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| Related Documents |  |  |  |  |
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**PRINCIPLE**

Specimens from genital sites are sent to the clinical microbiology laboratory for detection of microorganisms from females presenting with clinical syndromes such as cervicitis, vulvovaginitis, urethritis, bacterial vaginosis (BV), pelvic inflammatory disease [PID]), endometritis, or genital ulcers and from males exhibiting urethritis, epididymitis, prostatitis, or genital ulcers. Specimens are also submitted from pregnant females to diagnose the presence of organisms that may cause disease in the neonate. Less commonly, specimens are sent from children and postmenopausal women. The syndromes that are associated with female and male genital tract infections are listed in **Tables 3.9.1–1 to 3.9.1–3**, at the back of this procedure, with the major pathogens for each syndrome indicated.

For surgically collected specimens and those from normally sterile sites, a routine wound and abscess culture will detect most bacterial pathogens, as long as selective media for *Neisseria gonorrhoeae* are included. Anaerobic cultures are often indicated for these specimens and mycoplasma cultures may be appropriate in selected cases.

The human vagina is lined with 25 layers of epithelium cells. Many indigenous microorganisms colonize these surfaces. Accurate diagnosis of genital infections from the male and female genitalia depends on the separation of microbial pathogens from the normal genital microbiota. The microorganisms colonizing the female genital tract have been studied extensively and include lactobacilli, C*orynebacterium* spp., *Gardnerella vaginalis,* coagulase-negative staphylococci, *Staphylococcus aureus, Streptococcus agalactiae, Enterococcus* spp., *Escherichia coli,* anaerobes, and yeasts. For prepubescent females, diphtheroids and coagulase negative staphylococci predominate; lactobacilli predominate in the adult female. Postmenopausal women are generally colonized with fewer lactobacilli but have a greater number of *Enterobacteriaceae* than premenopausal women. They also lack the presence of yeasts and mycoplasmas. Many adult female genital tract infections arise from endogenous microorganisms, the pathogenicity of which has been activated by host factors and other microorganisms. Various viruses, including herpes simplex virus (HSV), human papillomavirus (HPV), and human immunodeficiency virus, may also influence the receptivity of the host surface to microorganisms.

The male urethra normally contains relatively few skin microbiota, consisting of coagulase-negative staphylococci, micrococci, *Corynebacterium* spp., and viridans group streptococci. Because the agents of disease have diverse culture and detection requirements and selective media are often needed, *a “routine” genital culture, with the intent to “detect what is there,” rarely has an indication.* Occasionally such specimens are appropriate for prepubescent or postmenopausal women. For certain pathogens, such as *N. gonorrhoeae, Haemophilus ducreyi,* or *S. agalactiae* (group B streptococcus [GBS]), cultures are ordered specifically for those pathogens, with proper collection and selection media. Because of the special nature of cultures for these pathogens, procedures to detect them are not included in this procedure.

To diagnose vulvovaginitis and BV, routine bacterial cultures are nothelpful. Gram stain is useful to diagnose BV by using the Nugent scoring system or assessing for clue cells. A wet mount and a yeast culture and Trichomonas culture are the recommended tests to diagnose vaginitis. It should be noted that by performing only a wet mount, without yeast or *Trichomonas* culture, 50% of either of these agents of vaginitis will be missed. Alternatively, a sensitive DNA probe assay is available via Reference Laboratory that combines the detection of yeasts, *Trichomonas,* and *G. vaginalis* as a marker for BV. For primary syphilis, a dark-field exam is useful (via reference laboratory) but is rarely performed.

For the recognition of toxic shock syndrome (TSS), isolation of *S. aureus* is difficult and not sufficient for the diagnosis. Further characterization of the strain is necessary to confirm the diagnosis. Testing acute- and convalescentphase sera for antibodies to the exotoxin (TSST-1) in a reference laboratory can be helpful. Most patients with TSS lack antibodies at the onset of infection but then produce them in response to the infection. Greater than 90% of women have antibodies to the exotoxin.

