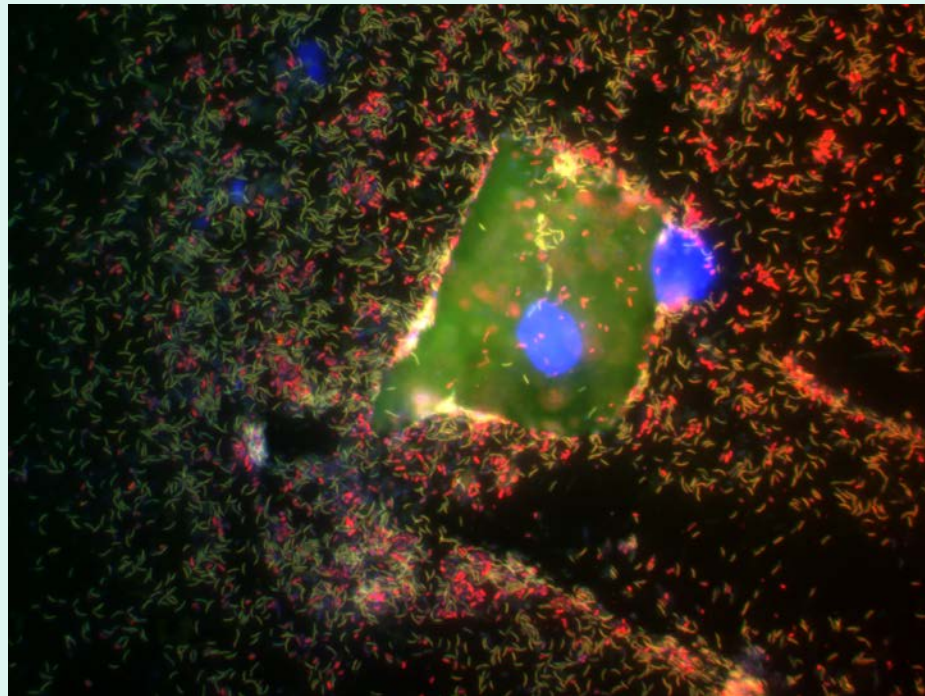


The Genital Microbiome in Studies of Bacterial Vaginosis



Jeanne Mrazzazzo, MD, MPH

University of Washington

August 2015

DISCUSSION

- Bacterial vaginosis (BV)
 - Clinical importance
 - Implications for STI/HIV transmission; reproductive health
 - Traditional guidance for treatment trials
 - Endpoint definition; FDA guidance
- Implications of microbiome approach for BV
 - Endpoint definition
 - Pathogenesis & natural history
 - Sexual behavior (BVAB transmission)

Benefits of an Optimal Vaginal Environment

Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

Maria G. Dominguez-Bello^{a,1,2}, Elizabeth K. Costello^{b,1,3}, Monica Contreras^c, Magda Magris^d, Glida Hidalgo^d, Noah Fierer^{e,f}, and Rob Knight^{b,9}

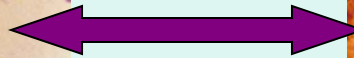
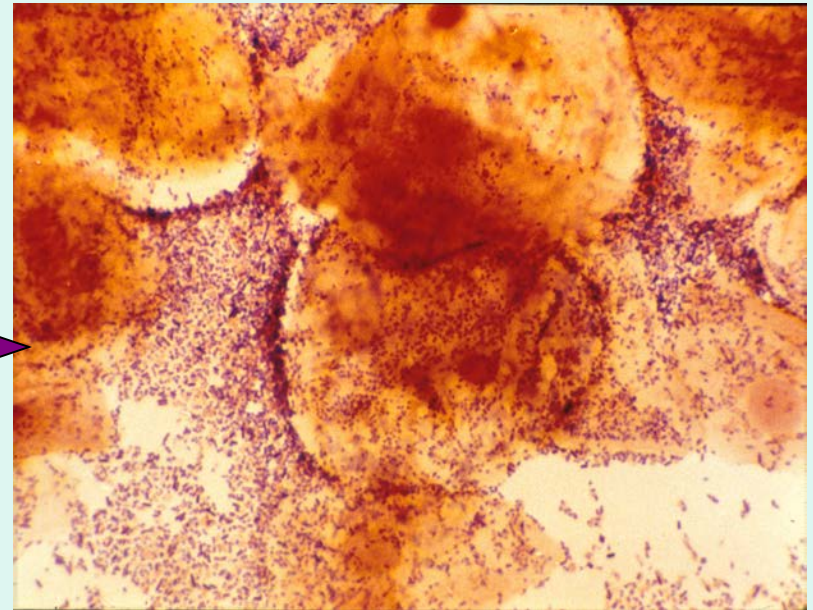
- Protection from clinical BV, PID, other pathogens
 - HIV (60% increase), chlamydia, GC, trichomoniasis
- Optimal birth outcomes (short-term)
 - Normal birth weight
 - Normal timing of delivery
 - Fewer pregnancy-associated infections
- Optimal health outcomes (long-term)
 - Transfer of maternal microbiota to infant
 - Lower rates of autoimmune diseases (asthma), metabolic disorders (Dominguez-Bello 2010; Torrazza 2011; Neu 2011)
 - Mediated by rapid colonization of skin, gut, genital tract with maternal microbiota

Gram Stain (Nugent score) & Clinical Criteria (Amsel's) Define BV & Cure

Nugent = 0



Nugent 7 -10



$\text{pH} < 4.7$

“Normal” discharge
No odor with KOH
No clue cells

**Amsel's
Criteria**

$\text{pH} \geq 4.7$

Homogenous discharge
Amine odor with KOH
Clue cells

Vaginal Fluid Gram Stain (Nugent Criteria)

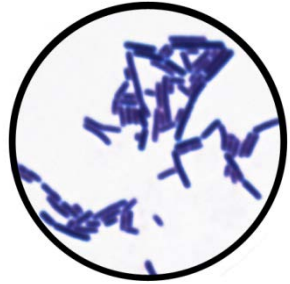
← BV Negative (0-6) → ← BV Positive (7-10) →

<p>Gram positive rods “<i>Lactobacillus</i> types”</p> <p>0-3</p>	<p>4-6</p>	<p>Gram negative or Gram variable rods or coccobacilli “<i>Gardnerella</i> or <i>Bacteroides</i> types” Curved Gram negative rods “<i>Mobiluncus</i> types” 7-10</p>
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No BV

Intermediate flora

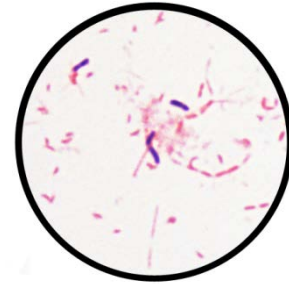
BV



Gram Positive

Advantages

- Simple sample collection
- Efficient storage and transport
- Standardized scale for interpretation



