

## Laboratory Testing of Genital Tract Specimens

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## Cast of Characters

Classic	Modern	MSM	Rare in US
<i>N. gonorrhoeae</i>	<i>C. trachomatis</i>	<i>Shigella</i>	<i>H. ducreyi</i>
<i>T. pallidum</i>	HSV	HBV	LGV
<i>Phthirus pubis</i>	HIV	<i>Campylobacter</i>	<i>C. granulomatis</i>
<i>Sarcoptes scabiei</i>	<i>T. vaginalis</i>	<i>Giardia</i>	Molluscum contagiosum
	<i>M. hominis</i>		
	<i>U. urealyticum</i>		
	<i>M. genitalium</i>		
	HPV		
	CMV		
	HCV		
	EBV		
	(Bacterial vaginosis)		
	(yeast vaginitis)		
	(Group B strep)		

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## The Vaginal Life Cycle

	Newborn	Premenarche	Menstrual	Postmenopause
Estrogen	Yes	No	Yes	No
Glycogen	Yes	No	Yes	No
pH	<4.5	>4.5	<4.5	>4.5
Predominant Endogenous flora	<i>Lactobacillus</i>	Coag-neg staph, enterococcus, <i>E. coli</i>	<i>Lactobacillus</i> ± <i>G. vaginalis</i>	Coag-neg staph, enterococcus, <i>E. coli</i>

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## Characteristics of the Vagina and Cervix in Women of Reproductive Age

	Vagina	Cervix
pH	<4.5	7.0
Epithelial Cells	Squamous	Columnar
Pathogens/Syndromes	Bacterial vaginosis <i>Candida</i> species <i>Trichomonas vaginalis</i>	<i>Neisseria gonorrhoeae</i> <i>Chlamydia trachomatis</i>

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## Vaginitis/Vaginosis

- Published guidelines recommend testing only for
  - Bacterial vaginosis 40-50%
  - Yeast vaginitis 20-25%
  - Trichomoniasis <5-75%
- 25% of women with vaginitis have >1

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## Other organisms

- Organisms such as *E. coli*, *Klebsiella*, and *Enterococcus* are part of the endogenous flora or are present as fecal contaminants and do not cause vaginitis
- Culture for or reporting the presence of these organisms, e.g., is inappropriate and will lead to unnecessary antimicrobial therapy

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## Bacterial Vaginosis

### Most common of the vaginitides

- OB clinic patients 10-26%
- Other clinic populations 12-25%
- STI clinic patients 32-64%

Symptoms*	BV	Normal
<i>n</i>	69	242
Present	46.4	31.8
Absent	53.6	68.2

\* Discharge, odor or irritation

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## BV Sequelae

### OB Complications

- Preterm delivery
- Premature rupture of membranes
- Amniotic fluid infection
- Chorioamnionitis
- Postpartum endometritis
- Premature labor
- Low birth weight

### GYN Complications

- Pelvic inflammatory disease
- Postabortal pelvic inflammatory disease
- Posthysterectomy infections
- Mucopurulent cervicitis
- Endometritis
- Increased risk of HIV

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## BV Clinical Characteristics

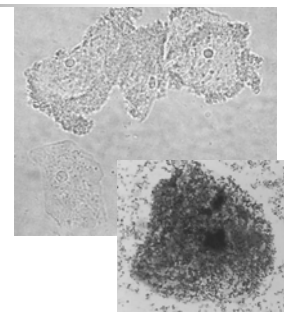
3 of these 4 needed for a clinical diagnosis

- vaginal fluid pH >4.5
- homogeneous discharge
- "fishy" odor on addition of KOH
- clue cells

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## BV Clinical Characteristics (cont.)

- Clue cells – vaginal epithelial cells so coated with bacteria that the cell borders are obliterated. Were originally described on wet mount.
- Watch out for pseudo clue cells



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## Common Endogenous Flora

	URT	GU(F)	GI	Skin
<b>Facultative Anaerobes</b>				
<i>Streptococcus</i>	X	20%		
<i>Enterococcus</i>		28	X	
<i>Staph aureus</i>		2-10		X
<i>Lactobacillus</i>	X	100		
<i>Gardnerella</i>		70		
<i>Corynebacterium</i>		40		X
<i>Enterobacteriaceae</i>		18	X	
<i>Neisseria/Haemophilus</i>	X			

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## Common EF (cont.)

