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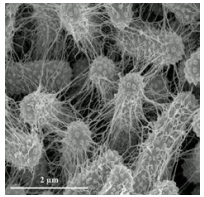
Disentangling Common Vaginal Infections: How Highly Sensitive Rapid Point-of-Care Testing Can Help Improve Patient Outcomes



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University of Alabama at Birmingham

Webinar, August 17, 2022

SOUTHEAST STD/HIV PREVENTION TRAINING CENTER

CONNECTING·PROVIDERS·PRACTICE·& PATIENTS

Disclosures (Research Support, Consulting Fees, and Honoraria)

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- Centers for Disease Control (CDC) – Consultant for 2021 STI Treatment Guidelines, National STD Curriculum 2nd Edition
- Lupin Pharmaceuticals - Consultant
- Roche, BioTechN, Abbott, Scynexis - Scientific Advisory Board Member
- Visby Medical

Speaker and Reviewer Honoraria:

- Lupin Pharmaceuticals
- Cepheid
- Abbott Molecular
- DynaMed
- Visby Medical

Learning Objectives

- Distinguish between BV and trichomoniasis in terms of epidemiology, pathogenesis, and adverse health outcomes
- Review clinical symptoms associated with both vaginal infections, while highlighting that either can be asymptomatic
- Discuss traditional and novel diagnostic methods for BV and trichomoniasis
- Highlight how highly sensitive rapid, point-of-care (POC) testing could improve time to diagnosis and potentially make a difference in patient outcomes

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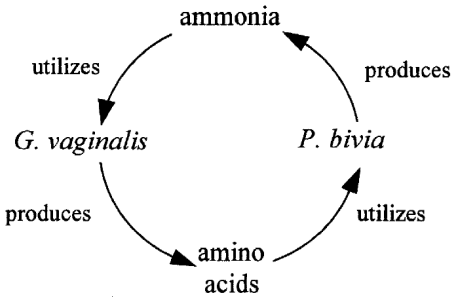
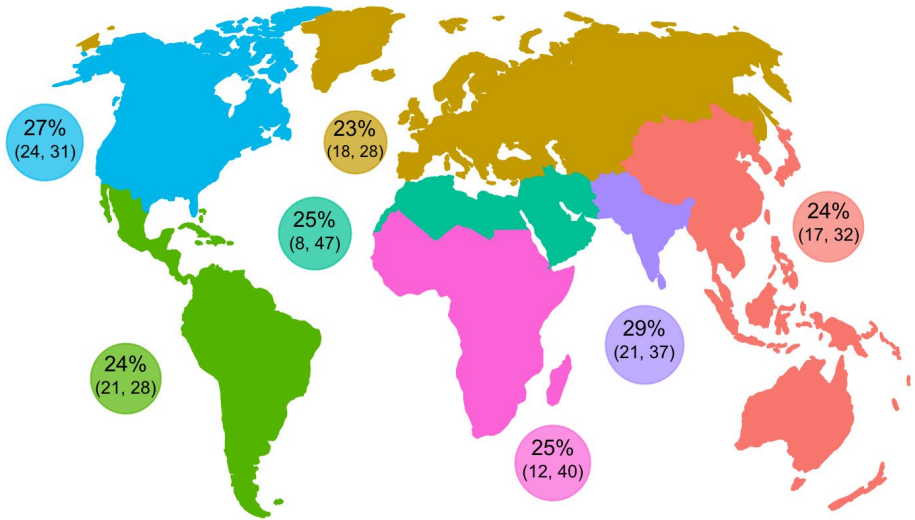
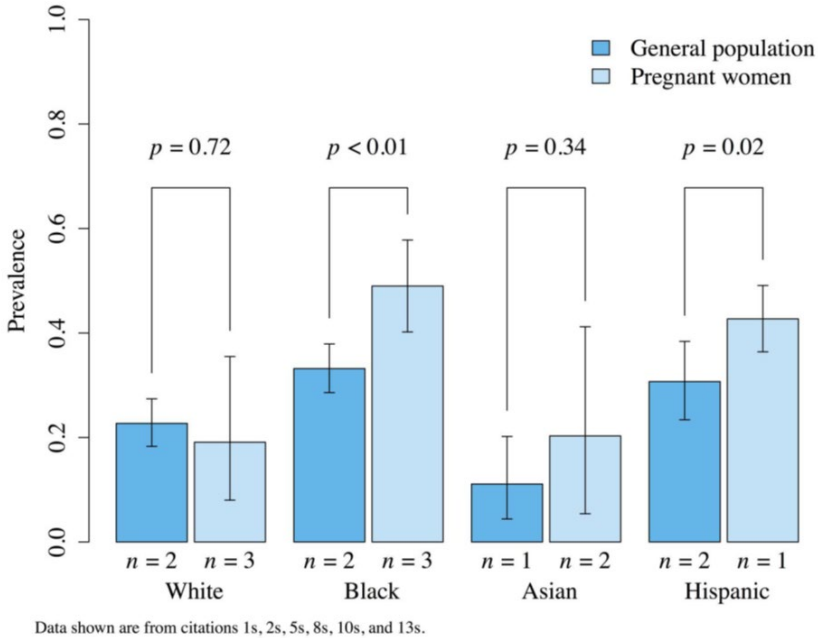
Epidemiology, Pathogenesis, and Adverse Consequences of BV and *T. vaginalis*



Global Prevalence of BV – the most common cause of vaginal discharge





Data shown are from citations 1s, 2s, 5s, 8s, 10s, and 13s.