Gram Negative

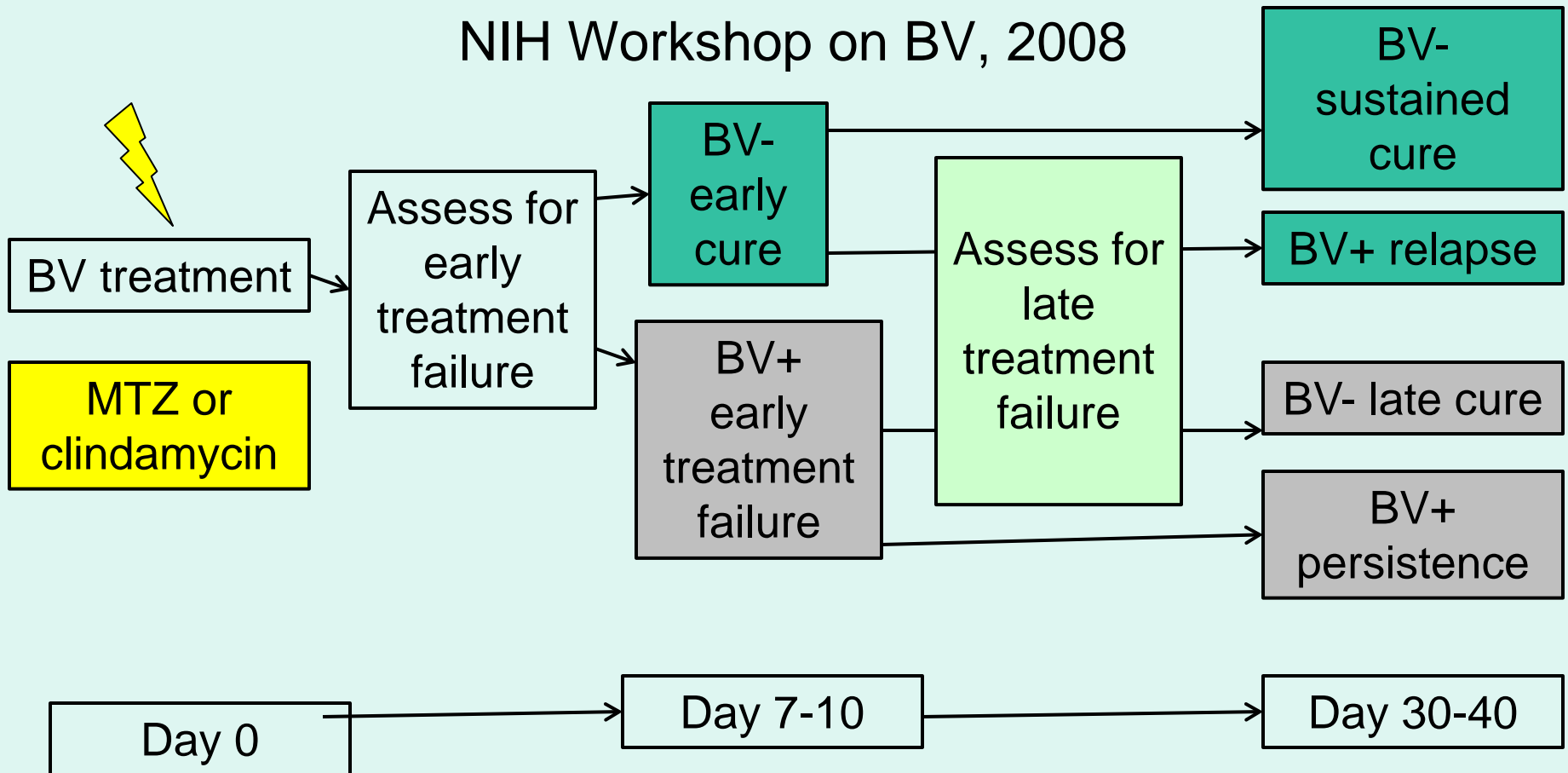
Widely Used Diagnostic Tool – Gold Standard for BV Diagnosis

Spiegel CA, Amsel R, Holmes KK. 1983. J Clin Microbiol 18:170-177

Nugent RP, Krohn MA, Hillier SL. 1991. J Clin Microbiol 29:297-301.

BV TREATMENT TRIALS: Proposed Timeline & Terminology

NIH Workshop on BV, 2008

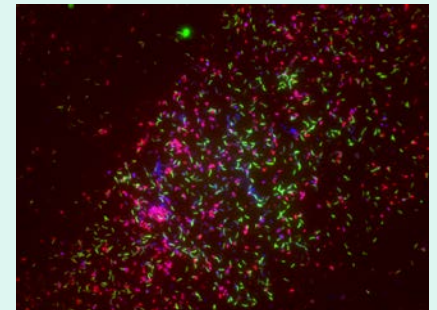


FDA Guidance for BV Trials

- BV definition at enrollment:
 - All 4 Amsel criteria plus confirmation by Nugent score (≥ 7)
 - NOTE: symptoms not required
- 1998: clinical cure = absence of all Amsel criteria; therapeutic cure = clinical cure plus Nugent score < 4 at 21-30 days after starting treatment
- On review in 2012, definition of clinical cure was modified to exclude Amsel's pH criteria

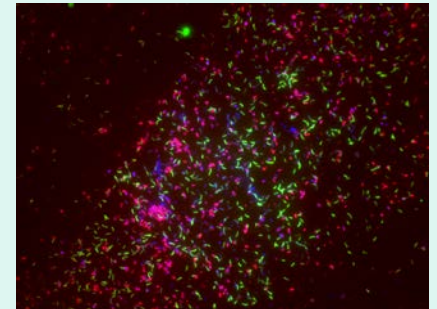
FDA Guidance for BV Trials

- BV definition at enrollment:
 - All 4 Amsel criteria plus confirmation by Nugent score (≥ 7)
 - NOTE: symptoms not required
- Clinical cure:
 - Absence of KOH odor, clue cells, abnormal discharge
 - Symptom resolution (additional; not well standardized)



Are These Relevant in the Era of Molecular Approaches?

- BV definition at enrollment:
 - All 4 Amsel criteria plus confirmation by Nugent score (≥ 7)
 - NOTE: symptoms not required
- Clinical cure:
 - Absence of KOH odor, clue cells, abnormal discharge
 - Symptom resolution (additional; not well standardized)



Key Questions

- Nugent Criteria: Do bacterial species other than those previously described (*Lactobacillus*, *Gardnerella*, *Bacteroides*, *Mobiluncus*) contribute to bacterial morphotypes observed by Gram stain?
- Amsel Criteria: Do all BV-associated bacteria contribute equally to these signs?
 - Quantity? Identity?

The Effects of Intravaginal Clindamycin and Metronidazole Therapy on Vaginal *Mobiluncus* Morphotypes in Patients With Bacterial Vaginosis

PAUL NYIRJESY, MD,* MATTHEW J. MCINTOSH, PhD,† JANA I. STEINMETZ, MS,†
ROBERT J. SCHUMACHER, PhD,† AND JAMES L. JOFFRION, BS, PMP†

