Female Genital Tract: What Diagnostic Testing is Appropriate?

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1. Introduction

In order to set out clinically relevant policies for the work-up of female genital specimens in the laboratory, it is important to understand that the resident microbial population of the vagina changes with alterations in the hormonal milieu[1,2] and or the use of medications that alter the normal flora of the vagina such as broad spectrum antibiotics.

At birth the mucosal surface of the vagina is stratified squamous epithelium (as is the cervix) that is under the influence of maternal estrogen and as a result, the pH is low. Lactobacilli are the predominant flora of the neonate's vagina and colonization by maternal organisms is established during delivery[1]. By 6 weeks of age, the vaginal epithelium thins and the pH rises due to the decline in neonatal maternal hormone levels[2]. Vaginal flora may now include gram-positive cocci such as *Staphylococcus epidermidis* and diphtheroids, but anaerobic organisms such as *Bacteroides, Peptococcus* and *Peptostreptococcus* spp. predominate. *Gardnerella vaginalis* and yeasts may also be isolated in approximately 10% of infants and young girls. **Unless estrogen is administered during menopause, the vaginal flora reverts back to this premenarchal state**.

At menarche, estrogen levels rise and a larger number and a greater variety of organisms colonize the vagina. The cervix transitions from stratified squamous epithelium to columnar epithelial cells. Lactobacilli are the predominant flora in the vagina of most women, but *Corynebacterium* spp. and S. *epidermidis* are also usually present[1,2]. Anaerobes are still found, but they are present in decreased numbers compared to the premenarchal female. C. *albicans* and other yeasts to a lesser extent are recovered in 30% of women. The composition of the normal flora of the vagina also fluctuates during the menstrual cycle, particularly the amount of lactobacilli present[3]. With the decrease of bacterial species found in the vagina towards the onset of menses, yeast may overgrow to cause vulvovaginitis towards the end of the menstrual cycle.

2. Genital Tract Infections

Infections may affect the vagina (vaginitis and bacterial vaginosis) or the cervix (cervicitis is most commonly caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*). The most common viral infectious agents that cause genital tract infections in women

include; Herpes Simplex virus (HSV) , Human Papilloma virus (HPV) and Human Immunodeficiency virus (HIV). This review will not address these viral pathogens.

2.1 Vaginitis: 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 acquisition of specific genital pathogens. Vaginitis is associated with an inflammatory response and PMNs contribute to the abnormal vaginal discharge that develops.

The 2 major causes of vaginitis are: *Candida albicans*, and *Trichomonas vaginalis*

2.1.1. Candidiasis 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. 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[7]. Cultures may be necessary to reliably detect smaller amounts of yeast in vaginal specimens, but should be done at the request of the physician in women who have recurrent infections, and/or in women 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 vaginitis.

2.1.2. *Trichomonas vaginalis* *T. vaginalis is* transmitted by sexual contact, and many women will be asymptomatic with this infection. The most common symptom reported by patients is an increased vaginal discharge. Since T *vaginalis is* a much less common cause of vaginitis, the laboratory may elect to perform diagnostic tests only on request of the physician. Currently the most sensitive diagnostic test for this pathogen is the Trichomonas antigen test[14],[15]. This test is not detrimentally affected by transit times as it does not require the organism to be viable.

Although samples received by the laboratory within 2-4 hours of collection, can have direct examination of a wet mount preparation of vaginal secretions – this method lacks the sensitivity of culture or antigen detection. Delays in the transportation of specimens may hinder the laboratory's ability to detect trichomonads using culture and culture is very labour intensive requiring repeated microscopic reads.

2.2. Bacterial vaginosis (BV) BV is suspected in women with vaginal discomfort and a malodorous discharge, and is clinically diagnosed based on the finding of 3 or more of the following criteria:

- thin homogenous discharge;
- vaginal fluid, pH of >4.5 (litmus test);
- positive "Whiff" test = amine odour with addition of 10% KOH to vaginal secretions
- presence of clue cells (epithelial cells covered with bacteria such that the cell border is obscured) on wet mount preparation of vaginal secretions[4,5].

