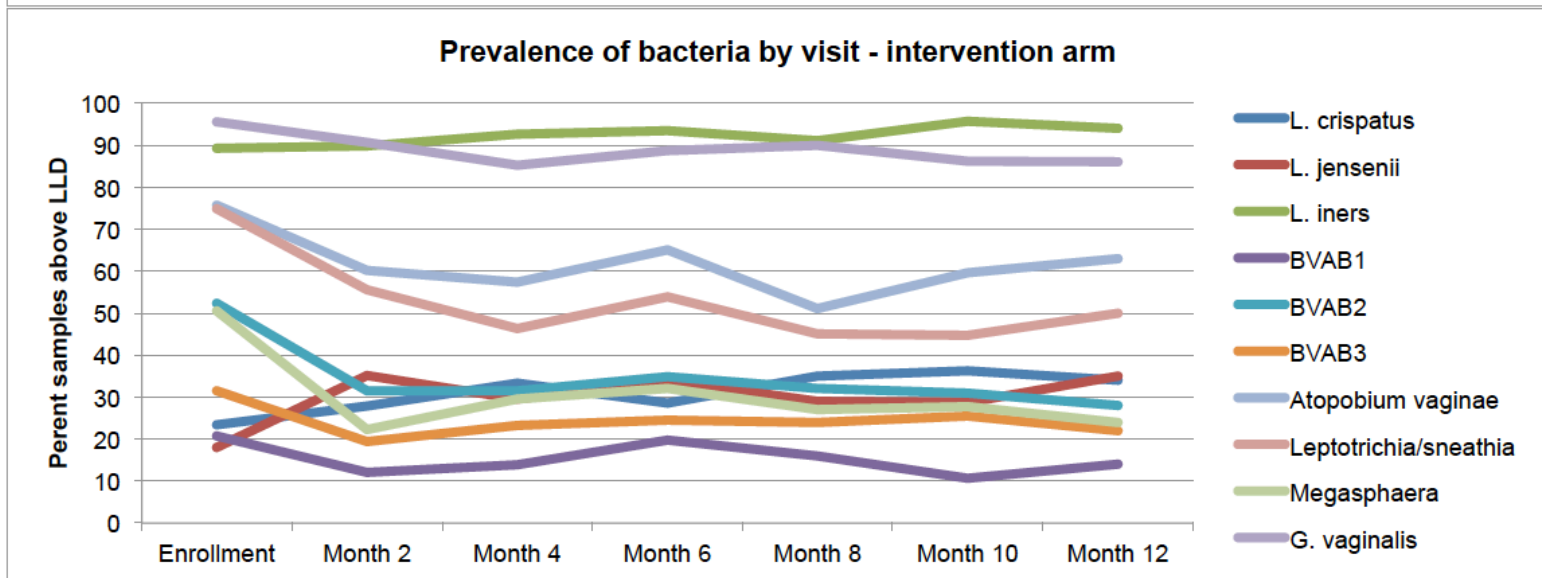
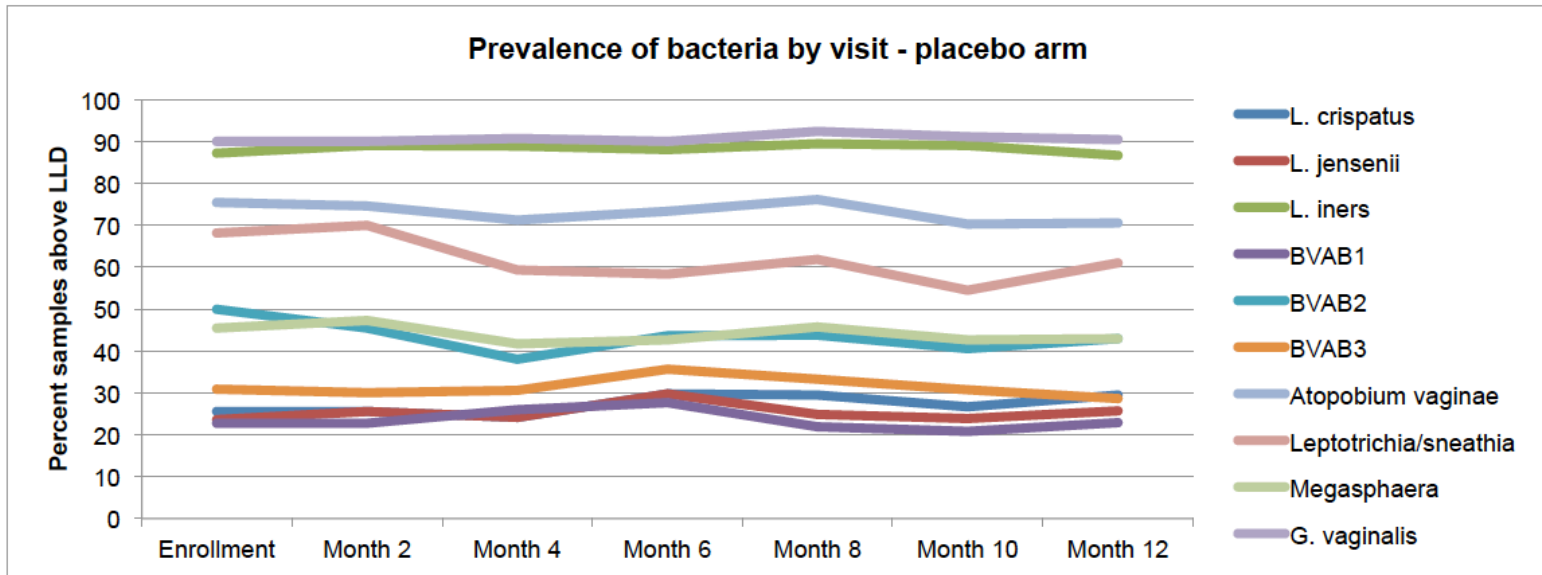
Bacteria	Placebo ¹ N=630		Intervention ¹ N=616		RR ²	(95% CI)	p-value	aRR ³	(95% CI)	p-value
BVAB1	149	(23.7)	89	(14.5)	0.63	(0.41, 0.96)	0.03	0.65	(0.47, 0.90)	0.009
<i>Megasphaera</i>	276	(43.8)	167	(27.1)	0.62	(0.46, 0.82)	0.001	0.67	(0.51, 0.87)	0.003
<i>A. vaginae</i>	458	(72.7)	366	(59.4)	0.81	(0.71, 0.93)	0.004	0.84	(0.73, 0.96)	0.009
BVAB2	267	(42.4)	194	(31.5)	0.74	(0.56, 0.97)	0.03	0.78	(0.60, 1.00)	0.05
<i>Leptotrichia/Sn.</i>	384	(61.0)	304	(49.4)	0.81	(0.67, 0.96)	0.02	0.82	(0.69, 0.97)	0.02
<i>M. indolicus</i>	198	(31.4)	142	(23.1)	0.74	(0.54, 1.02)	0.07	0.77	(0.57, 1.04)	0.09
<i>G. vaginalis</i>	572	(90.8)	541	(87.8)	0.96	(0.91, 1.03)	0.25	0.95	(0.90, 1.01)	0.08
<i>L. iners</i>	558	(88.6)	571	(92.7)	1.05	(0.98, 1.12)	0.15	--	--	--
<i>L. crispatus</i>	173	(27.5)	199	(32.3)	1.20	(0.91, 1.56)	0.19	1.19	(0.91, 1.54)	0.20
<i>L. jensenii</i>	161	(25.6)	197	(32.0)	1.30	(0.96, 1.76)	0.09	1.29	(0.96, 1.73)	0.09