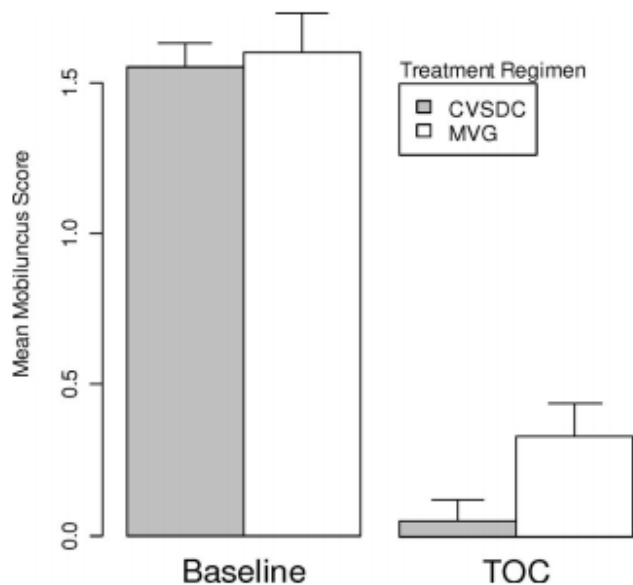
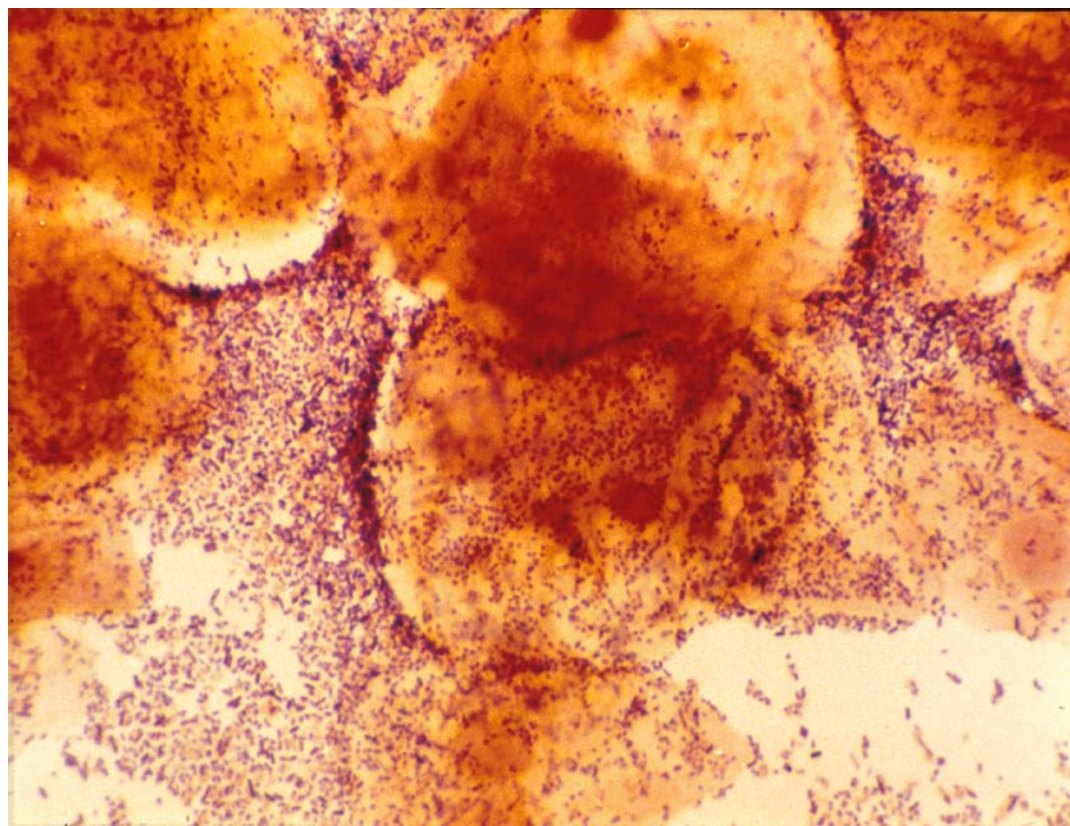


Fig. 1. Mean *Mobiluncus* scores (0–2) in each treatment group at the entry visit (baseline) and the test-of-cure (TOC) visit. 2 = more than 5 morphotypes observed per oil immersion field; 1 = <1 to 4 morphotypes observed; 0 = no morphotypes observed. Mean *Mobiluncus* scores at the TOC visit in the MVG group were significantly higher in the MVG group than in the CVSDC group ($P = 0.0471$). CVSDC indicates clindamycin vaginal single-dose cream; MVG = metronidazole vaginal gel.

“Although current data suggest that there are important differences between women diagnosed with BV with or without the presence of *Mobiluncus* morphotypes, it is currently unknown whether these variations can be directly attributed to *Mobiluncus* species or to some other unknown organisms for which these Gram stain findings are a marker.”

More Than Meets the Eye: Associations of Vaginal Bacteria with Gram Stain Morphotypes Using Molecular Phylogenetic Analysis

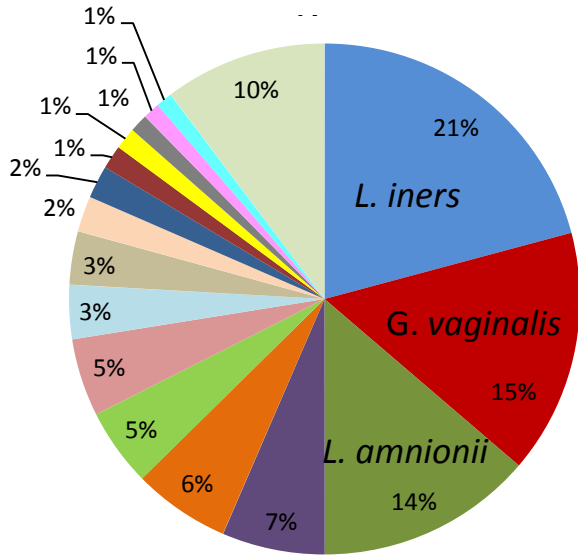
Sujatha Srinivasan^{1*}, Martin T. Morgan², Congzhou Liu¹, Frederick A. Matsen², Noah G. Hoffman³, Tina L. Fiedler¹, Kathy J. Agnew⁴, Jeanne M. Mrazo⁵, David N. Fredricks^{1,5,6*}



Women with BV

Broad-Range PCR and Pyrosequencing

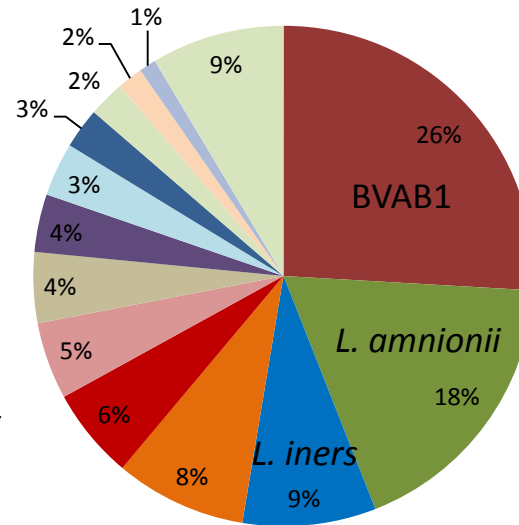
n = 91



Nugent 7-8

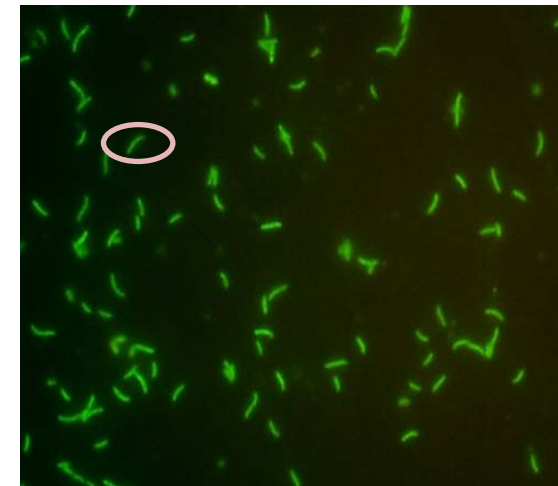
- *Lactobacillus iners*
- *Gardnerella vaginalis*
- *Leptotrichia amnionii*
- *Atopobium vaginae*
- *Megasphaera sp. type 1*
- *Prevotella bivia*
- *Sneathia sanguinegens*
- *Prevotella genogroup 2*
- *Prevotella genogroup 1*
- BVAB2
- *Eggerthella*
- BVAB1
- *Aerococcus christensenii*
- *Parvimonas micra*
- *Prevotella disiens*
- *Veillonella montpellierensis*
- Other

