

G084 Vaginal smear for Gram staining: 2+ (1-5/oif) epithelial cells, 2+ (2-10/oif) yeast, 3+ (11-50/oif) gram-positive bacilli. Negative for Bacterial Vaginosis

HISTORY This Gram smear challenge was sent to category A, B, C, and C1 laboratories as a vaginal sample collected from a 30-year old female with vaginitis. A representative view of the Gram smear may be seen on the CMPT web site.

CMPT QA The sample was made to be consistent with a **negative bacterial vaginosis sample**. It contained 2+ (1-5/oif) epithelial cells, 2+ (2-10/oif) yeast, and 3+ (11-50/oif) gram-positive bacilli. The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E¹.

GRADING (maximum grade = 4) A grade of 4 was assigned to the cellular component only.

CELLULAR COMPONENT (grade=4) The 15 reference laboratories reported the cellular component as follows: 10 reported no neutrophils and either 2+, 3+ or 4+ epithelial cells and 4 reported no neutrophils seen therefore the cellular component was considered suitable for grading. One reference laboratory reported 2+ neutrophils and 4+ epithelial cells and is asked to return the slide to CMPT for review.

Of 121 laboratories that processed the sample, 117 (97%) reported no neutrophils seen. The four laboratories that reported clue cells in varying amounts received a grade of zero as reporting clue cells could lead the clinician to suspect BV and treat accordingly. The four laboratories that reported neutrophils received a grade of zero as there were no neutrophils in the sample. Laboratories are asked to return their slide to CMPT for review if needed. Four laboratories were graded as zero for not submitting a report. Table 1 summarizes the reports received and the grades assigned.

BACTERIAL/ORGANISM COMPONENT (ungraded) There was a variety of reporting styles submitted by the reference laboratories for the bacterial component and these are listed in Table 2. Due to lack of consensus between the reference laboratories the bacterial/organism component was not suitable for grading.

Table 3 shows the variety of organisms and reporting styles submitted by participants. Out of 121 laboratories submitting a report only 47 (39%) specifically reported a BV negative result, 39

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-GRADING-Maximum grade =

Cellular component: (laboratories receiving grades of 4/4): 94.5% (70/74) of category A laboratories, 88% (30/33) of category B laboratories, and 79% (15/19) of category C/C1 laboratories

NOTE: For future challenges of this type the Microbiology Advisory Committee requests the CMPT Gram smear form be revised to reflect these recommendations for reporting Vaginal Gram Smears on women of reproductive age—

‘Due to the wide variety of Gram smear results from vaginal samples that can be considered normal, specific bacterial types need not be reported, but may be listed as “organisms resembling normal urogenital flora”. Yeast should always be reported with an added comment such as “*Candida* species are normal flora in the genital area of 30-40% of women. The presence of yeast must be correlated with the clinical picture.” Smear results that score ≥7 in the Nugent scoring system should be reported as “consistent with bacterial vaginosis”. It is acceptable to NOT report cells, or bacteria, and ONLY report presence or absence of yeast, and whether smear results are consistent with BV or not’.

Reported Component	Number of Labs
1+, 2+ or present yeast, BV negative	4
yeast present, Nugent score 4	1
1+ yeast, 3+ gram positive bacilli	1
1+, 2+ or present yeast, BV inconclusive, equivocal, indeterminate or intermediate	6
2+ yeast, Comment A “smear shows altered vaginal flora, which frequently represents a transitional stage. If signs and symptoms persist, repeat testing recommended.”	1
2+ yeast, 3+ gram positive bacilli, 3+ gram positive cocci, Comment A	1
2+ yeast, BV positive	1

Cells reported	A	B	C/C1	# labs	Grade
no neutrophils seen (no cells seen [1A])	16	5	1	22	4
no neutrophils, 1-5, 6-10, 13/oif, 1+, 2+, 3+, 4+ epithelial cells	52	24	14	90	4
no neutrophils seen, 1.7/oif epithelial cells	1	0	0	1	4
no neutrophils, 4+ epithelial cells, 2+ clue cells/clue cells present	1	0	1	2	0
no neutrophils, 1+ clue cells (1B) or clue cells present (1A)	1	1	0	2	0
1+ neutrophils, 1+, 2+, 4+ epithelial cells	1	2	0	3	0
2+ neutrophils, 4+ epithelial cells	1	0	0	1	0
no report	0	1	3	4	0
no slide received; not noticed until completion date therefore unable to request another slide.	1	0	0	1	0
Gram smears not routinely processed	0	0	1	1	ungraded
TOTAL	74	33	20	127	

(32%) reported BV inconclusive results, and 12 (10%) reported BV positive; and 117 (97%) reported yeast.