Effect of PVI intervention on high concentrations of species (≥ROC cut-off)

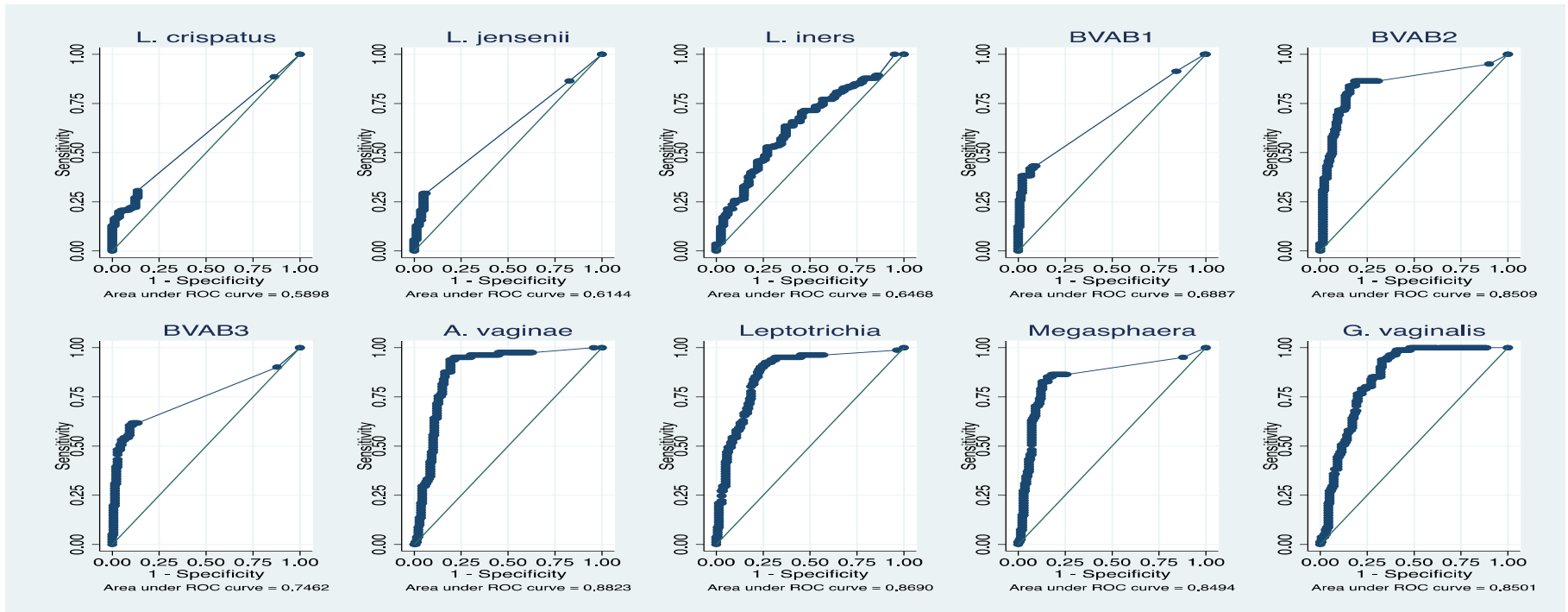
Bacteria	Placebo ¹ N=630		Intervention ¹ N=616		ROC ⁴ cut-off	RR ²	(95% CI)	p-value	aRR ³	(95% CI)	p-value
BVAB1	92	(14.6)	47	(7.6)	7.11	0.50	(0.28, 0.91)	0.02	0.54	(0.33, 0.90)	0.02
<i>Megasphaera</i>	205	(32.5)	115	(18.7)	6.44	0.57	(0.40, 0.81)	0.001	0.60	(0.43, 0.84)	0.003
<i>A. vaginae</i>	283	(44.9)	174	(28.3)	7.14	0.62	(0.48, 0.80)	<0.001	0.63	(0.50, 0.81)	<0.001
BVAB2	203	(32.2)	133	(21.6)	5.61	0.66	(0.48, 0.92)	0.02	0.70	(0.52, 0.96)	0.03
<i>Leptotrichia/Sn.</i>	174	(27.6)	123	(20.0)	7.21	0.70	(0.51, 0.97)	0.03	0.72	(0.53, 1.00)	0.05
<i>M. indolicus</i>	127	(20.2)	92	(14.9)	5.88	0.73	(0.49, 1.08)	0.12	0.75	(0.51, 1.09)	0.13
<i>G. vaginalis</i>	209	(33.2)	162	(26.3)	8.84	0.78	(0.61, 1.01)	0.06	0.80	(0.63, 1.03)	0.08
<i>L. iners</i>	438	(69.5)	489	(79.4)	7.17	1.14	(1.04, 1.26)	0.008	1.13	(1.03, 1.24)	0.01
<i>L. crispatus</i>	173	(27.5)	199	(32.3)	2.15	1.20	(0.91, 1.56)	0.19	1.19	(0.91, 1.54)	0.20
<i>L. jensenii</i>	157	(24.9)	194	(31.5)	2.30	1.32	(0.97, 1.79)	0.08	1.31	(0.97, 1.77)	0.08

¹Data presented are N (%). ²RR from generalized estimating equations with a log link and exchangeable correlation structure and clustered by participant. ³RR from generalized estimating equations with a log link and exchangeable correlation structure and clustered by participant, with adjustment for study site. ⁴Log-10 value at highest correctly classified concentration using ROC curves
Convergence not achieved for *L. iners* model with adjustment for site.

Bacteria >LLD by study visit



Bacterial quantity and BV by Nugent score at enrollment



Bacteria	Area under the ROC curve (95% CI)		Cut point at highest % correctly classified (\log_{10})	% correctly classified	Sensitivity	Specificity
<i>L. crispatus</i> ²	0.59	(0.53, 0.65)	2.15	51.3%	30.7%	86.4%
<i>L. jensenii</i> ²	0.61	(0.55, 0.67)	2.30	52.9%	29.3%	93.8%
<i>L. iners</i> ²	0.65	(0.57, 0.72)	7.17	64.7%	77.1%	43.2%
BVAB1	0.69	(0.62, 0.76)	7.11	76.0%	38.3%	97.9%
BVAB2	0.85	(0.79, 0.91)	5.61	84.2%	84.0%	84.3%
BVAB3	0.75	(0.67, 0.82)	5.88	79.6%	53.1%	95.0%
<i>Atopobium vaginae</i>	0.88	(0.83, 0.93)	7.14	85.5%	93.8%	80.7%
<i>Leptotrichia/sneathia</i>	0.87	(0.82, 0.92)	7.21	81.5%	84.0%	80.0%
<i>Megasphaera</i>	0.85	(0.79, 0.91)	6.44	85.5%	82.7%	87.1%
<i>G. vaginalis</i>	0.85	(0.80, 0.90)	8.84	78.7%	76.5%	80.0%