### Anaerobes

	URT	GU(F)	GI	Skin
<i>Bacteroides fragilis</i>		4-40	X	
<i>Prevotella/Porphyromonas</i>		12-33		
<i>Fusobacterium</i>	X	7-28		
<i>Veillonella</i>	X	0-27		
<i>Clostridium/Eubacterium</i>		0-13	X	
<i>Actinomyces</i>	X			
<i>Peptostreptococcus</i>	X	low	X	
<i>Propionibacterium</i>		0-8		X

CID 32:e69-77, 2001

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## BV-associated Flora

	Normal	BV
<i>Lactobacillus</i>	High	Low
<i>Gardnerella vaginalis</i>	Varies	High
<i>Prevotella/Porphyromonas</i>	Low	High
<i>Peptostreptococcus</i>	Low	High
<i>Mobiluncus</i> species	Low	High
<i>Fusobacterium</i>	Low	Higher
<i>Mycoplasma hominis</i>	Low	Higher
<i>Atopobium vaginae</i>	Low	Higher
BVAB (NEJM 353:1899, 2005)	Low	High
Total CFU/mL	$10^5$ - $10^7$	$10^{10}$ - $10^{11}$

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## Culture not recommended because...

- there is no single pathogen
  - is an alteration in relative numbers of EF
- 50% of women with 3-4+ *G. vaginalis* have BV and the other 50% do not
- culture for associated flora is slow and costly; some are uncultivable
- reporting the presence of Enterobacteriaceae, *S. aureus* or *Enterococcus* may lead to unnecessary antibiotic exposure
- there are better methods for laboratory diagnosis of BV

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## Gram Stain Diagnosis

- predominance of lactobacilli  $\pm$  gardnerella = normal
- mixed small gram-positive and gram-negative rods  $\pm$  curved rods = BV

\*\*Kopeloff modification of the Gram stain with basic fuchsin counterstain\*\*

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## Gram Stain Diagnosis (cont.)

Score: 0, no cells present      1+, <1 cell/OIF      2+, 1-5 cells/OIF  
 3+, 5-30 cells/OIF      4+, >30 cells/OIF

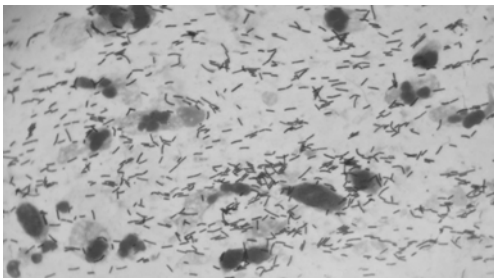
### Nugent Scoring System

Score	<i>Lactobacillus</i> morphotype	<i>Gardnerella</i> and <i>Prevotella</i> morphotypes	<i>Mobiluncus</i> morphotype
0	4	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

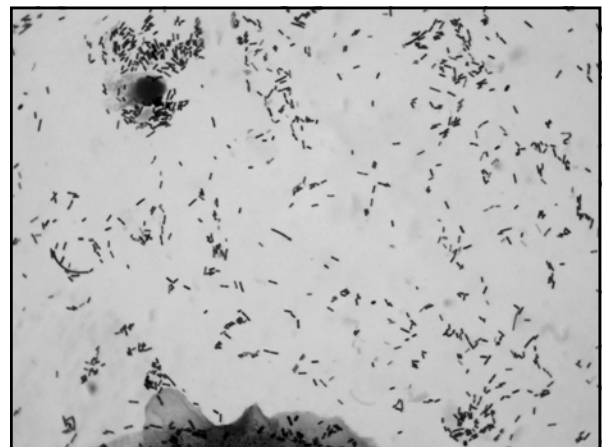
Interpretation: 1-3, normal; 4-6 intermediate (altered vaginal flora); 7-10, BV

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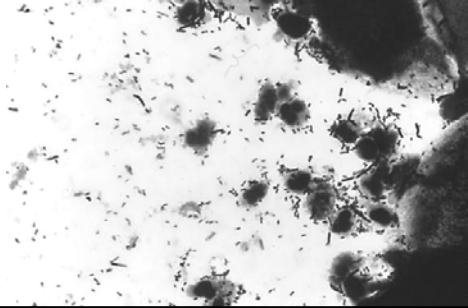
## Normal Vaginal Gram Stain