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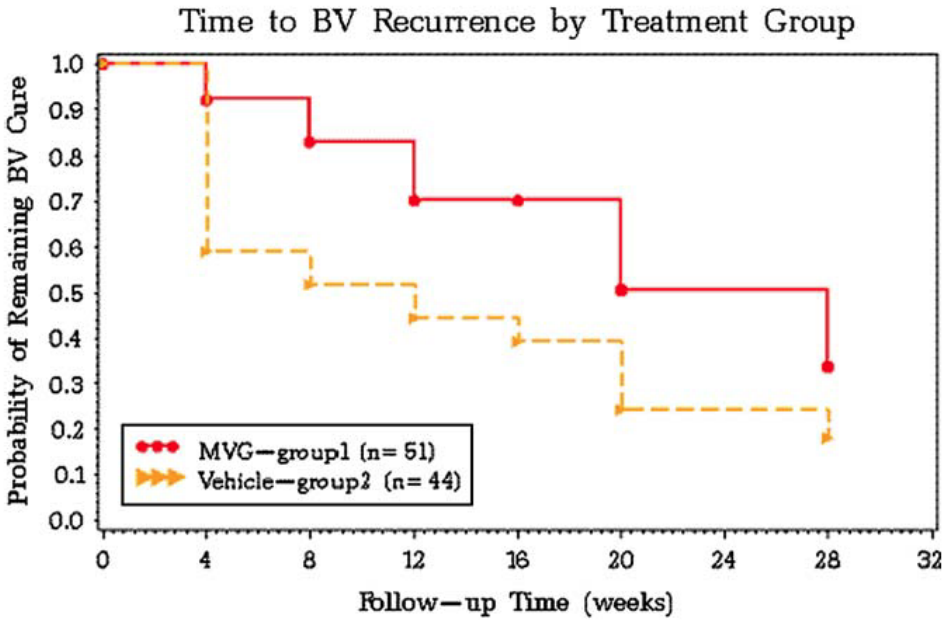
Sex Transm Dis 2019;46(5):304-311

BV

- U.S. prevalence 30% per NHANES data[1]
- Associated with preterm birth, low birth weight, post-operative gyn infections, and increased risk for acquisition and transmission of HIV and STIs[2]
- Characterized by depletion of lactic acid-producing lactobacilli and increases in facultative (*Gardnerella vaginalis*) and strict anaerobic bacteria (*Prevotella* bivia, *Atopobium vaginae*, BVAB1-3, *Megasphaera* spp., *Sneathia* spp., etc.) which form a biofilm on the vaginal mucosa[2]

[1]*Obstet Gynecol* 2007;109(1):114-20; [2]Bacterial Vaginosis, STD 4th edition, 2008

Despite Treatment, BV Recurrence Is Common



At 28-weeks, BV recurrence occurred in **75%** of women on placebo compared to **51%** of women on suppressive metronidazole therapy

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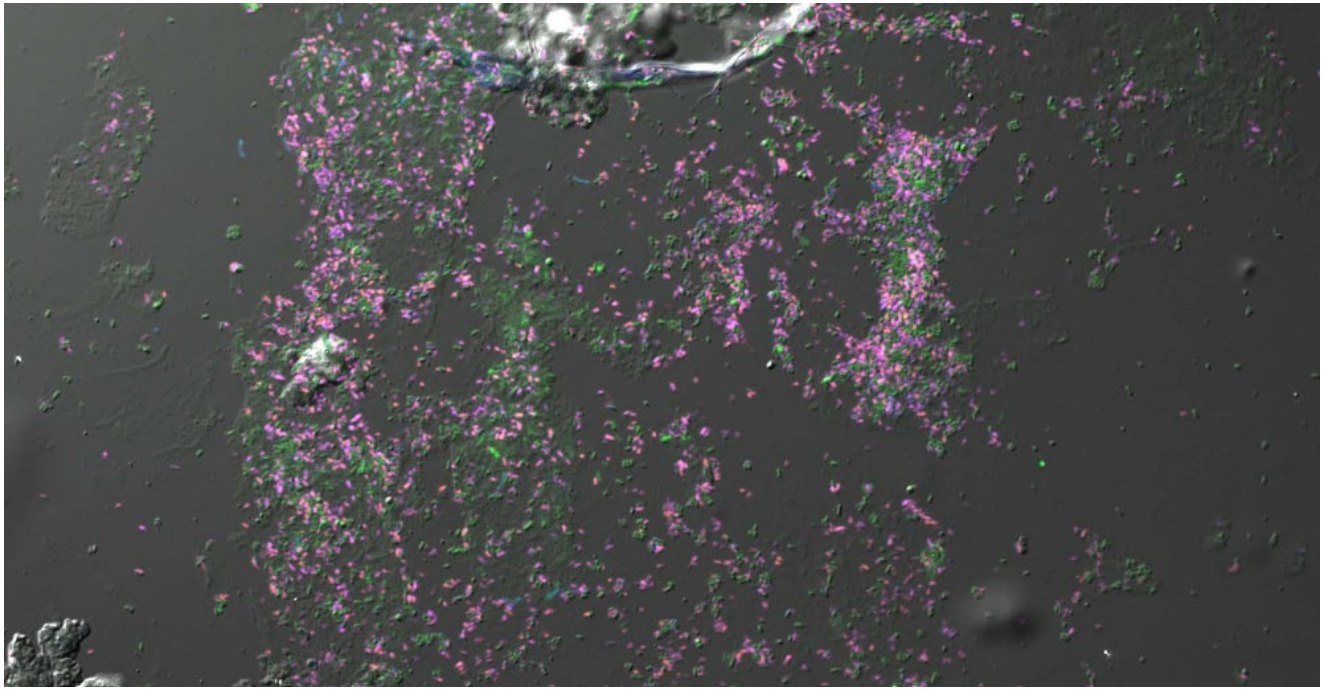
Am J Obstet Gynecol 2006;194(5):1283-9