Unlike vaginitis in BV there is seldom an inflammatory response and often PMNs will not been detected on microscopy.

The laboratory can provide confirmation of BV by examination of a Gram smear of vaginal secretions (Table 2). Nugent [6] have established a specific microscopic grading system for the diagnosis of BV based upon the presence tiny gram variable coccobacilli/gram negative rods with vacuoles suggestive of *Gardnerella/Bacteroides*, curved anaerobic rods suggestive of *Mobiluncus spp.* as well as a corresponding decrease in the normal amounts of lactobacilli present[6]. Cultures are not helpful in the diagnosis, because they do not distinguish between bacterial species of the normal vaginal flora and infection.

BV and Candida infections are by far the most common infections diagnosed in menarchal women. There is NO value in submitting a vaginal swab in the absence of abnormal discharge [i.e. do NOT submit vaginal swabs when women are having routine pre-natal screening, PAP testing or routine physical examination].

3. Cervicitis

The two bacterial pathogens that are most commonly associated with cervicitis are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. **There IS value in submitting a cervical swab to screen for C. trachomatis in asymptomatic sexually active women.**

3.1. *Neisseria gonorrhoeae* and ***Chlamydia trachomatis*:** The premenarchal girl (<12 years of age) is susceptible to vaginal infections due to sexually transmitted pathogens such as N. *gonorrhoeae* and *C. trachomatis* because of the characteristics of the vaginal epithelium [9]. It is therefore important that cultures for *N. gonorrhoeae* be done in pediatric patients. **However, in postmenarchal girls and women, vaginal specimens should not routinely be cultured for *N. gonorrhoeae* or tested for *C. trachomatis* infection since the primary site of infection by these organisms in adult women is the cervix and there is, therefore, a high false-negativity rate of testing vaginal specimens in postmenarchal patients[8].** The preferred sample for optimal laboratory diagnosis of these sexually transmitted infections in postmenarchal girls and women is cervical swabs, and the laboratory should educate physicians that cervical samples must be submitted in order to reliably diagnose cervicitis due to these important pathogens. Currently Nucleic Acid amplification Tests (NAATs) are the optimal diagnostic tool for detection of *C. trachomatis* and *N. gonorrhoeae* (one cervical swab can be used for detection of both pathogens). The superior sensitivity of the NAAT test is because it is not detrimentally affected if organisms die during transit (unlike culture methods).

Many sexually active women who are infected with *C. trachomatis* are asymptomatic. Therefore, screening of sexually active women using NAAT testing of a cervical swab is recommended.

4. Other significant organisms in the Female Genital Tract

4.1. Group B Streptococci: Group B Streptococci (GBS) colonization should only be looked for in pregnant women. It causes early onset sepsis in newborns and pre-natal screening of pregnant women is recommended so that intra-partum antibiotics can be given if the mother is found to be a GBS carrier. The recommended time for screening in the prenatal period is between 36-38 weeks of gestation [16,17]. The physician should collect a SINGLE vaginal-rectal swab for culture[10]. Although vaginal swabs may still be cultured, there is decreased yield if the rectum is also not sampled. All GBS vaginal/rectal cultures should be enriched using Todd-Hewitt broth containing selective antibiotics (Colistin (10 µg/mL) and Nalidixic Acid (15 µg/mL). Only the NAAT test for GBS screening (Cepheid GeneXpert and Cepheid Smart GBS (formerly Strep-IDI)) have received US Food and Drug Administration (FDA) clearance for GBS detection. The test can be used for intra-partum testing on women of unknown GBS status or as a pre-natal screen for GBS.

4.2. Toxic Shock: Cultures for **group A streptococci** and ***Staphylococcus aureus*** should be done in patients suspected of having toxic shock syndrome and retrieval of any amount should be reported. In addition, the isolates should be sent to a reference laboratory for detection of TSST-1 production. If associated with purulence of vaginal secretions, predominant amounts of group A streptococci should also be reported since this organism can cause vaginal infections in young girls and more rarely in postmenopausal women.