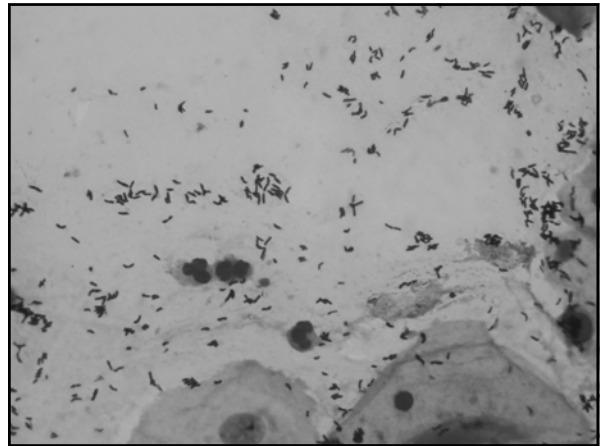
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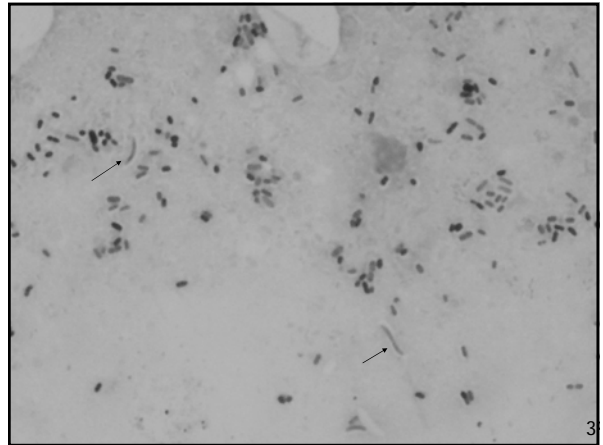
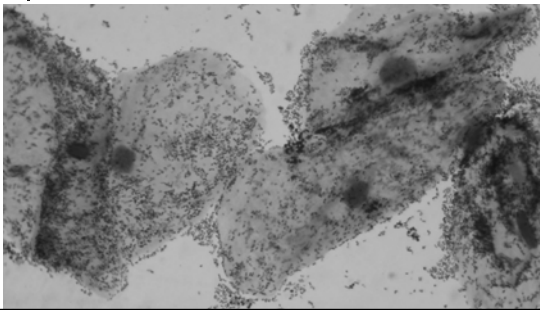
## Intermediate Gram Stain



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## BV



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## Affirm VP<sub>III</sub> Instrument



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## Affirm method

- a DNA probe test for *T. vaginalis*, *Candida* spp., and *Gardnerella vaginalis*
- single swab treated to release and stabilize RNA
- capture probes on a probe analysis card
- after exposure of card to sample, exposed to detection probes with bound enzyme
- in the presence of enzyme substrate a blue color develops to indicate a positive test

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## BV – Other Diagnostic Methods

- Affirm VP<sub>III</sub>
  - vs. *G. vaginalis* culture: sens 97%; spec 71%
  - vs *G. vaginalis* culture and pH >4.5: sens 94%; spec 81%

JCM 32(1):148-152, 1994

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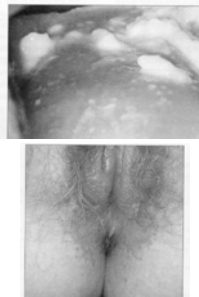
## BV – Other Diagnostic Methods

- Biochemical tests (point-of-care tests)
  - FemExam (Litmus Concepts) STD 30(6):483-9, 2003
    - I – pH and amines: 71.4% sensitive, 72.8% specific
    - II – proline iminopeptidase: 70.0 % sensitive, 80.9% specific
    - I & II: 91.0% sensitive, 61.5% specific
  - BVBlue (Gryphus Diagnostics)
    - sialidase (*Bacteroides*, *Gardnerella*, *Prevotella*)
    - 91.7/88% sensitive, 97.8/95% specific vs. Gram stain
    - JCM 41(5):1925-8, 2003; JCM 43(2):584-7, 2005

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## Vulvovaginal Candidiasis (aka Yeast Vaginitis)

- 2<sup>nd</sup> most prevalent
- Estimated 13 million cases/year
  - 70-75% 1 episode in lifetime
  - 40-50% 2 episodes in lifetime
  - 5-8% multiple episodes
- Signs and symptoms
  - Curd-like discharge
  - Vaginal itching (pruritus)



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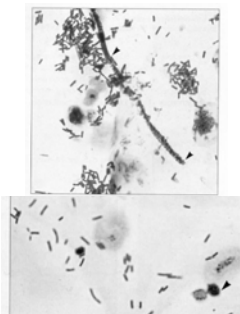
## Vulvovaginal Candidiasis (cont.)

- Acute
  - *Candida albicans* (95%); *C. glabrata* (5%)
- Chronic
  - *C. albicans* (68%), *C. glabrata* (16%), *Saccharomyces cerevisiae* (6%) *C. tropicalis* (4%)

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## Diagnostic Methods

- Wet mount (KOH or calcofluor white)
  - estimated at 50% sensitivity (KOH)
  - False negative KOH more likely if *C. glabrata*.
- Affirm VP<sub>III</sub> vs. culture
  - 79% sensitive, 85% specific



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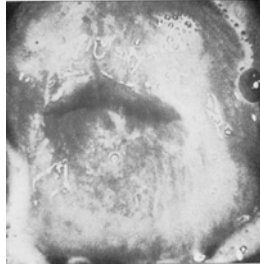
## When is vaginal culture for yeast appropriate?