**SPECIMEN COLLECTION, TRANSPORT, AND HANDLING**

**A. Specimen collection**

**Female specimens**

a. Amniotic fluid

1. Aspirate fluid by catheter at cesarean section or at amniocentesis.
2. Order **Culture Body Fluid**

b. Bartholin cyst

1. Decontaminate the skin with and aspirate material from the duct(s). NOTE: Bartholin glands are small mucus-secreting glands located beneath the posterior portion of the labia majora.
2. Order **Culture Wound** **and** **Culture Anaerobe**.

c. Cervical

1. Clear away vaginal mucus and exudate with large swab. Moisten speculum with warm water, not lubricants, which can be antibacterial. Using a small swab (not cotton or wood shaft) inserted through a speculum, sample endocervical canal. Avoid the vaginal walls during collection.
2. **See Appendix A for ordering options.**

d. Culdocentesis

1. After cleaning the vaginal wall with surgical disinfectant perform transvaginal puncture of the cul-de-sac to aspirate fluid. NOTE: The cul-de-sac is the pouch between the anterior wall of the rectum and the posterior wall of the uterus. Collection is often done to diagnose PID without more invasive laparoscopy; however, results may not correlate with more invasive testing.
2. Order **Culture Wound** **and Culture Anaerobe**.

e. Endometrium

1. Insert endometrial suction curette or catheter-protected Dacron swab through the cervical os and transfer beyond the cervical opening into the uterine cavity. Collect sample from within the cavity.
2. Order **Culture Wound** **and Culture Anaerobe**.

f. Fallopian tubes and pelvic cavity

1. Collection: obtain aspirates and biopsy samples during laparoscopy. Also sample the pelvic peritoneum. Biopsies often yield better diagnostic specimens.
2. Order **Culture Wound** **and** **Culture Anaerobe**.

h. Vagina

1. Collect fluid from the vagina with sterile pipette or Dacron swab. Successful self-collection of vaginal swabs can be done.
2. **See Appendix A for ordering options.**

i. Vulva

1. Collect only if pain, erythema, or edema is present.
2. Clean the surface of the lesion with 0.85% NaCl and collect by one of the methods below.
	1. Sample exudate or area of erythema with swab for yeast culture.
	2. If there is a vesicle present, collect for HSV culture.
		1. Unroof vesicle.
		2. Collect fluid with a sterile swab *or a*spirate vesicular fluid with a 26- to 27-gauge needle and syringe.
		3. Then scrape the base of the vesicle with a sterile scalpel blade, and collect specimen with a Dacron swab by vigorously rubbing the base of the vesicle.
	3. If there is a crust on the lesion, gently remove it.
		1. Moisten swab with saline and collect specimen by vigorously rubbing the base of the lesion for *H. ducreyi* culture.
		2. Alternatively, gently abrade the lesion with a sterile scalpel or needle until serous fluid emerges. (Try to avoid bleeding.) Irrigate with saline.
		3. For *H. ducreyi* culture, rub the base vigorously with a sterile swab or aspirate fluid with flamed smoothed Pasteur pipette or needle and syringe.
		4. For *Treponema pallidum,* wipe away fluid, blood, and debris with sterile gauze. Apply gentle pressure to the base of the lesion until clear fluid is expressed. Touch a slide to the fluid, and cover the fluid on the slide with a coverslip. If no exudate is present, add a drop of saline to the lesion or insert a needle and syringe at the lesion base, aspirate, and then draw a drop of saline into the needle. Express the material onto a slide.
3. Order ***T. pallidum* dark-field microscopy**, ***H. ducreyi* culture**, or **HSV culture** or request **yeast culture** for most cases showing only erythema or edema.

**Male specimens**

a. Epididymis or testicular fluid

NOTE: The specimen of choice for diagnosis of infected epididymis is urethral culture. If that does not yield a diagnosis, collect first-voided and midstream urine, and compare the yield from smear and culture of each specimen. Collect testicular fluid only if the diagnosis cannot be made otherwise.

1. Disinfect skin surface with surgical disinfectant. Use a needle and syringe to aspirate material from the epididymis or testicles.
2. Choose from the following tests.
	1. Routine **Wound (aerobic) culture** for bacteria, most commonly members of the family *Enterobacteriaceae* or pseudomonads and generally encountered in men over 35 years of age.
	2. M*ycobacterium tuberculosis,* generally occurring after involvement of the prostate or seminal vesicles.
	3. ***Chlamydia trachomatis* and *N. gonorrhoeae* NAAT test**.