LABORATORY EXAMINATION OF VAGINAL SMEARS

Laboratory confirmation of the presence of moderate to heavy amounts of yeast can be provided by the direct microscopic examination of vaginal secretions either using a wet mount preparation or either a Gram stain or a Calcofluor white stained smear. Provided the technologist examines the entire slide, direct examination reliably confirms the presence of yeast overgrowth in the vagina. Cultures may be necessary to detect smaller amounts of yeast in vaginal samples, but should be done *at the request of the physician* in women who have recurrent infections, and or who are symptomatic and have no other obvious cause of vaginitis. *C. albicans* is the species most commonly found in the vagina, although other yeast species can cause vaginitis⁴.

The standard scoring system termed the “Nugent score” is an accepted technique using microscopic examination of a Gram stained smear of vaginal discharge for determining bacterial vaginosis⁷. The Nugent score is calculated by quantifying the presence of *Lactobacillus* species, *Gardnerella/Prevotella*, and *Mobiluncus* or their combination. A “Nugent score” of 7 or greater is defined as being consistent with bacterial vaginosis. Values of 6 or less do not correlate with bacterial vaginosis, and should not be interpreted as being consistent with incipient bacterial vaginosis. However, if clue cells are present a score of 4-6 (as well as ≥ 7) can be reported as “smear consistent with BV”. (See p. 4 for Nugent Score Tables)

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Organisms reported	A	B	C/C1	Total
1+, 2+ or present yeast, BV negative (Nugent Score: 3 [Interpretation 0-3 normal] (1C)	23	15	3	41
yeast present, Nugent score of 2 (3+ gram-positive bacilli, 2+ gram-positive cocci) (1); yeast present, Nugent score of 4 (1+ <i>Gardnerella/ Bacteroides</i> sp., 1+ <i>Lactobacillus</i> sp.) (1); 2+ yeast, Nugent score of 4 (4+ gram-positive cocci) (1); yeast, Nugent score 6 (1C1)	3	0	1	4
BV negative, no yeast seen (1A); no yeast seen, BV negative (1A, 1B)	2	1	0	3
0-2/oif, 1+, 2+ yeast (3A); yeast present, normal bacterial vaginal flora (1A); 1+ yeast, 3+ gram-positive bacilli (1A); 2+ yeast, 1+ gram-positive bacilli, 2+ gram-variable bacilli, 3+ gram-positive cocci (1A); 1+, 2+ yeast, 4+ gram-positive bacilli, 3+, 4+ gram-positive cocci, 1+, 4+ gram-negative bacilli (2B); 1+ yeast, 2+ gram-positive cocci, 3+ gram-negative bacilli, 2+ gram-negative cocci (1B); 1+, 2+ yeast, 3+, 4+ gram-positive bacilli (1C) & 2+, 3+, 4+ gram-positive cocci (3C) & 1+ gram-negative bacilli (2C1)	6	3	6	15
1+, 2+ or present yeast, BV indeterminate, inconclusive, equivocal, borderline +/- clinical correlation required	18	6	0	24
All with Comment A: “smear shows altered vaginal flora, which frequently represents a transitional stage. If signs and symptoms persist, repeat testing recommended”. 2+ yeast, 3+ gram-positive bacilli, 3+ gram-positive cocci (1A); 1+, 2+ yeast, 3+ mixed organisms +/- (2A); 2-10/oif, 1+, 2+ or present yeast (8A); yeast present, 5-30/oif lactobacilli, 5-30/oif gram-negative bacilli (1A); 2+ yeast, comment A (1B, 1C);	12	1	1	14
4+ <i>Gardnerella/Bacteroides</i> , no <i>Lactobacillus</i> , consistent with BV	1	0	0	1
1+, 2+ or present yeast, BV positive	6	3	2	11
1+ yeast, 3+ gram-positive bacilli, 3+ gram-positive cocci, 2+ gram-negative cocci, snnp, refer	0	1	0	1
<1/oif yeast, <1/oif gram-positive bacilli, 1+ gram-positive cocci, 2+ gram-negative bacilli, 3+ gram-negative diplococci (1); 1+ yeast, 3+ gram positive bacilli, 2+ gram-positive cocci, 2+ gram negative diplococci, 2+ gram-negative bacilli (1); 2+ yeast, 4+ gram-positive coccobacilli, 4+ gram-positive cocci, 2+ gram-negative diplococci, (1B); 1+ yeast, 4+ gram-positive diplococci, 4+ gram-negative diplococci, refer for BV scoring (1C); 2+ yeast, 3+ gram positive cocci/coccobacilli, 3+ gram-negative diplococci/coccobacilli (in epi's) (1C); 2+ yeast, 3+ gram-positive bacilli, 2+ gram-negative diplococci (1C1)	2	1	3	6
No report received	0	1	3	4
No slide received. It was not noticed until completion date therefore unable to request another slide.	1	0	0	1
not normally processed, refer	0	0	1	1
Total	74	33	20	127