- Symptomatic patient
- KOH wet mount negative
- No other diagnosis
  - Chromagar Candida
  - If >10 colonies, then "In a symptomatic patient with no other genitourinary infection, treatment for yeast vaginitis is warranted."

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## *T. vaginalis* vaginitis (cont.)

- Third most common; STI
- 170 million annually; 8 million in N.A.
- Prevalence <5-75%
- Up to 50% asymptomatic
- Signs and symptoms
  - Copious, greenish discharge
  - Strawberry cervix
- Sequelae
  - increased PID, infertility, post surgical infections, HIV transmission, preterm birth



Clin Micro Rev 17(4):794-803, 2004

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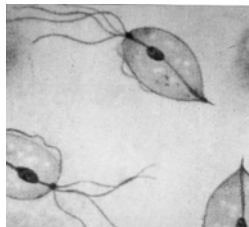
## *T. vaginalis* in men

- >50% asymptomatic
- symptomatic infections
  - urethritis (nongonococcal urethritis)
  - prostatitis
  - male factor infertility?

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## *T. vaginalis* Microscopic Diagnosis

- Microscopy
  - saline wet mount
    - 50-60% sens in women, hi specificity
    - ~30% sens in men, low specificity
  - Pap smear
    - 50 % sensitivity, 90% specificity
    - low PPV in low prevalence pop



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## *T. vaginalis* Culture Diagnosis

- In-Pouch TV
  - 85-95% sens in women, >95% spec
  - ~60 % sens and high specificity in men (urethral swab or FVU sediment)



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## *T. vaginalis* Antigen Detection

- OSOM *Trichomonas* Rapid Test (Genzyme Diagnostics, Cambridge, MA)
  - 10 min point-of-care test
  - 83.3% sensitive 98.8% specific vs. culture
  - (wet mount 71.4% sensitive)
    - JCM 43(2):664-7, 2005

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## *T. vaginalis* Molecular Methods (cont)

- Affirm
  - For *Trichomonas*: Sensitivity 90.5%; Specificity 99.8%
- PCR
  - >90% sensitive and >95% specific for females
  - >90% sensitive and specific for males
  - Culture 70% sensitive vs. PCR

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## When is it appropriate to perform a bacterial CULTURE?

- group B strep
  - 35-37 wk gestation
  - rectovaginal sample
  - enrichment (followed by culture or AccuProbe)
    - Trans-Vag broth
    - LIM
    - StrepB Carrot broth
  - PCR
    - direct
    - post enrichment
  - NOT direct antigen tests

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## When...to...culture? (cont.)

- toxic shock syndrome
  - 2-10% have *S. aureus* as endogenous flora
  - 10% of those are TSST-1 producers
- desquamative inflammatory vaginitis (DIV)
  - uncommon, purulent vaginitis
  - mean age 41.8 years
  - associated with Group B strep
    - patients respond to clindamycin

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## Do NOT culture for...

- Endogenous or contaminating flora
  - *Gardnerella vaginalis*
  - Enterobacteriaceae
    - *E. coli*
    - *Klebsiella* species
    - *Proteus* species
  - *Pseudomonas*
  - *Enterococcus*

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## When...to...culture? (cont.)

- pediatric patients
  - Group A strep
  - *Shigella* species, esp. *S. flexneri*
  - *Campylobacter jejuni/coli*
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*

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## When...to...culture? (cont.)

- Female upper tract infections( e.g., pelvic inflammatory disease (PID), salpingitis, post hysterectomy, post abortion)
- upper tract sample or cervical swab
- culture for known pathogens
  - *S. aureus*, *Streptococcus pyogenes* (BAP, CNA)
  - *Neisseria gonorrhoeae* (GC-lect, Choc)
  - Enterobacteriaceae (EMB/MAC)
  - *Bacteroides fragilis*, *Clostridium sordellii* (BBE/LKV, EYA)

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## Sources

- [www.guidelines.gov](http://www.guidelines.gov)
- Management of women of reproductive age attending non-genitourinary medicine settings complaining of vaginal discharge. J Fam Plan. Reprod. Health Care 32(1):33-42, 2006
- Brigham and Women's Hospital. Common gynecologic problems: a guide to diagnosis and treatment. Boston (MA): Brigham and Women's Hospital; 2003

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*Thank you!!*



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