n = 25

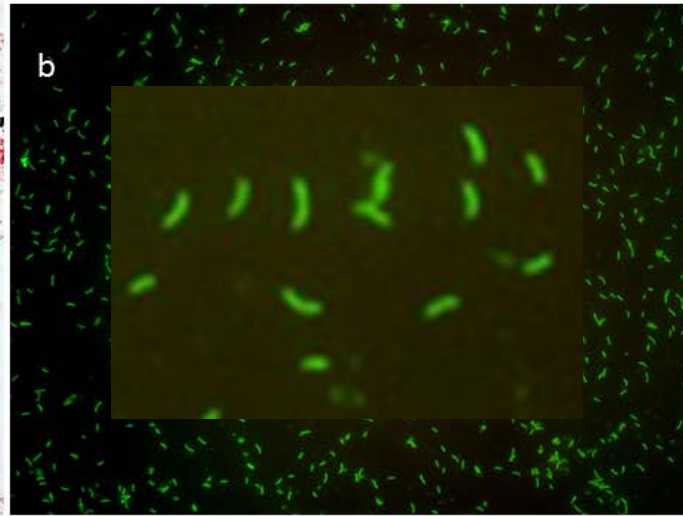
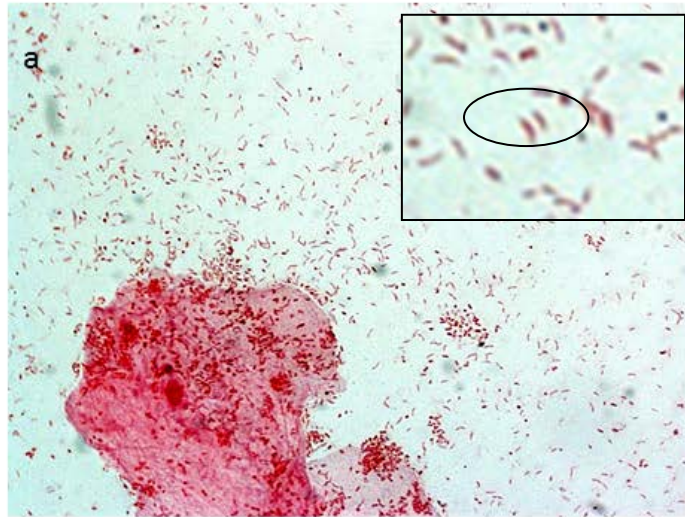


Nugent 9-10

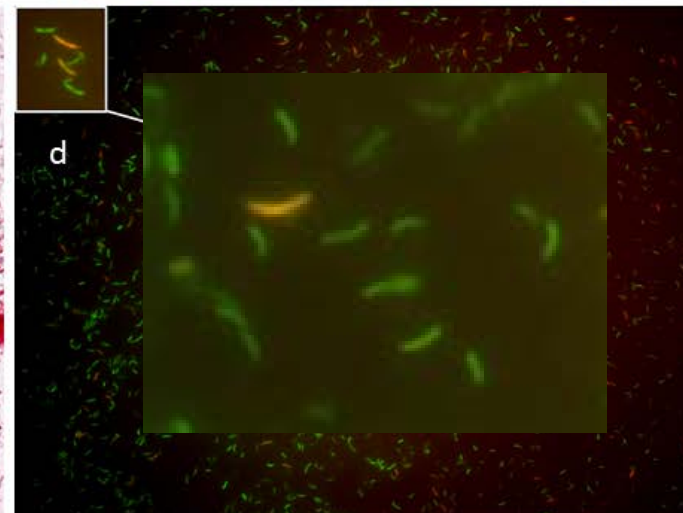
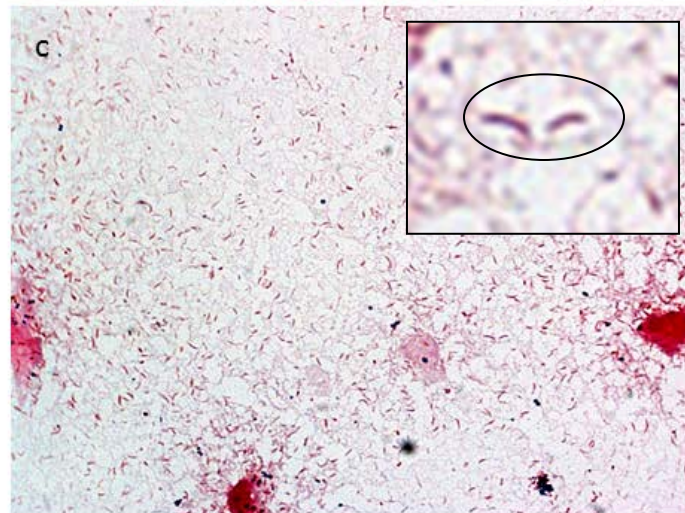
- BVAB1
- *Leptotrichia amnionii*
- *Lactobacillus iners*
- *Megasphaera sp. type 1*
- *Gardnerella vaginalis*
- *Sneathia sanguinegens*
- *Prevotella genogroup 1*
- *Atopobium vaginae*
- *Prevotella genogroup 2*
- *Eggerthella*
- *Prevotella genogroup 3*
- BVAB2
- *Lactobacillus crispatus*
- Other



Fluorescence Micrographs & Gram Stains of Vaginal Fluid Smears for 2 Women with Nugent Score =10



qPCR	
<i>Mobiluncus</i> DNA	2.5×10^5
BVAB1 DNA	2.4×10^9
FISH	
<i>Mobiluncus</i> cells	<1
BVAB1 cells	661