b. Penile lesion or vesicle

1. Clean the surface of the lesion with 0.85% NaCl and collect by one of the methods below.
	1. If there is a vesicle present, collect for HSV culture.
		1. Unroof vesicle
		2. Collect fluid with a sterile swab *or*
		3. Aspirate vesicular fluid with a 26- to 27-gauge needle and syringe.
		4. Then scrape the base of the vesicle with a sterile scalpel blade, and collect specimen with a Dacron swab by vigorously rubbing the base of the vesicle.
	2. If there is a crust on the lesion, gently remove it.
		1. Moisten swab with saline and collect specimen by vigorously rubbing the base of the lesion for *H. ducreyi* culture.
		2. Alternatively, gently abrade the lesion with a sterile scalpel or needle until serous fluid emerges. (Try to avoid bleeding.) Irrigate with saline.
			1. For *H. ducreyi* culture, rub the base vigorously with a sterile swab or aspirate fluid with flamed smoothed Pasteur pipette or needle and syringe.
			2. For *T. pallidum,* wipe away fluid, blood, and debris with sterile gauze. Apply gentle pressure to the base of the lesion until clear fluid is expressed. Touch a slide to the fluid, and cover the fluid on the slide with a coverslip. If no exudate is present, add a drop of saline to the lesion or insert a needle and syringe at the lesion base, aspirate, and then draw a drop of saline into the needle. Express the material on to a slide.
2. **Order *T. pallidum* dark-field microscopy, *H. ducreyi* culture, or HSV culture.**

c. Prostate

1. After the patient urinates, perform a digital massage through the rectum.
2. Have patient pass prostatic secretions in the urethra by urinating into a cup. Alternatively, pass the urethral genital wire swab or a bacteriological loop several centimeters into the urethra.
3. Sequential **urine cultures** may be used to diagnose the location of a lower urinary tract infection in men a urethral swab may also be collected for detection of ***N. gonorrhoeae* and *Chlamydia trachomatis*****NAAT**and other urethritis primary pathogens.

**Male or female cultures**

a. Rectal cultures

1. Insert swab past anal sphincter, move swab from side to side, allow 10 to 30 s for absorption, and withdraw.
2. If contaminated with feces, recollect.
3. **Order *N. gonorrhoeae* culture.**

b. Throat cultures

1. Depress tongue gently with tongue depressor.
2. Extend one or two sterile swabs (one for antigen test and one for culture) between the tonsillar pillars and behind the uvula, avoiding the tongue, inner cheeks, and uvula.
3. Sweep the swabs back and forth across the posterior pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain sample.
4. **Order *N. gonorrhoeae* culture.**

c. Urethral discharge

1. Express exudate onto swab from distal urethra.
2. If there is no exudate, collect 1 h after urination. Wipe area clean, insert a urethrogenital swab 2 to 4 cm into the endourethra, gently rotate the swab, leave it in place for 1 to 2 s, and withdraw it.
3. **Order *N. gonorrhoeae* and *Chlamydia* NAAT*.***

Abscess material (e.g., bubo, lymph node, etc.)

1. Disinfect skin with surgical disinfectant.
2. Aspirate the lesion with needle and syringe.
3. **Order Wound Culture with Gram and Culture Anaerobe,** and if indicated from a lymph node, *Chlamydia* or *H. ducreyi* culture.

**B. Transport medium**

1. For transport for specific organisms, refer to the separate procedures. Otherwise, submit swab in Amies transport tube.
2. Place immediately on ice or in the refrigerator until and during transport. NOTE: Previous literature indicates that *N. gonorrhoeae* does not survive well at refrigeration temperatures, but recent studies indicate otherwise.
3. Label specimens and accompanying requisition with patient name, hospital medical record number, room number or clinic location, other patient demographics, and date, time, and site of collection.
4. Indicate the pathogens sought on requisition or computer entry. “Routine genital/urogenital culture” from sexually active patients is not recommended. These are rarely indicated and are performed mostly from prepubescent or postmenopausal females.

**C. Rejection criteria**

1. Vaginal swabs from women in childbearing years for “routine genital culture” are not recommended. Using a form similar to that in **Appendix A** require that the disease or agent sought be ordered specifically.
2. Reject specimens not received in transport medium or in a timely fashion since the agents of genital infections lose viability easily.

**MEDIA Inoculation**

CHOC

Martin Lewis

BAP

CNA

MAC (if invasively collected)

**NOTE:** For invasively collected specimens, refer to other procedures in this handbook for culture, including anaerobic cultures.