BV is a Biofilm Community – likely plays a role in high rates of recurrence after treatment



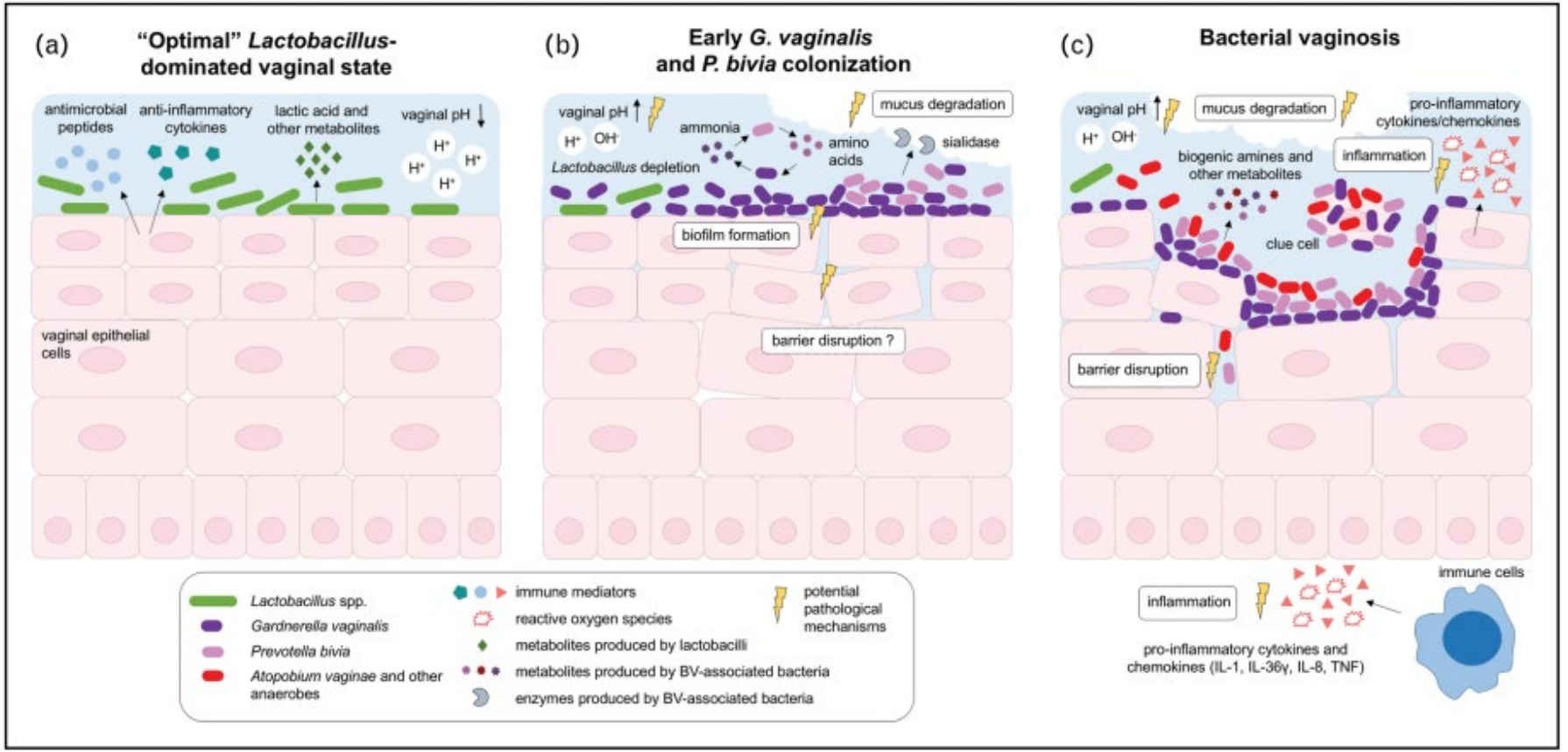
Biofilm mainly composed of *Gardnerella vaginalis* (60-95% biofilm mass), *Atopobium vaginae* (1-40%), and *Lactobacillus* spp. (0.01-5%)

Obstet Gynecol 2005;106:1013–1023

Epidemiology of BV Strongly Supports Sexual Transmission of BV-Associated Bacteria[1]

- BV associated with inconsistent condom use and increased numbers of recent and lifetime sexual partners[2]
- Women with BV have an earlier median age of sexual debut than women without[2]
- Most significant risk factor for incident BV is a new sexual partner while that for recurrent BV is a regular sexual partner[2]
- The penile microbiota of male partners is significantly more similar to the vaginal microbiota of their female partners, compared to non-partner women, regardless of circumcision status[3]

[1]*Curr Infect Dis Rep* 2013;15:130-135; [2]*J Infect Dis* 2016:214 (Suppl 1); [3]*Microbiome* 2016; 4:16