4.3 Foreign body infections of the vagina may also occur in young girls and postmenopausal women and typically these infections are caused by an increase in the vaginal anaerobic flora[12]. Gram stain of the vaginal secretions in these cases should be done to confirm an increase in the numbers and types of anaerobes present, but anaerobic cultures of vaginal secretions are not useful since the normal vaginal flora cannot be distinguished from infection.

4.4 Pre-pubescent females: As mentioned previously prior to menarche the cervix in pre-pubescent females is undifferentiated squamous epithelial cells similar to the vagina. As such from this population it is acceptable to submit a vaginal swab when abnormal discharge is detected. The organisms that should be evaluated from such a sample include; Beta-hemolytic streptococci, yeast, *S. aureus*, *H. influenzae*, and predominant heavy growth of *E. coli* or *Pseudomonas aeruginosa. Neisseria gonorrhoeae* should also be evaluated even if there is no indication of sexual abuse – as this genital pathogen can be frequently missed [18]. Although rare, *Salmonella* and *Shigella* spp. may be significant vaginal pathogens in children[13]. A predominant or pure growth of an *Enterobacteriaceae* should therefore be worked-up in a child with purulent vaginal secretions.

4.5 Ureaplasma and Mycoplasma: Mycoplasma and Ureaplasma may have a role in recurrent miscarriage and infertility but because they are frequently carried asymptomatically in the vagina the pathogenicity of these organisms remains controversial. Laboratories should not routinely culture them without discussion with the ordering physician and consultation with a gynecologist should be advised.

The laboratory can influence the effective treatment of most patients with vaginitis by providing a rapid direct microscopy result. **By also encouraging physicians to collect**

genital samples in adult women from the actual site of infection (i.e., cervix NOT vagina) and not culturing and reporting normal vaginal flora, the laboratory can not only enhance the diagnosis of sexually transmitted diseases such as *N. gonorrhoeae,* but also curtail unnecessary antibiotic use.

Table 1 is a summary of the appropriate specimen collection for the female genital tract.

Table 1. Sample Submission and Work-up for Female Genital Samples.*

Clinical Diagnosis	Suspected pathogen(s)	Sample to Submit	Reporting Protocol
Vaginitis [abnormal vaginal discharge] **	*Candida albicans* *Candida* species	Vaginal swab in routine transport media.	Gram stain microscopy to assess yeast and PMNs. (No culture needed.) Report PMNs with quantitation and report yeast as present or absent.
	Trichomonas vaginalis	Vaginal swab placed in transport media for Antigen testing Or: A vaginal swab placed in Trichosel tube for culture	*Trichomonas vaginalis* antigen: is reported as present or absent. Culture: Wet preps performed and *T. vaginalis* reported if present (interim assessment made on receipt and at 48 hours with a final assessment at 5 – 7 days).
Bacterial vaginosis [abnormal vaginal discharge]	Altered normal vaginal flora.	Vaginal swab in routine transport media.	Gram stain prepared and Nugent score to assess for bacterial vaginosis. (See Table 2 for scoring)
Toxic shock associated with tampon use *	*Staphylococcus. aureus*	Vaginal swab in routine transport media.	Culture and report *S. aureus* if present in any amount.
Cervicitis [abnormal cervical discharge]	*Chlamydia trachomatis* *Neisseria gonorrhoeae*	Cervical swab collected using Nucleic Acid Test (NAAT) kit. Culture for *N. gonorrhoeae* not recommended as NAAT is optimal due to better sensitivity).If suspected treatment failure do both NAAT and culture.	Molecular amplification testing and report if *N. gonorrhoeae* or *C. trachomatis* is detected.