**Procedure**

**Inoculation**

**NOTE:** Use of a biosafety cabinet will avoid contamination of cultures as well as protect laboratory processing personnel.

1. If the specimen is not received on plates, inoculate plates from the swab in transport medium.
2. See **Appendix A** for tests for reference to specific organism procedures.
3. For specimens from wounds, abscesses, and normally sterile sites, refer to the wound and abscess procedure or body fluid culture procedure.
4. For cervical, vaginal, or other noninvasive genital source specimens submitted for unusual culture requests, inoculate the first five media listed above.

**Direct smear**

1. Prepare a Gram stain from the swab after plate inoculation, if requested.
2. Stain slide and, for women in childbearing years, observe for evidence of clue cells from vaginal specimens, for yeasts, and for evidence of other bacteria associated with WBCs.

**Incubation**

1. Incubate plates at 35-37°C in (Chocolate in 5% CO2 to provide the proper atmosphere and moisture).
2. Observe for growth after 18 to 24 h of incubation. Hold negative plates for up to 72 hours.

**Culture examination**

1. Observe plates after 24 h for growth of abnormal microbiota.
2. Correlate growth with Gram stain result, if available, to determine the extent of workup.
3. Identify the following pathogens if present, using rapid identification kits as possible.
4. *Streptococcus pyogenes*
5. *S. agalactiae*
6. *Listeria monocytogenes*
7. *N. gonorrhoeae*
8. *Candida albicans.* Mention the presence of other yeasts. *Candida glabrata* has been implicated as a cause of vulvovaginitis.

**NOTE:** Do not examine cervical or vaginal specimens for other *Enterobacteriaceae,* as these microorganisms are normally found in the female genital tract.

1. Identify the following only if the specimen was invasively collected or there is heavy growth and they are the predominant microorganism in the culture.
	1. *Haemophilus* spp. **Refer to Table 3.9.1-6**
	2. Gram-negative rods. **Refer to Table 3.9.1-6**
		1. Enteric gram-negative rods (exception: *Enterobacteriaceae* are part of the normal microbiota of the vagina and should not be reported).
		2. *Pseudomonas* spp. and other non-glucose-fermenting GNRs
		3. *Pasteurella bettyae* (CDC group HB-5). **NOTE:** *P. bettyae* has been associated with genital infections, especially in neonates. It is an indole-positive gram-negative rod, but unlike *E. coli,* it is catalase negative and oxidase variable and does not grow or grows as pinpoint colonies on MAC.
		4. *Capnocytophaga* spp. **NOTE:** This group of organisms have been associated with genital infections. They are catalase-negative, oxidase-negative glucose-fermenting gram-negative rods that do not grow on MAC.
		5. *Campylobacter fetus*
	3. *S. aureus*
	4. *Streptococcus pneumonia*
	5. *Neisseria meningitides*
	6. *G. vaginalis*
		1. For vaginal specimens, do *not* use selective medium to isolate this organism, because the importance of its isolation is determined by the quantity compared to that of lactobacilli in the culture. *G. vaginalis* grows well on both CNA and CHOC.
		2. When present in quantities less than the other normal microbiota, it should be included as part of normal vaginal microbiota. However, for children report its presence regardless of the quantity present.
		3. If it is the predominant microorganism from the female vaginal tract and is isolated in 3 to 4 + quantities (third or fourth quadrant on the plate), report its presence.
		4. Identification
			1. Colonies appear pinpoint and transparent, with no greening of the agar
			2. Gram-variable to gram-negative small, pleomorphic coccobacilli that do not elongate into filaments or chains
			3. Catalase negative.

**NOTE:** The API CORYNE strip accurately identifies this microorganism. It is not necessary to confirm the identification with tests other than colony morphology, catalase, and smear, if the direct Gram stain is consistent with diagnosis of BV.

**Susceptibility testing**

1. Antimicrobial susceptibility testing should be performed on all aerobic bacterial isolates from pelvic or amniotic fluid or tissue specimens that are considered to be primary pathogens.
2. Anaerobes isolated from sterile site / invasive collections such as pelvic or amniotic fluid or tissue specimens considered to be primary pathogens should have antibiotic susceptibility testing sent out.
3. N. *gonorrhoeae* isolated from any genital site should have antimicrobial susceptibility testing performed.
4. Fastidious gram-negative coccobacilli or rods that do not grow on MAC should have a beta-lactamase test performed.
5. Hold positive culture plates for at least 7 days should further testing be indicated.