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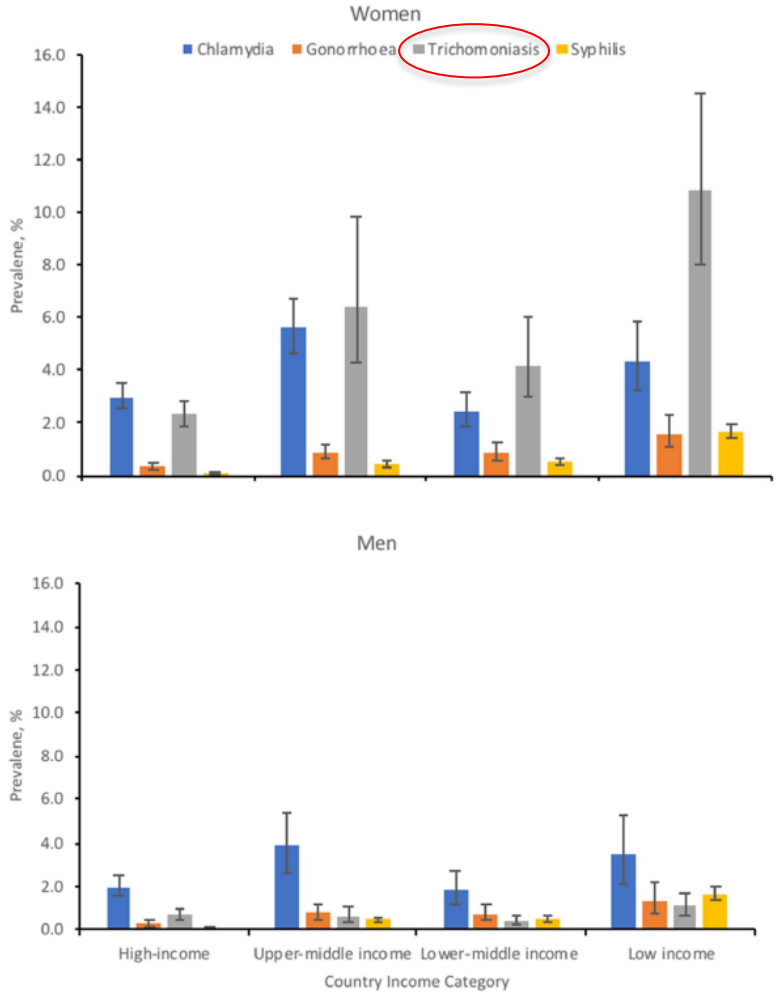
One Hypothetical Model of BV Pathogenesis

Curr Opin Infect Dis 2020;33(1):59-65

Global Prevalence of Chlamydia, Gonorrhea, Trichomoniasis, and Syphilis in Adult Women and Men, 2016

Fig. 2. **Prevalence estimates of chlamydia, gonorrhoea, trichomoniasis and syphilis in adults, by World Bank classification, 2016**



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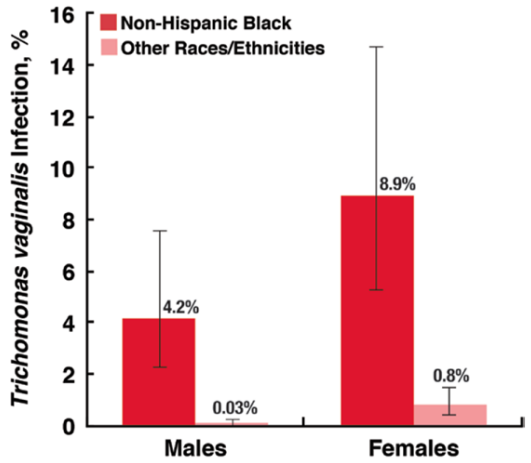
Bull World Health Organ 2019; 97(8): 548-562

Epidemiology of *T. vaginalis* in U.S. Women and Men, NHANES 2013-2014[1]

- Prevalence among U.S. women (1.8%) and men (0.5%) ages 18-59 (urine specimens tested with the Hologic Gen-Probe Aptima *T. vaginalis* NAAT)



- *T. vaginalis* significantly associated with female sex, black race, older age, <high school education, being below the poverty level, and having ≥2 sexual partners in the past year
  - **Racial disparity for *T. vaginalis* in the black population exceeds that for chlamydia, HSV-2, and HPV**
- Prevalence estimates exceed estimates of *T. vaginalis* burden in other high-income countries (i.e. UK)[2]

NAAT = Nucleic acid amplification testing

[1]*Clin Infect Dis* 2018; 67(2):211-217, [2]*Sex Transm Infect* 2018; 94(3):226-229

Epidemiology of *T. vaginalis* at the Jefferson County Health Department (JCDH) STD Clinic

Birmingham, AL

- In 2012, the JCDH STD clinic initiated screening for all women and men presenting to the clinic using a *T. vaginalis* NAAT
- Clinical and laboratory data of men (n=2,514) and women (n=3,821) receiving a *T. vaginalis* NAAT between 2012-2013 reviewed
- *T. vaginalis* prevalence: 20.2%; 27.0% in women and 9.8% in men
- Correlates of *T. vaginalis* in women: age >40, African American race, WBC on wet mount, elevated vaginal pH, positive whiff test, co-infection with gonorrhea
- Correlates of *T. vaginalis* in men: age >40, African American race, ≥5 PMNs/HPF on urethral Gram stain
- *T. vaginalis* NAAT detected 1/3 more infections in women than wet mount alone

WBC = White blood cell PMN = polymorphonuclear neutrophils HPF = high power field

Clin Infect Dis 2014; 59:834-41

Clinical Consequences of Untreated Trichomonas Infection

- Urethritis, penile discharge, and dysuria
- Increased risk of HIV
- Infertility
- Increased risk of prostate cancer?

