

Detailed Collection Procedures for Clinical Microbiology

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BLOOD CULTURES

The procedure for obtaining blood cultures from a central line will be followed by RN's. The procedure for obtaining blood cultures from peripheral sites will be followed by RN's or PCT's. The same person should draw the complete set of blood cultures.

PURPOSE: To obtain blood without contaminating the specimen for identification and/or confirmation of causative organisms in bacteremia and septicemia.

EQUIPMENT: 2 sets of B.C. or 1 Isolator tube (to be drawn at the same time) – 4 bottles.
 **If applicable, refer to unit specific guidelines for obtaining blood cultures.
 2 Blood Culture Prep Kits (available CSS or Distribution Center) containing:

- Blood culture (skin) prep kit
- Disposal bag
- Aerobic bottle
- Anaerobic bottle
- Blood culture bottle adaptor
- Tourniquet
- Alcohol applicator

- 2 – 20ml syringe (30ml if blood cultures for Histoplasma also)(35 ml if both blood cultures for Histoplasma and AFB requested)
- 2 – Sterile needles or vacutainer blood culture adaptor with butterfly needle
- 1 – Isolator tube if culture for Histoplasma is ordered (Obtain from CSS)
- 1 – MB bottle if AFB blood culture is ordered (Call Microbiology 686-6880 and sent via tube system)
- 2 – Band-Aid
- 2 – Microbiology slips/Sunrise transmittal slip
- Patient labels
- Gloves

KEYPOINT: Butterfly needle must be used if using vacutainer adaptor. Do not use straight vacutainer needle.

NURSING ACTION: Identify patient by comparing ID band to lab request. Determine sites and ports the cultures are to be obtained from. Wash hands with 2% CHG.

How to Collect - Blood Cultures

Key Points

1. Procedure to obtain 2 sets B.C.

KEYPOINT: Inoculate blood culture bottle prior to other lab tubes in multiple sample draws. Consider any bottles that are dropped or knocked to be damaged. Do not use!

Blood culture bottles should be stored away from light.

KEYPOINT: If catheter colonization is suspected, do not draw from the same site as the first set – Ex: (Other extremity, implantable port, Hickman). Do not choose site above an I.V. site, but selection below I.V. site is acceptable.

2. Prep the skin using the blood culture prep kit.

- a. Open the kit and remove the sterile antiseptic applicator. Gently squeeze the applicator to release the antiseptic into the sponge.
- b. Thoroughly scrub the site in a back and forth and up and down motion for 30 seconds. Allow to dry for 30 seconds.

KEYPOINT: Palpate site before prepping skin and visually target as site cannot be touched again unless you use a sterile glove or a glove that has been prepped with an alcohol pad.

KEYPOINT: If drawing from central or arterial line, clean each infusion cap or leur-lock connection with a separate blood culture prep kit. Allow to dry before collecting blood.

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KEYPOINT: For immunosuppressed patients, do not waste any blood prior to obtaining cultures from central venous catheter. To discard or not to discard is based on physician preference and the type of sepsis being assessed.	
3. Vigorously wipe septa of the blood culture bottles (or Isolator tube) with 70% alcohol. Allow to dry completely.	KEYPOINT: The septum of the blood culture bottle (or Isolator tube) is not sterile and must be disinfected before injecting blood.
4. Apply gloves and collect a full 10 ml per bottle (adults) and 1 – 1.5 ml (1 ml minimum) per bottle in neonates. 5. If blood cultures for Histoplasma are to be done, draw an additional 10 ml for a total of 30 ml for adults. 6. If also drawing for AFB blood culture, draw an additional 3 – 5 ml for a total of 35 ml for adults	KEYPOINT: If drawing with a winged butterfly set you must inject the blue topped Aerobic bottle first then the purple topped Anaerobic bottle. If drawing with a syringe, either directly or from a central line, then you must inject the purple topped Anaerobic tube first then the blue topped Aerobic tube last.
7. Inject 10 ml of blood into one bottle. If using vacutainer adaptor, be careful not to over fill the bottle. 8. Inject remaining 10 ml of blood into other bottle. 9. Inject 10 ml into Isolator tube (if blood cultures for Histoplasma are ordered). 10. Inject 3 – 5 ml into MB bottle (For AFB blood culture)	KEYPOINT: <u>DO NOT</u> change needles between drawing the specimen and inserting the specimen into collection container.
KEYPOINT: If you are able to obtain only 8-10 ml: inoculate ONLY the aerobic bottle and send to the lab. Note on the transmission slip or lab request that only one bottle was sent due to volume.	
11. Mix blood in bottles by inverting bottles 4-5 times. 12. Place patient label on each bottle. 13. Align the lab label with the bottle label covering the 5 ml incremental marks, but allowing the fluid level to be seen. 14. The Bolded label number is nearest the cap or septum end.	KEYPOINT: Put your initials, site, and time drawn on labels. KEYPOINT: If blood is drawn from a CVL, flush according to unit specific guidelines.
15. Fill out microbiology specimen transmission slip.	KEYPOINT: Put your signature, site, and time drawn on slip. Check box for B.C. or culture for Histoplasmosis as ordered. State probable diagnosis, type of antibiotics, if applicable, on slip. If from central line, record lumen drawn from.
16. Send samples immediately to lab.	
17. Second set of blood cultures should be drawn from a separate site 5-30 minutes after the first set or according to unit/clinic specific protocol.	