**REPORTING RESULTS**

1. From surgical specimens and those from normally sterile sites, report the pathogens isolated. For cultures with mixed microbiota, grouping of pathogens may be indicated, e.g., “Mixed enteric rods or mixed anaerobes present.”
2. If no pathogens are isolated but normal microbiota is present, report as “Normal genital microbiota isolated” for vaginal and cervical specimens and as “Normal cutaneous microbiota isolated” for male urethral specimens.
3. Do not list genera and species of normal microbiota individually.
4. If specific pathogens are requested, report “No [pathogen name] isolated.”
5. Quantitate pathogens.
6. Notify caregiver of pathogens of serious significance in pregnant females (e.g., *L. monocytogenes, Group B Strep*).

**INTERPRETATION**

1. The presence of any microorganism from a normally sterile site is generally considered significant.
2. Isolation of sexually transmitted disease pathogens (e.g., *N. gonorrhoeae* and *H. ducreyi*) is considered clinically significant from any genital site. Isolation of certain organisms such as *S. pyogenes* and *Clostridium perfringens* from soft tissue as well as endometrial tissue or transvaginal fluid samples should also be considered clinically significant.
3. The presence of other pathogens may or may not be a cause of disease and must be evaluated with consideration of relative amounts and symptoms or other conditions (e.g., pregnancy) in the patient.

**LIMITATIONS**

1. Many agents of disease are difficult to culture, and the lack of isolation may not indicate that the pathogen is not the cause of disease.
2. Communication between the laboratory and the physician is necessary to provide the appropriate cultures for the disease present, since agents such as *Ureaplasma* and *H. ducreyi* may not grow on routine laboratory media.
3. Unless selective media and incubation are used, a routine genital culture will not detect carriage of GBS in all cases.
4. Because of the difficulty in evaluating the significance of *G. vaginalis* in culture, unless it is clearly predominant and numerous, BV is best diagnosed by Gram stain.
5. The presence of fastidious gram-negative rods in genital specimens may or may not indicate infection. Cases have been reported, but they are infrequent.

**REFERENCE**

Clinical Microbilogy Procedures Handbook. Guidelines for Performanc of Genital Cultures. 3.9. Updated March 2007.

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|  | **Genital Specimen Lab Orders** |
| **Appendix A** |
| **Diagnosis** | **Recommended Test** | **Specimen** | **Additional Information** |
| Bacterial Vaginosis | Gram Stain | Smear of vaginal discharge | No further culture needed if clue cells are present along with amine order |
| Candidiasis | Fungal Culture with smear | Swab moistened with vaginal fluid submitted in 1 ml sterile saline |   |
| Trichomonas infection | Wet Prep | Swab moistened with vaginal fluid submitted in 1 ml sterile saline | Deliver immediately to the laboratory for testing |
| Chancroid | Haemophilus ducreyi culture | Aspirate and scraping of base of ulcer in transport medium | Contact Lab prior to collation for transport media provision |
| Chlamydia Infection | Chlamydia Nucleic Acid Amplification Test (NAAT)  | Collect cervical swab for females; Urethral swab for males; First Void Urine for Males (first void urine is acceptable for females but not preferred) | NAAT from urogenital sources require special collection device. Contact Lab prior to collation for transport media provision. |
| Gonorrhea | GC Culture and Gram OR GC Nucleic Acid Amplification Test (NAAT)  | Collect cervical swab for females; Urethral swab for males; First Void Urine for Males (first void urine is acceptable for females but not preferred); Throat and Rectum collections - GC Culture | Routine culturette is required for culture. NAAT tests require special collection device. Contact Lab prior to collation for transport media provision. |
| Herpes Infection | HSV Culture | Aspirate and scraping of base of vesicle | Contact Lab for M4 Viral Culture Media. Specimen should be placed immediately into culture medium. |
| Genital Mycoplasma infection | Ureaplasma culture | Vaginal or Cervical swab for females or urethral swabs for males | Test should only be ordered for symptomatic patients and not as a screening test. Contact Lab for M4 Viral Culture Media. Specimen should be placed immediately into viral medium. |
| Prevention of neonatal Group B streptococcal (GBS) disease | GBS Culture | Swab from vaginal and anal area collected at 35 to 37 weeks gestation |   |
| Syphillis | Diagnosis is generally done by serologic means |   |   |