- Vaginitis, cervicitis, endometritis, increased risk of post-gyn surgical infection
- Premature rupture of membranes, low infant birth weight, long-term developmental problems
- Increased risk of HIV
- Increased risk of HPV, cervical cancer
- Fallopian tube damage, infertility

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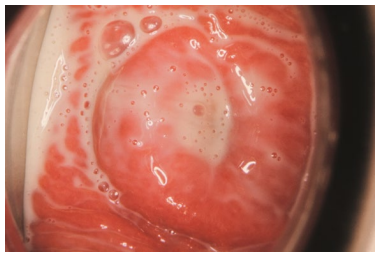
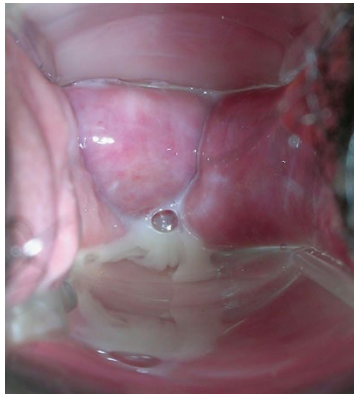
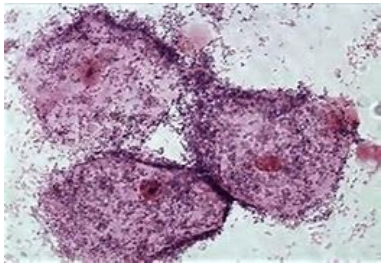
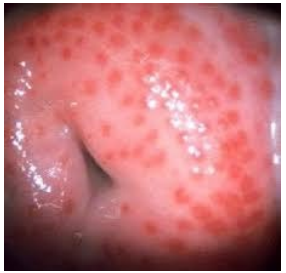
Traditional and Novel Diagnostic Methods for BV and *T. vaginalis*









POLL QUESTION #1

How long do BV test results currently take to come back at your clinic?

A. <30 mins

B. 30 min to 1 hour

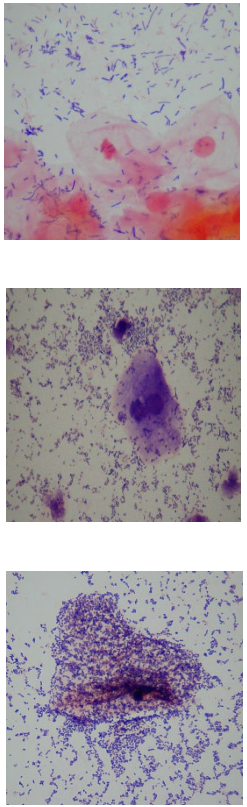
C. 1-12 hours

D. 12-48 hours

E. >2 days

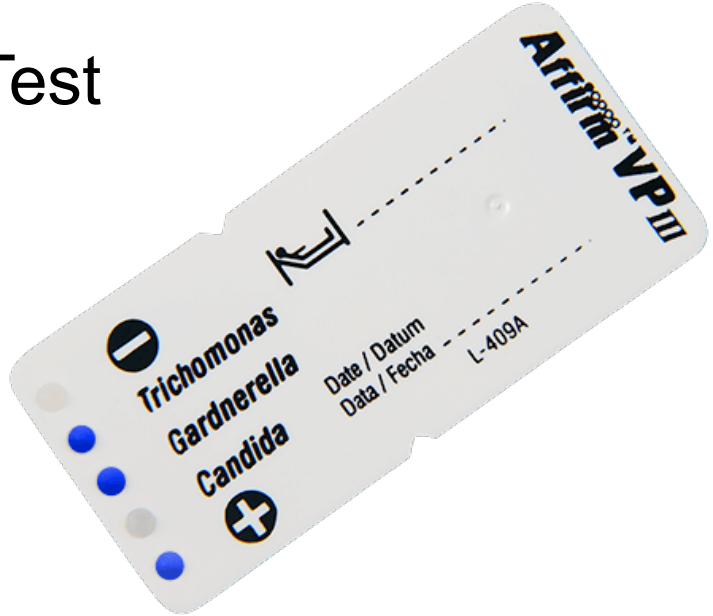
Traditional BV Diagnosis

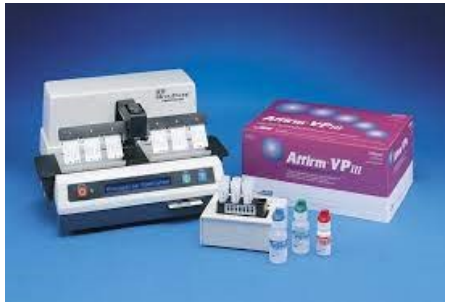
- POC: Amsel criteria[1] (clinical criteria)
  - Homogenous vaginal discharge, vaginal pH>4.5, positive whiff test, >20% clue cells/hpf
  - 3 out of 4 criteria needed for diagnosis
- (POC): Nugent score[2] (vaginal Gram stain)
  - **0-3**: lactobacillus predominate vaginal flora
  - **4-6**: intermediate flora with emergence of *G. vaginalis*
  - **7-10**: disappearance of lactobacilli with numerous *G. vaginalis* and strict anaerobes