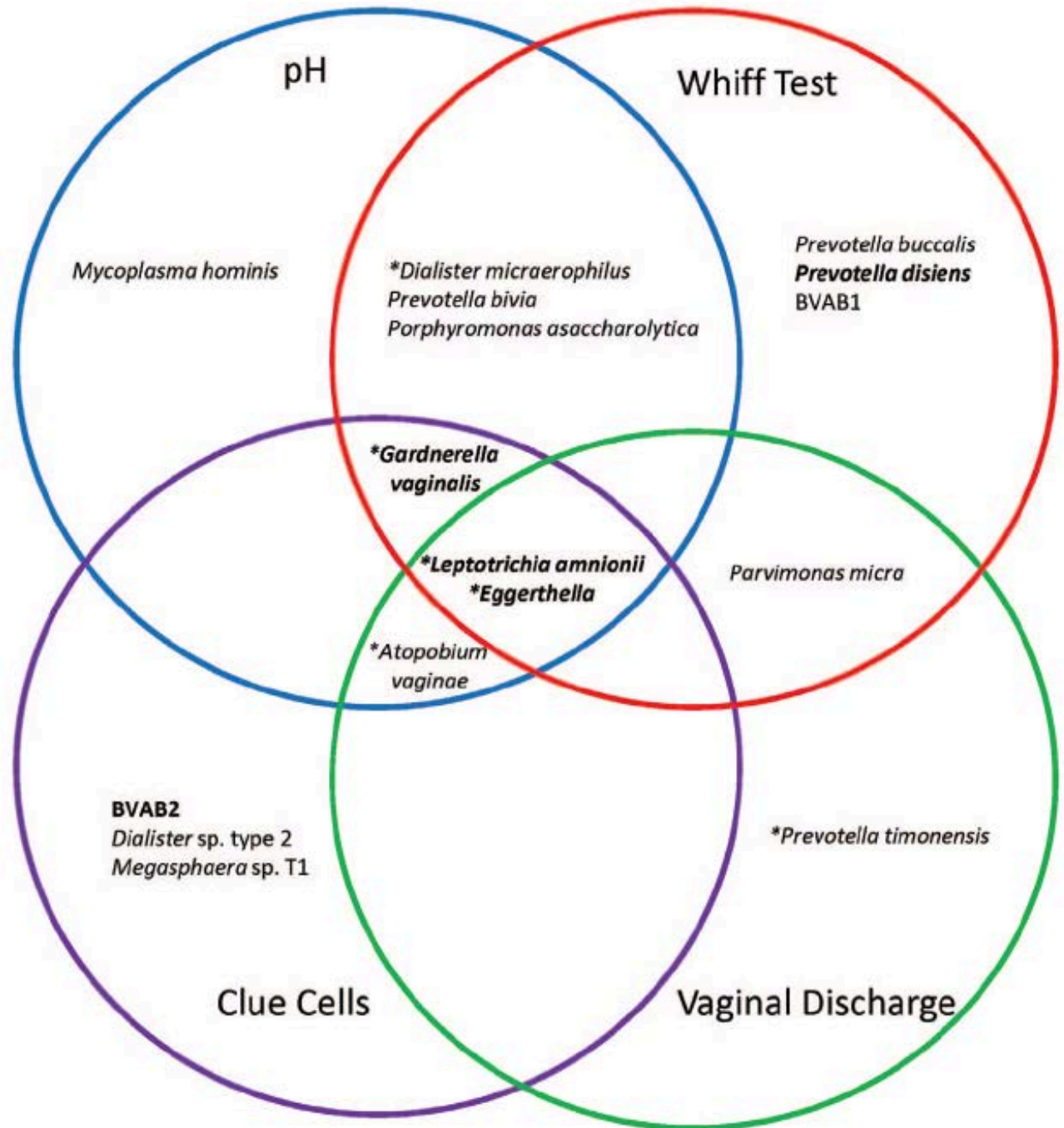
qPCR	
<i>Mobiluncus</i> DNA	1.3×10^7
BVAB1 DNA	5.1×10^8
FISH	
<i>Mobiluncus</i> cells	145
BVAB1 cells	908

Conclusions

- Separate lines of evidence suggest that curved GNR designated Mobiluncus morphotypes on Gram stain are more likely BVAB1 in our study population:
 - Broad-range PCR with pyrosequencing
 - Species-specific quantitative PCR.
 - Fluorescence in situ hybridization
- *Prevotella* and *Porphyromonas* spp. were significantly associated with the Bacteroides morphotype, whereas *Bacteroides* species were rare
- Implications:
 - Treatment studies monitor response by Gram stain
 - Natural history studies examine transitions in the microbiota by Gram stain
 - This needs evaluation!

Bacterial Taxa Associated with Amsel's Criteria

- *Leptotrichia amnionii* & *Eggerthella* sp. associated with each criteria
- Stars denote bacteria present in >75% of women with BV
- Taxa in bold denote those associated with Amsel's criteria as a composite unit



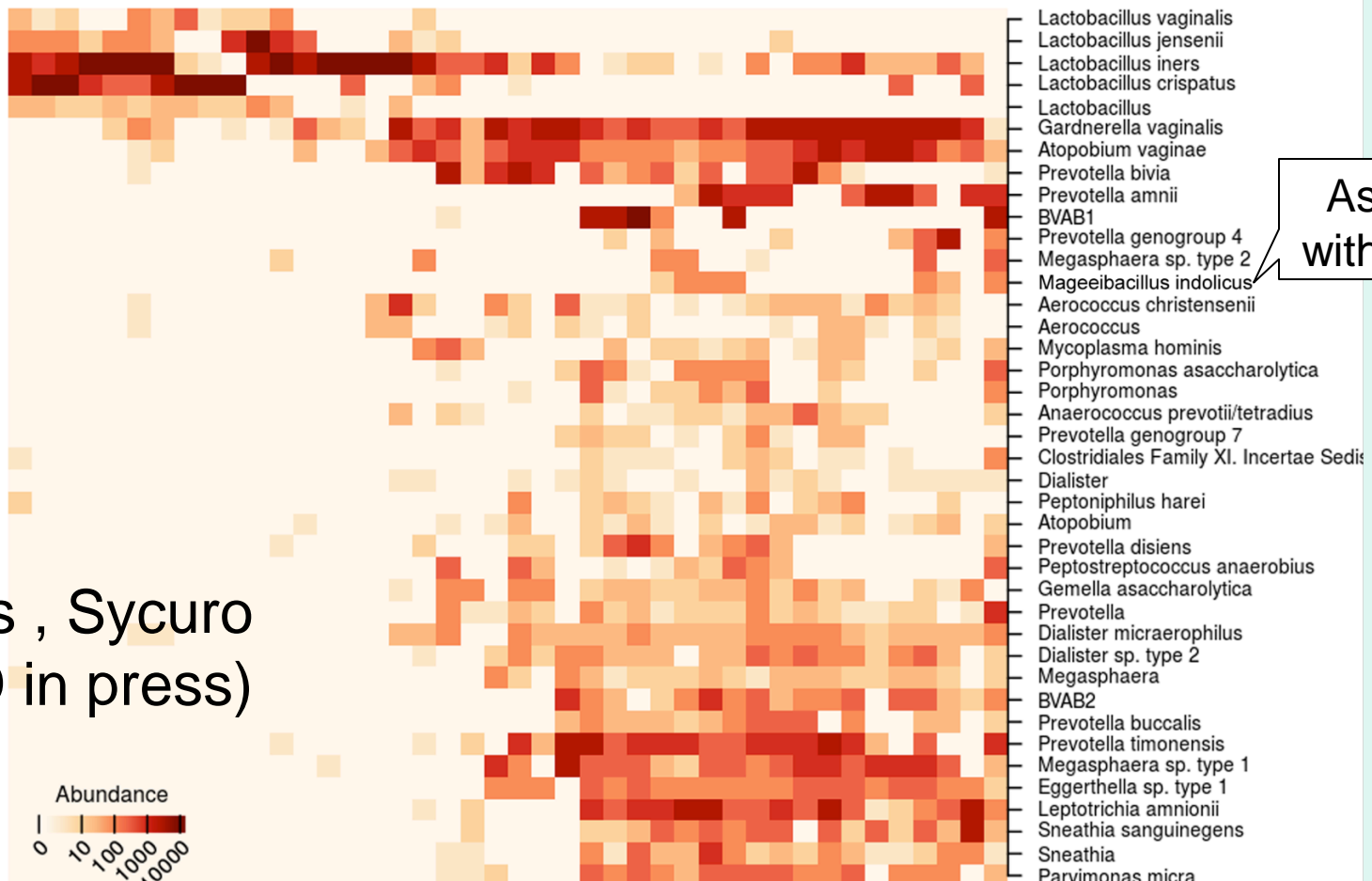
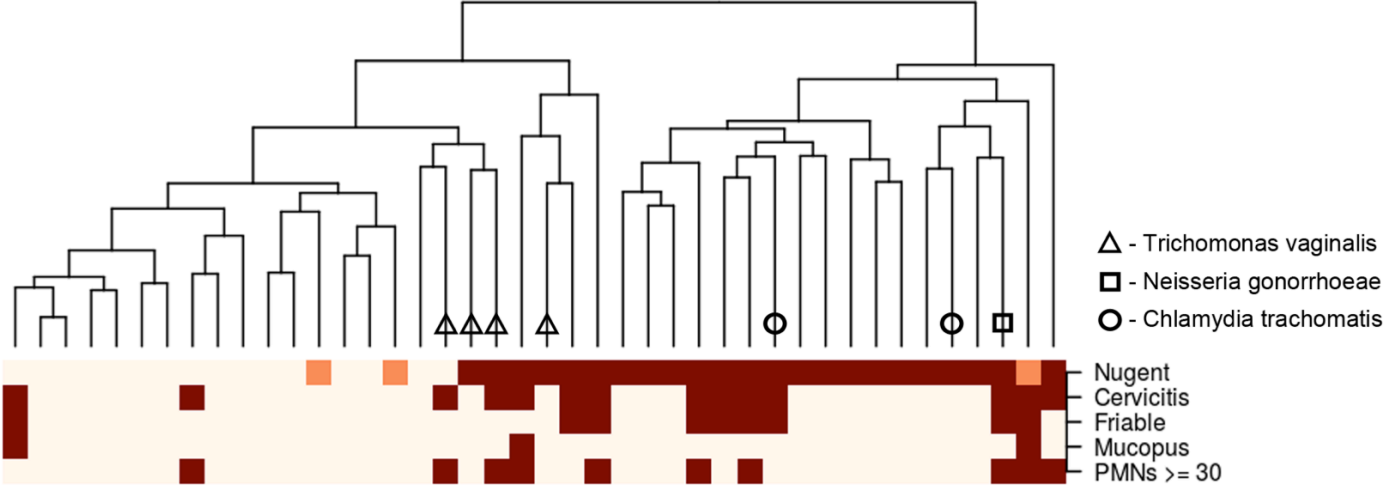
Cervicitis in Women Attending an STD Clinic: Association with Specific BVAB