CLUE CELLS A smear of vaginal discharge from a BV patient typically shows "clue cells" with mixed flora consisting of large numbers of small gram-negative rods and gram-variable rods and coccobacilli. Lactobacilli morphotypes are absent or greatly reduced. Clue cells can be recognized as different from normal vaginal epithelial cells either on saline mount or on Gram stain. In clue cells, the cell borders are obscured by adherent small coccobacilli. Clue cells are a critical component of the Amsel clinical criteria for bacterial vaginosis, which includes vaginal pH, KOH "whiff test," clue cells, and appearance of the vaginal discharge. The Nugent score and the Amsel clinical criteria strongly correlate for the diagnosis of bacterial vaginosis².

The presence of *Atopobium* in vaginal samples has the potential to add confusion to the Nugent scoring system, as the genus contains organisms formerly classified as anaerobic *Lactobacillus* species⁹ and could be interpreted as normal¹⁰. In addition, some related species are thought to readily decolorize and may appear to be gram-negative or variable. A simplified assessment of Gram-stained smears has also been proposed. When interpreting the intermediate state of BV the image area examined in microscopes requires standardization^{9,11}. Note: A Zeiss FL30 microscope from the early 1990s with an area of 0.0165 mm², was used as the reference microscope for Nugent scoring¹¹.

CLINICAL SIGNIFICANCE Vaginitis is one of the most common infections seen in primary care. The vast majority of vaginal infections are caused by either proliferation of organisms such as *Candida albicans* as part of the normal commensal flora, or the sexual transmission of specific organisms. There are three major causes of vaginitis: 1. bacterial vaginosis (BV), 2. Candidiasis, and 3. *Trichomonas vaginalis*. Effective treatment of vulvovaginal infections requires accurate diagnosis based on history, physical exam, and microscopic examination of a vaginal sample⁶. BV and *Candida* infections are by far the most common infections diagnosed in menarchal women and are further discussed below⁴.

1. Bacterial Vaginosis (BV) Bacterial vaginosis (BV) (also termed non-specific vaginitis) is the most common cause of abnormal vaginal discharge in women of childbearing age. This condition predisposes women to increased susceptibility to sexually transmitted diseases, including human immunodeficiency virus (HIV) infections and adverse pregnancy outcomes when bacteria (*Mobiluncus*) ascend to the upper genital tract⁹. The term "vaginosis" is preferred over "vaginitis" because the latter implies an inflammatory process, which is rarely present. The causative organism for this condition was proposed as *Haemophilus vaginalis*, later named *Gardnerella vaginalis*. The role of this organism has further been supplemented by the role of *Mycoplasma hominis* and anaerobic bacteria, in particular *Prevotella*, *Peptostreptococcus*, *Porphyromonas*, and *Mobiluncus* species². It is thought that a shift to a symptomatic BV state may simply be due to a decline in the levels of "beneficial" lactic acid and hydrogen peroxide-producing lactobacilli and/or an increase in the levels of gram-negative anaerobes^{9,10}. A variety of events can contribute to the development of BV in which a mixture of the organ-

isms listed above are usually present in concentrations 100-1000 times greater than in the healthy vagina².