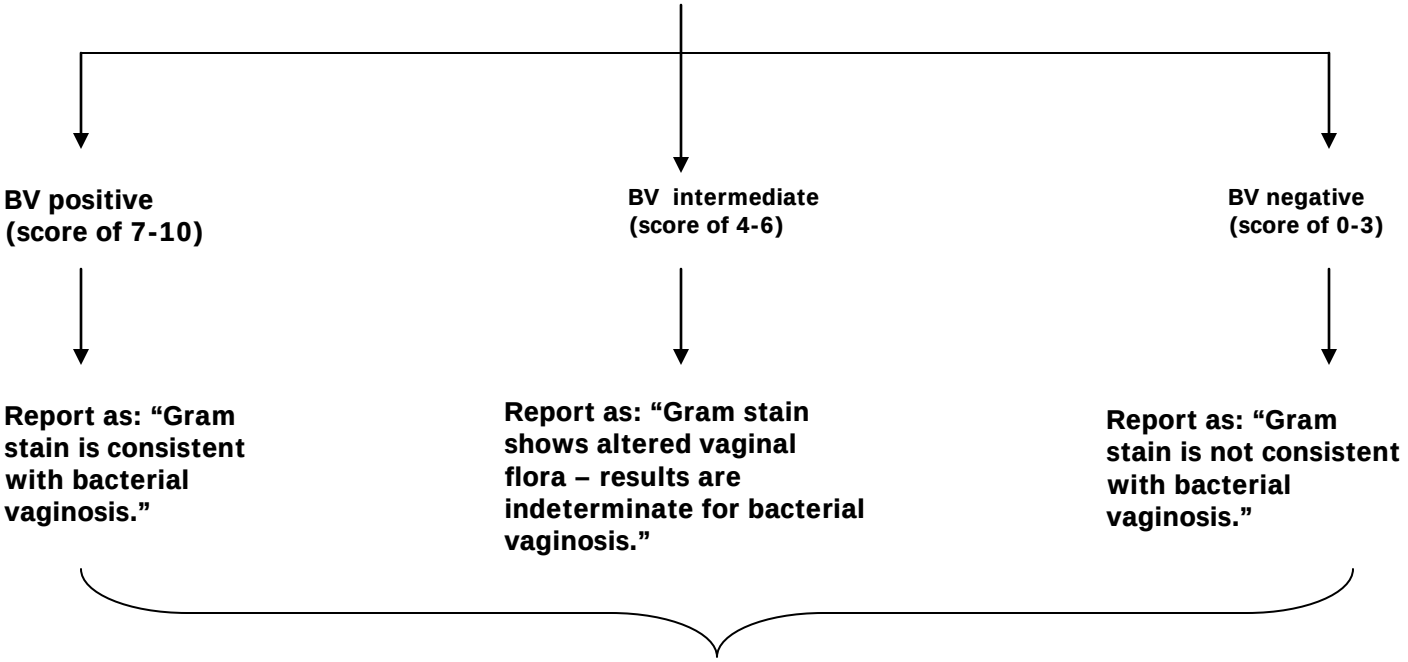
* If a diagnosis other than vaginitis or bacterial vaginosis is suspected (e.g. Toxic Shock), please ensure that it's indicated on the specimen requisition.

** In pre-pubescent females assess culture for: Beta-hemolytic streptococci, yeast, *S. aureus,* Salmonella, Shigella, *H. influenzae*, *N. gonorrhoeae*, as well as a predominant or pure growth of *E. coli/*Enterobacteriaceae or *P. aeruginosa.* If none of these pathogens are isolated report as: "No pathogens associated with pre-pubescent vaginal discharge detected."

Table 2 – Scoring Vaginal Gram Stain for Bacterial Vaginosis

Organism (Morphotype)	Number / Oil Immersion Field	Score
Lactobacillus-like (parallel-sided, gram-positive rods)	>30 5–30 1–4 <1 0	0 1 2 3 4
Mobiluncus-like (curved, gram-negative rods)	>5 <1–4 0	2 1 0
Gardnerella / *Bacteroides*-like (tiny, gram-variable coccobacilli and rounded, pleomorphic, gram-negative rods with vacuoles)	>30 5–30 1-4 <1 0	4 3 2 1 0

Assess smear for the presence and amount of: Lactobacilli, *Gardnerella/Bacteroides* and Curved Gram Negative Rods. Score as indicated above and add the scores together.



Patient ≥ 60 years of age: Add comment:

Gram stains of vaginal smears for bacterial vaginosis in individuals who are post menopausal has not been standardized, clinical correlation is therefore suggested in these patients.

[Table adapted from Bailey and Scott; Diagnostic Microbiology [19]]

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