Table 3. Multivariate Analyses of the Relationship Between the Presence and Load of Specific Bacterial Species in Seattle Participants and Cervicitis

Presence Alone	Detection in the Cervix		Detection in the Vagina	
	aOR (95% CI) [†]	p-value*	aOR (95% CI) [†]	p-value*
<i>Mageibacillus indolicus</i>	4.38 (0.84–23.68)	0.086	2.93 (0.57–14.78)	0.232
<i>Lactobacillus jensenii</i>	0.17 (0.02–0.89)	0.032	0.41 (0.06–2.17)	0.401

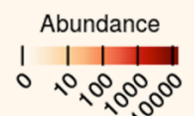
Presence and Load [‡]	Detection in the Cervix		Detection in the Vagina	
	aOR (95% CI) [†]	p-value*	aOR (95% CI) [†]	p-value*
<i>Mageibacillus indolicus</i>	1.53 (0.98–2.44)	0.064	1.63 (1.08–2.56)	0.019
<i>Lactobacillus jensenii</i>	0.61 (0.29–1.02)	0.061	0.68 (0.40–1.02)	0.068

Gorgos , Sycuro (STD in press)



Associated with friability

Gorgos , Sycuro
(STD in press)



BV & Increased HIV Acquisition

- Overall, increased risk of ~60% (Atashili 2008)
- Loss of H₂O₂ (directly virucidal)
- Activation of CD4 by alkaline pH
- Upregulation of cytokines that promote local HIV replication (TNF-alpha, IL-1 beta) & increased shedding
 - HIV shedding increased with intermediate flora or BV (Rebbapragada 2008; Coleman 2007; Sha 2005; Tanton 2011)
 - Not in all prospective studies (Wang 2001; Moreira 2009)
 - Successful BV treatment: decreases in IL-1 beta, IL-8, RANTES & activated CD4 T-cells at endocervix, including CCR5 and CD69 expression (Rebbapragada 2008)



Bacterial Vaginosis in HIV-Infected Women Induces Reversible Alterations in the Cervical Immune Environment

Anuradha Rebbapragada, PhD, Kathryn Howe, PhD,* Charles Wachih, MCChB,†
Christopher Pettengell, BSc,* Sherzana Sunderji, BSc,* Sanja Huibner, BSc,* T. Blake Ball, PhD,‡
Francis A. Plummer, MD,‡ Walter Jaoko, PhD, MBChB,† and Rupert Kaul, MD, PhD*†§*

REVIEW ARTICLE

Vaginal microbiota and its role in HIV transmission and infection

Mariya I. Petrova^{1,2}, Marianne van den Broek^{1,2}, Jan Balzarini³, Jos Vanderleyden¹ & Sarah Lebeer^{1,2}

¹KU Leuven, Centre of Microbial and Plant Genetics, Leuven, Belgium; ²University of Antwerp, Department of Bioscience Engineering, Antwerp, Belgium; and ³KU Leuven, Rega Institute for Medical Research, Leuven, Belgium

Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Analysis among African Couples


Craig R. Cohen^{1,2*}, Jairam R. Lingappa^{3,4,5}, Jared M. Baeten^{3,4,6}, Musa O. Ngayo⁷, Carol A. Spiegel⁸, Ting Hong³, Deborah Donnell⁹, Connie Celum^{3,4,6}, Saidi Kapiga¹⁰, Sinead Delany¹¹, Elizabeth A. Bukusi^{1,3,5,7}

Model	HR	Adjusted HR*
Primary analysis		
Pre-visit BV	3.62 (1.74-7.52)	3.06 (1.35-6.95)
Sensitivity Analyses		
Current visit BV	5.30 (2.21-12.74)	3.97 (1.67-9.43)
More severe BV status	7.19 (2.59-19.94)	6.98 (2.12-23.0)

*Fixed covariates: age, geographic region, partner HSV-2 status, circumcision, randomization assignment and STD;

Time-dependent covariates: pregnancy, hormonal contraception, plasma HIV-1 RNA, unprotected sex with study partner, CD4 count, outside partners, no. of sex acts with study partner, genital ulcer disease.