2. Candida vulvovaginitis Symptomatic *Candida* vulvovaginal infections in women of reproductive age are so common that it is estimated that 75% of women will have one or more episodes before the age of 40.¹² The less frequent occurrence of infection among children and postmenopausal women suggests that colonization is hormone related. Estrogen promotes elevated glycogen production in the vaginal epithelium, contributing a nutrient source for *Candida* species⁵. *Candida albicans* may be readily isolated from approximately 20% of healthy women with no vulvovaginal symptoms. There are various events that may trigger the *Candida* to transform yeast forms into mycelia that are capable of invading the mucous membrane, and eliciting clinical symptoms. Some of these triggers include pregnancy, diabetes, estrogen, antibiotic use, and decreased cell-mediated immune response⁵. Vaginal candidiasis can be clinically diagnosed when patients typically have vulvovaginal pruritis and/or superficial burning and increased amounts of thick or curd-like white, non-foul smelling discharge.

Normal Vaginal Flora The "normal" vaginal microbial ecosystem is variable and fluctuates with a woman's age, hormonal status, immune status, sexual activities, use of medications and other vaginal products. There is also evidence to suggest that there may even be differences due to ethnicity. Cultures will usually show lactobacilli, streptococci, staphylococci, yeast, and various gram-negative bacteria; however, gene amplification technology has resulted in identification of additional non-culturable bacteria, often in high concentrations. The various microbes influence the presence of one another and contribute to a woman's unique vaginal ecosystem^{5,12}. At birth, the mucosal surface of the vagina is under the influence of maternal estrogen and consequently has a low pH. Lactobacilli are the predominant organism in a neonate's vagina. By the age of 6 weeks, as maternal estrogen declines, vaginal pH rises in the neonate, and flora changes to include a variety of anaerobic organisms and skin flora such as diphtheroids and coagulase-negative staphylococci. *Gardnerella* and *Candida* species may also be present⁴. Unless estrogen is administered after menopause, the vaginal flora reverts to the premenarchal state⁴.

One major influence on vaginal flora is pH which, in turn, is dependant on hormonal status. An acidic pH of <4.5 is normal in most asymptomatic females of reproductive age. Lactobacilli have a selective advantage and contribute to the acidic condition. They also influence the vaginal ecosystem by the production of hydrogen peroxide and bacteriocins that inhibit the growth of other organisms. Some organisms such as anaerobic streptococci and *Candida* species are also able to tolerate acidic conditions and may be present⁵.

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While the commensal relationship between the ammonium-producing *Prevotella bivia* and the utilization of ammonium by *G. vaginalis* has been demonstrated previously, it is also possible that other associations exist ¹⁰. *Atopobium* species, *Atopobium*-related species, and others species detected may be involved directly in BV or simply react opportunistically when vaginal conditions change. There are reports of *Atopobium vaginae* causing an ovarian abscess and other isolates that originated from blood and amniotic membrane infections ¹⁰. In a 2007 study, *A. vaginae*, identified by polymerase chain reaction, was found to be present in a majority of patients with BV, but absent in most women without BV ⁵. Additional information regarding vaginal samples will be published in an upcoming *CMPT Connections* article.

TREATMENT Metronidazole and clindamycin are used for the treatment of BV. Clindamycin has higher activity against *G. vaginalis* and *A. vaginae* than metronidazole, but not all *A. vaginae* isolates are metronidazole resistant ¹⁰.

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Laboratory examination of vaginal smears and the determination of the Nugent Score						
N Score = The sum of the scores for each bacterial morphotype listed below. (Note Number of Organisms seen / 100X objective)						
<i>Lactobacilli</i>	SCORE	<i>Gardnerella, Bacteroides</i>	SCORE	Curved gram-negative bacilli	SCORE	Sum=*N-SCORE
30 or >	0	0	0	0	0	0
5-30	1	<1	1	<1	1	3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	30 or >	4	30 or >	2	10

*Interpretation of Nugent Score		
If N Score is:	AND:	Then Report:
0-3		Smear NOT consistent with BV
4-6	Clue Cells NOT present	
4-6	Clue Cells ARE present	Smear consistent with BV
≥ 7		