[1]*Am J Med* 1983;74:14-22; [2]*J Clin Microbiol* 1991;29:297-301; Nugent photos courtesy of Charles Rivers, PhD, MSPH

Affirm VP III Test

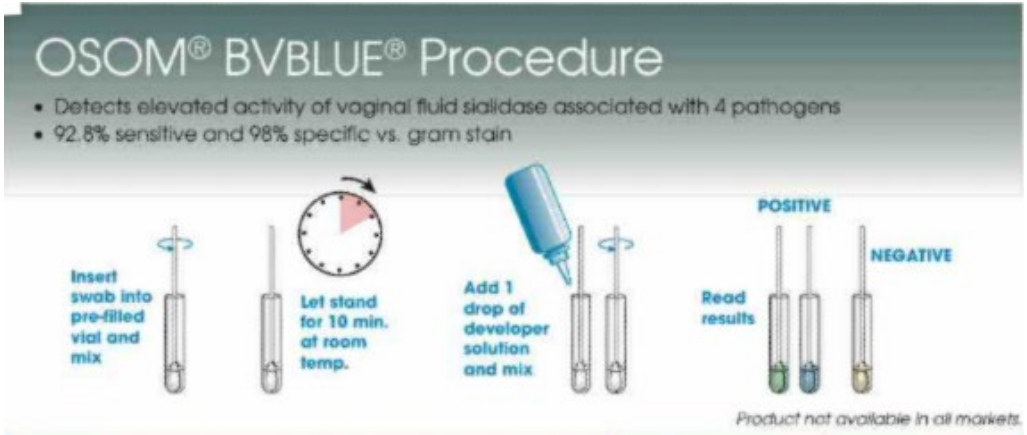




- DNA probe test detecting high concentrations of *G. vaginalis* nucleic acids
- Can also diagnose *Candida* spp. and *T. vaginalis* (not FDA-cleared for *T. vaginalis* diagnosis in men)
- Results available in <1 hour

- Most useful in symptomatic women in conjunction w/vaginal pH and presence of amine odor
- 97% sensitivity, 81% specificity compared with Nugent score
- Moderate CLIA complexity

POC: OSOM® BV BLUE® Test



- Detects vaginal sialidase activity produced by *G. vaginalis*, *Prevotella*, *Bacteroides*, and *Mobiluncus* spp.

- 92.8% sensitive, 98% specific versus Nugent score

POC: FemExam Test Card

- Measures vaginal pH, trimethylamine (metabolic by-product of *G. vaginalis*), and proline aminopeptidase
  - Eliminates the need for pH paper and KOH for whiff test
- Sensitivity 91%, Specificity 61% compared with Nugent
- Primarily used in resource-poor settings
- Not a preferred diagnostic method for BV

Novel BV Molecular Diagnostics

- Advantageous over microscopy and POC tests as they are based on detection of specific bacterial nucleic acids
- Objective, able to detect fastidious BVAB, enable quantitation, and are ideal for self-collected vaginal swabs
- Most useful in symptomatic women, two of which are FDA-approved

J Clin Microbiol 2018;56(9); 2021 CDC STI Treatment Guidelines

Current BV NAAT Tests in the U.S. – only for use in symptomatic women

5 quantitative multiplex PCR tests are currently available that detect certain BVAB as well as *Lactobacillus* spp.[1-4]

	BD MAX™ Vaginal Panel	Hologic Aptima® BV	LabCorp NuSwab® VG	Quest Diagnostics™ SureSwab® Bacterial Vaginosis	MDL OneSwab® BV Panel PCR w/ Lactobacillus Profiling by qPCR
Regulatory	**Cleared**	**Cleared**	LDT	LDT	LDT
Gardnerella vaginalis	Y	Y	-	Y	Y
***Lactobacillus* spp.**	Y	Y	-	Y	Y
Atopobium vaginae	Y	Y	Y	Y	Y
BVAB-2	Y	-	Y	-	Y
Megasphaera-1 *(& -2)	Y	-	Y	Y*	Y*
Reported as	BV	BV	Species w/ interpretation guidance for BV	Species w/ interpretation guidance for BV	Species w/ interpretation guidance for BV
Reportable results	POS NEG	POS NEG	Score: Negative for BV (0-1) Indeterminate (2) Positive for BV (3-6)	Not supportive of BV Equivocal Supportive of BV	Normal microflora Transitional microflora Abnormal microflora

LDT = laboratory developed test

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[1]*J Clin Microbiol* 2012; 50: 2321-2329; [2]*Obstet Gynecol* 2017; 130:181–189; [3]*J Clin Microbiol* 2016; 54:1017–1024; [4]*J Clin Microbiol* 2020;58(2):e01643-19

POLL RESULTS

Q1. How long do BV test results currently take to come back at your clinic?

POLL QUESTION #2

How long do trichomonas test results currently take to come back at your clinic?