Log₁₀ HIV RNA concentration in plasma and female genital secretions compared by vaginal flora category

Modest increase: 0.2 log₁₀ 

Vaginal Flora	Log ₁₀ HIV Mean ± SD	P-Value vs. normal vaginal flora	P-Value* vs. normal vaginal flora
Genital HIV RNA			
Normal vaginal flora	3.04 ± 0.99	N/A	N/A
Intermediate vaginal flora	3.25 ± 1.01	0.0035	0.058
BV	3.23 ± 0.99	0.0023	0.095
Plasma HIV RNA			
Normal vaginal flora	3.81 ± 1.00	N/A	N/A
Intermediate vaginal flora	3.96 ± 1.07	0.037	N/A
BV	3.99 ± 1.07	0.0056	N/A

*After controlling for plasma HIV RNA

BV & Increased HIV Transmission

- Bacteria may activate Langerhans cells and CD4+ T-cells (Donoval, 2006; deJong 2009)
 - May involve direct stimulation by BVAB of relevant immune targets in male genitalia
 - BVAB / LB shared in male & female partners (Bukusi 2011; Gray 2009; Mrazek 2009)
 - Male circumcision changes microbiota of penis, and reduces women's risk of subsequent BV (Price 2010; Gray 2008; Liu 2013)

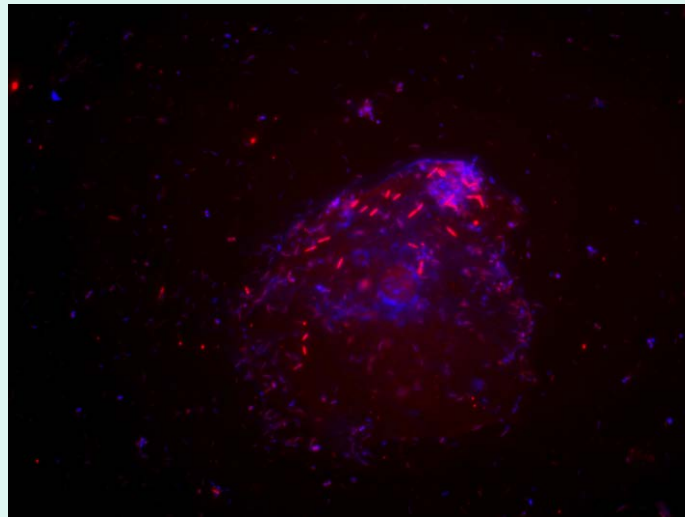
Some Answers, but...

- BV is a dysbiotic syndrome associated with acquisition of complex vaginal bacterial communities that include many uncultivated species
 - Heterogeneity may explain differences in treatment outcomes, relapse rates, & incidence of adverse sequelae
 - This heterogeneity – ideally measured with comprehensive, complementary approaches to defining bacterial communities—should be defined in both treatment trials & natural history studies
 - We can't neglect to study the behavioral & host factors involved

Acknowledgements:

UW-FHCRC Genital Health Study Team

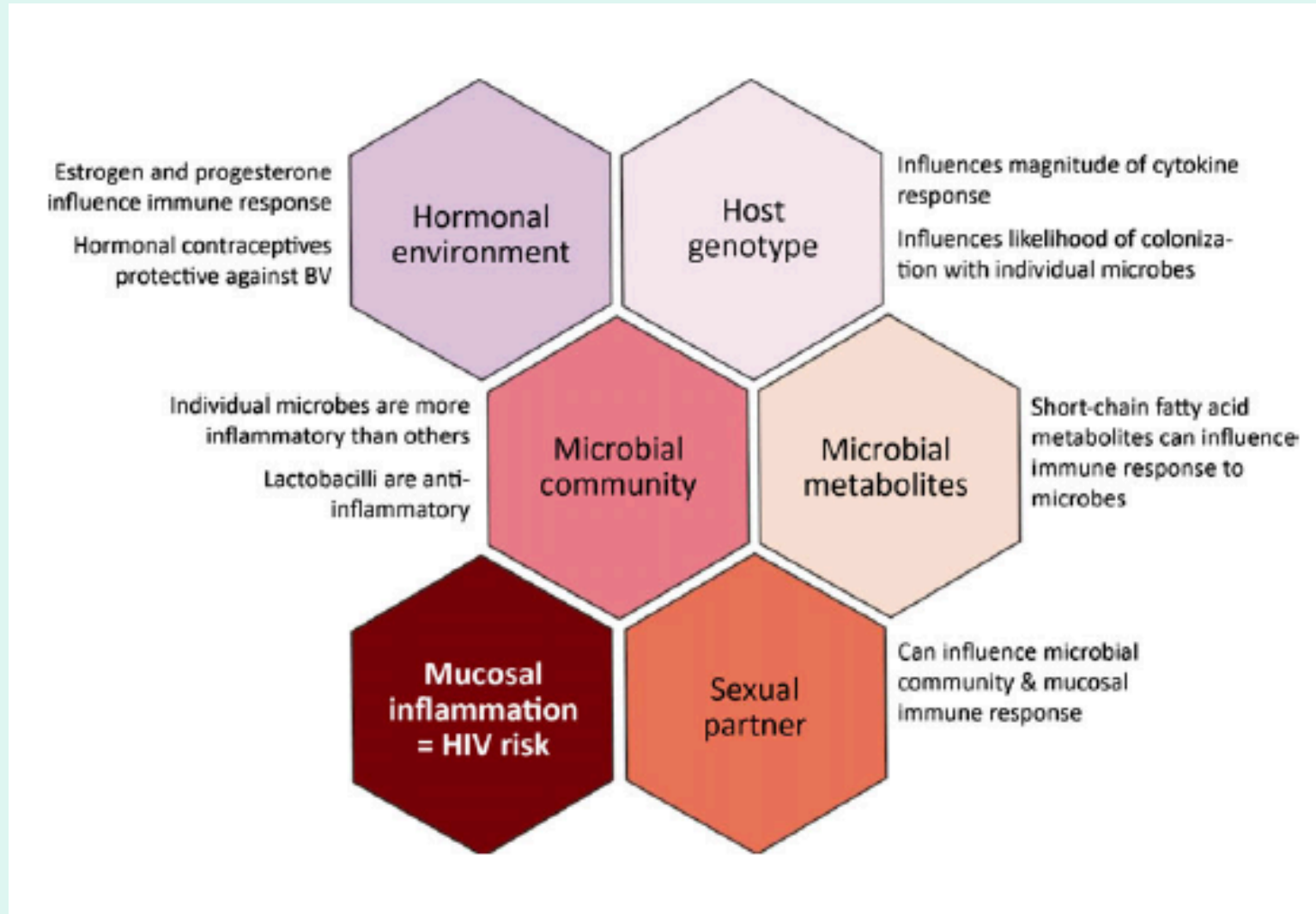
- **David Fredricks**
- **Kathy Ringwood**
- **Tina Fiedler**
- Kathy Thomas
- **Sujatha Srinivasan**
- Congzhou Liu
- Kathy Agnew
- Nancy Dorn
- Dana Kubulis
- Dwyn Dithmer
- Laura Sycuro



Thank you!