A. <30 mins

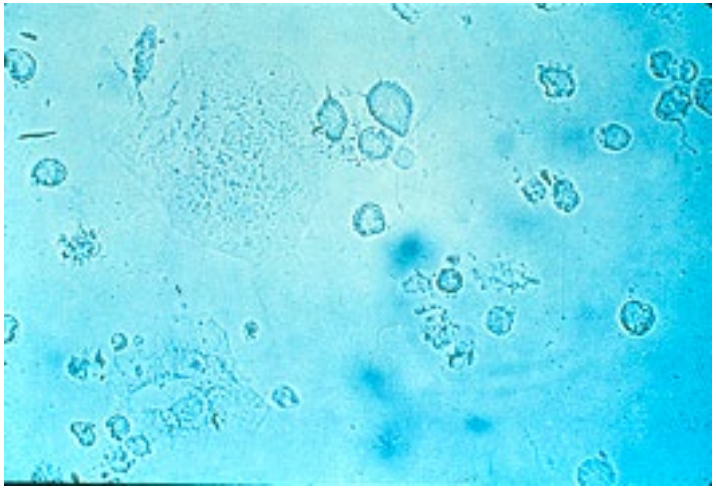
B. 30 min to 1 hour

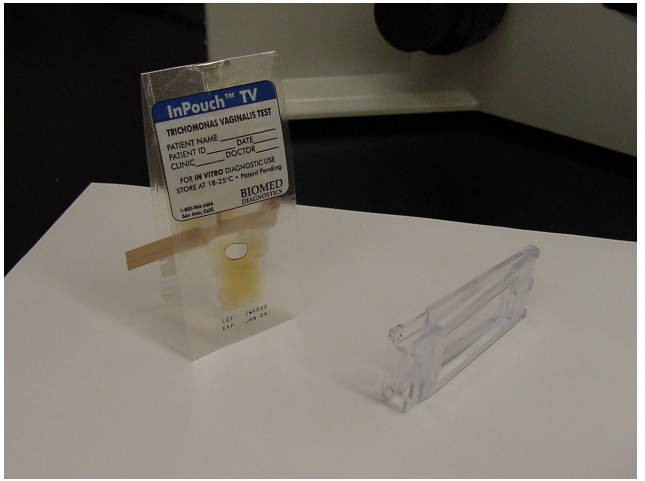
C. 1-12 hours

D. 12-48 hours

E. >2 days

Traditional *T. vaginalis* Diagnostics: Wet Mount and Culture



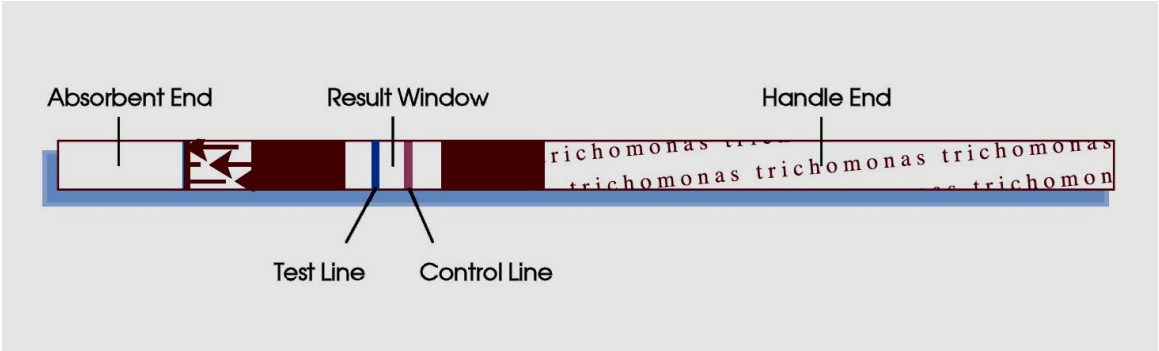


- POC test
- Must be performed in 10-20 minutes after specimen collection or trichomonads will lose viability
- Sensitivity 44-68%; Specificity 100%

- Need to inoculate InPouch within 1 hour of specimen collection (women: vaginal secretions; men: urethral swab, urine sediment, semen: multiple specimens from men recommended)
- Requires incubation at 37ºC; read over 5-7 days
- Sensitivity 44%–75%; Specificity 100%

Sex Transm Infect 2013; 89: 434–438; *Int J Curr Microbiol Appl Sci* 2016; 5:731–741

POC: OSOM® Test Stick: Rapid Antigen Test for *T. vaginalis*



- Performed on vaginal secretions: uses antibodies to detect trichomonas protein antigens; results in ≤10 minutes
- Sensitivity 82%–95%, specificity 97%–100%, compared to wet mount, culture, and TMA
- Not validated in men

TMA=transcription-mediated amplification

Sex Transm Infect 2013; 89: 434–438

Molecular Amplified Assays for *T. vaginalis* Diagnosis

- Solana Trichomonas Assay
  - Qualitative detection of *T. vaginalis* DNA from female vaginal and urine specimens from asymptomatic and symptomatic women
  - Sensitivity >98%, compared with NAAT for vaginal specimens, and >92% for urine specimens
  - Results < 40 minutes

- Amplivue Trichomonas assay
  - Rapid test for qualitative detection of *T. vaginalis* from vaginal specimens from symptomatic and asymptomatic women
  - Sensitivity of 90.7% and specificity of 98.9% compared with NAAT
  - Results in 45 – 50 minutes

T. vaginalis NAATs – Instrument-based

- Hologic Aptima *T. vaginalis* NAAT – 2011[1]
  - FDA-approved on vaginal swab, endocervical swab, ThinPrep Pap, and urine specimens in asymptomatic and symptomatic women; internally validate prior to use in men, no real-time results

- BD ProbeTec Qx *T. vaginalis* NAAT – 2014[2]
  - FDA-approved on vaginal specimens; internally validate prior to use in men, no real-time results
  - BD CTGCTV2 assay approved 2016 for *T. vaginalis* diagnosis in men using urine[3]

[1]*J Clin Microbiol* 2011; 49:4106–11; [2]*J Clin Microbiol* 2014;52(3):885-9; [3]*Sex Transm Dis* 2021;48(2):134-40

T. vaginalis NAATs – Instrument-based

- Cepheid Xpert® *T. vaginalis* NAAT – 2018[4]
  - FDA-approved in women and men; on-demand results within 40-65 minutes
- Roche Cobas® TV/MG assay – 2019[5]
  - FDA approved women and men (urine), no real-time results
- Abbott Alinity m STI Assay – FDA approved 5/4/22
  - 4 pathogen testing (GC/CT/TV/MG), <115 minutes to first result

[4]*J Clin Microbiol* 2018; 24;56(2); [5]*Expert Review of Molecular Diagnostics* 2020; 20(4):381-6

T. *vaginalis* NAAT – Instrument-free

- Visby Medical™ Sexual Health Testing Device - 2021
  - First, single-use, POC PCR device for the detection of chlamydia, gonorrhea, and trichomonas
  - FDA cleared for use in self-collected vaginal swabs from women at least 14 years old
  - Results available in <30 minutes without complex instrumentation
  - Sensitivity 99.2%, Specificity 96.9% for *T. vaginalis* diagnosis

Lancet Infect Dis 2021;21(5):668-76

POLL RESULTS:

Q2: How long do trichomonas test results currently take to come back at your clinic?

Follow-Up After BV and *T. vaginalis* Diagnosis

- Follow-up for BV is unnecessary if symptoms resolve; if symptoms recur after treatment, women should present to clinic for re-evaluation
- Re-testing after treatment for *T. vaginalis* is recommended for all sexually active women <3 months after initial treatment regardless of whether they believe their sexual partners were treated
- If re-testing at 3 months is not possible, clinicians should re-test whenever women next seek medical care <12 months after treatment

Case Presentation

- A 30-year-old African American female presents to the STD clinic with complaints of several days history of progressively worsening vaginal discharge over the past week. She reports a new male sexual partner whom she met on Bumble a few weeks ago. They have been having unprotected penile-vaginal sex in addition to oral sex. She has a Mirena IUD in place over the past 6 months and has not had periods over the past few months. She cannot remember when her last HIV/STI screening occurred.
- A pelvic exam reveals cervical discharge which is pooling in the posterior vaginal fornix. There is no uterine or adnexal tenderness on exam. Her IUD string is visible. There is some mild bleeding with endocervical specimen collection. No other abnormalities are noted.

Case Presentation (Cont.)

- Vaginal pH is 4.7. Wet mount results come back with no clue cells or trichomonads noted, however, there are many WBCs and a few RBCs. The KOH smear shows no yeast forms. Whiff test is borderline positive. A urine pregnancy test is negative.

POLL QUESTIONS #3&4:

3. What additional tests would you order?

A. Urine NAAT test for chlamydia, gonorrhea, trichomonas

B. Urine NAAT test for chlamydia, gonorrhea, trichomonas, Mycoplasma genitalium

C. Urine NAAT test for chlamydia, gonorrhea, trichomonas + blood test for HIV and syphilis

D. Would not order additional tests

4. Would you give her any treatment at this time?

A. Yes, Ceftriaxone 500 mg IM X 1 + Doxycycline 100 mg po BID X 7 days

B. Yes, Ceftriaxone 500 mg IM X 1 + Doxycycline 100 mg po BID X 7 days + Metronidazole 500 mg po BID X 7 days

C. Yes Metronidazole 500 mg po BID X 7 days

D. No, would not give her any treatment at this time, would wait for test results to come back

Case Presentation (Cont.)

- The patient was empirically treated for cervicitis with Ceftriaxone 250 mg IM X 1 and Doxycycline 100 mg po BID X 7 days per current 2021 CDC STI Treatment Guidelines.
- Given the presence of blood, it was unknown if her borderline elevated vaginal pH and whiff test were truly positive however no clue cells or trichomonads were seen on wet mount, thus, she was not treated for BV or trichomonas.
- Her Aptima STI NAAT test results came back 7 business days later positive for BOTH chlamydia and trichomonas.
- Our STD clinic NPs had difficulty contacting her via phone and she did not return for treatment of her trichomonas infection until 1 additional week later.
- *Could having a rapid and accurate POC test help in the decision-making process for this patient?*

POLL RESULTS

Q3. What additional tests would you order?

Q4. Would you give her any treatment at this time?

Key Take Home Points

- Both BV and *T. vaginalis* have high prevalence rates (esp. among African Americans) and are associated with significant adverse health outcomes, particularly in women
- *T. vaginalis* is a known STI; a large body of data suggests that BV is also an STI however the primary pathogen(s) remain controversial; this is an area of active research
- Novel diagnostic methods for both infections have recently been developed; time to test result and sensitivity of test result are important factors to consider
- Highly sensitive rapid, POC testing could help improve time to diagnosis of both BV and *T. vaginalis* and potentially make a difference in patient outcomes

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Thank you!

Questions/Comments?