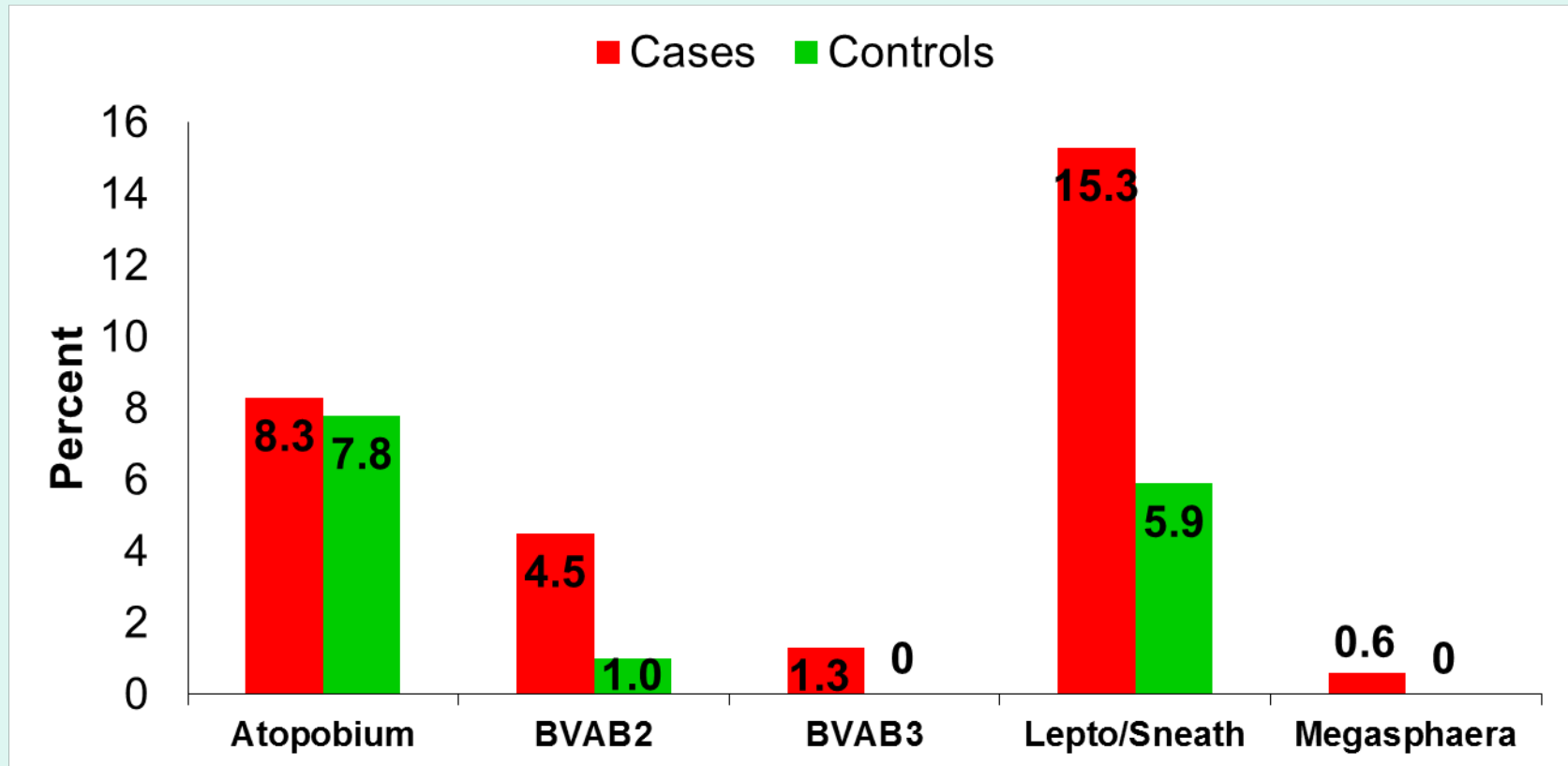
Complexity: Immunology in BV



Mitchell,
Marrazzo
2014

- Production of sialidase (IgA destruction), glycosidase, volatile amines; IL-8 increase variable; ?SCFA (Mirmonsef 2012)

Association of bacteria with NGU



OR
(95% CI):

1.1 (0.42-2.66)
p=1.0

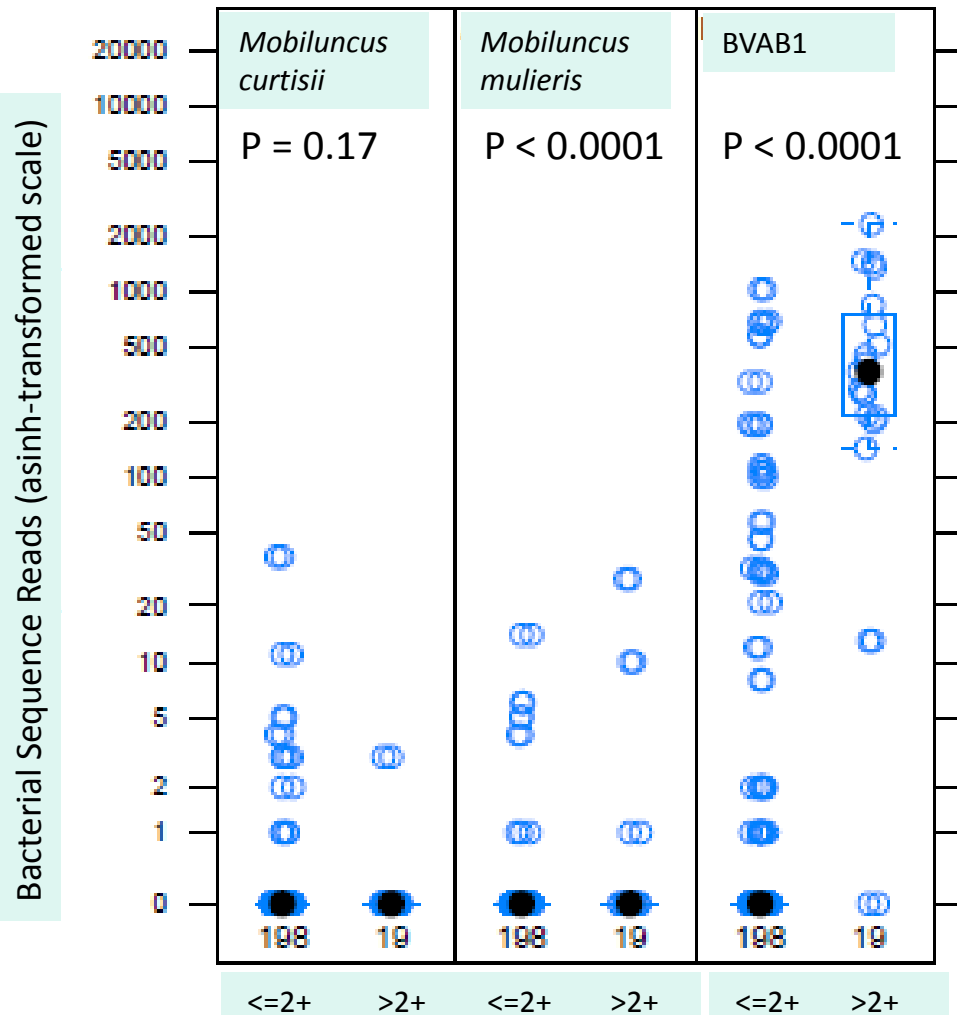
4.7 (0.57-39.05)
p=0.15

--

2.9 (1.1-7.4)
***p*=0.03**

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Association of *Mobiluncus* morphotype with BVAB1 sequence reads



Curved Gram-negative rods visualized by Gram stain are more likely to be the uncultivated bacterium, BVAB-1, rather than the widely accepted *Mobiluncus* spp.

Mobiluncus morphotype abundance (Curved Gram negative rods)

Women with BV

Species-Specific Quantitative PCR