A. Rejection criteria

1. Reject blood cultures that are received unlabeled.
2. Reject blood cultures that are mislabeled.
3. Do not process if the tube or bottle is cracked or broken.
4. **Labeled blood cultures are not rejected even if medium is expired, volume or number of bottles is insufficient, or bottles were received >12 h after collection.**
 - a Note these on culture comments and notify unit to check other bottles for expiration dates.

Body Fluid Cultures (except, Blood, CSF, and Urine)

A. Refer to Table 3.5–1 for commonly submitted body fluids and synonyms.

_ **NOTE:** Because the body may respond to infections with infiltration of fluid, some of these sites may have fluid accumulation only during infection.

Table 3.5.–1 Types of body fluids submitted for culture

<u>Fluid</u>	<u>Location</u>	<u>Synonym</u>	<u>Definition of synonym</u>
Joint	At the union of two bones	Synovial	Viscid fluid of the joint cavity
Pleural	Within the membrane surrounding the lungs	Empyema	Fluid with purulent exudate
		Thoracentesis	Fluid collected by aspiration following puncture of chest wall
Peritoneal	Within the membrane lining the abdominal cavity	Abdominal	Same as peritoneal
		Ascites	Abnormal accumulation of fluid in the cavity
		Paracentesis	Fluid collected by aspiration following puncture of cavity
		CAPD	Fluid from peritoneum of patient on CAPD
		PV fluid	Fluid from peritoneum of patient with a shunt tube inserted from the ventricles of the brain, under the skin into the peritoneal cavity
Pericardial	Within the membrane lining the cavity of the heart		
Cul-de-sac	A blind pouch between the anterior wall of the rectum and the posterior wall of the uterus	Culdocentesis	Fluid obtained by transvaginal puncture and aspiration of the cul-de-sac
Amniotic	Within the membrane of the fetus	Amniocentesis	Fluid obtained by puncture and aspiration of amniotic fluid

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Body fluid specimens collected by percutaneous aspiration for pleural, pericardial, peritoneal, amniotic, and synovial fluids

NOTE: Use care to avoid contamination with commensal microbiota.

- A. Clean the needle puncture site with alcohol first.
- B. Next, disinfect site with an iodine solution (1 to 2% tincture of iodine or a 10% solution of povidone-iodine [1% free iodine]). (If tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn.)
- C. Aseptically aspirate with syringe and needle to obtain pleural, pericardial, peritoneal, or synovial fluid.
- D. Vigorously cleanse the septum of each blood culture bottle with 70% alcohol.
- E. Immediately place up to 10 mL of the joint fluid or peritoneal fluid into each blood culture bottle (aerobic and anaerobic).
 - a. **10 mL/bottle maximum.**
- F. Retain some (0.5 ml) and placing in a sterile tube or cup for Gram stain and direct plating.
- G. Submit other fluids and the remainder of specimens **NOT** placed in blood culture bottles in a sterile, leakproof cup.

Drainage tube specimens

NOTE: Submission of drainage fluids, which may be contaminated with skin microbiota, is discouraged. Direct aspiration of the area being drained is preferred.

- A. Disinfect the collection tubing and aseptically aspirate fluid from the tubing.
- B. Submit in sterile, leakproof cup.
- C. **Do not inoculate blood culture bottles**, since they are unlikely to increase the yield of significant microbiota.

NOTE: Swabs afford the least desirable sample for culture, since the quantity of sample may not be sufficient to ensure recovery of a small number of organisms.

Specimen transport

- A. Submit to laboratory as soon as possible.
- B. Do not refrigerate.
- C. Label specimens with patient demographics and date, time, and site of collection: e.g., left knee joint fluid.
- D. **Record the patient diagnosis.**

Rejection criteria

- A. If only blood culture bottles are received, a Gram stain cannot be performed.
- B. Do not submit specimens from drains after they have been infused with antimicrobial agents.
- C. Contact physician if specimen is insufficient for the number of tests requested.
 - a. Routine bacterial culture will identify *Candida* species.
 - b. Fungal cultures of joint and abdominal specimens are rarely needed (*Blastomyces dermatitidis* and *Histoplasma capsulatum*) but are discouraged routinely.
- D. Invasively collected specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.

Catheter Tip Cultures

- A. Collect two blood cultures, one through the catheter and one from a peripheral site at the time the catheter is submitted for culture.
- B. Clean the skin with 70% alcohol prior to catheter removal.
- C. Observing aseptic technique, hold the exposed end of the catheter and carefully remove the catheter from the patient with a sterile instrument. Avoid contact with exposed skin.
- D. Holding the distal end over a sterile tube or specimen cup, cut the tip with a sterile scissors, dropping the last 2 to 3 in. into the tube or specimen cup.
- E. Avoid drying by sealing the tube and submit to the laboratory as soon as possible.
- F. Rejection criteria
 1. Do not accept urinary Foley catheter tips.
 2. Do not accept catheter tips which arrive in saline or transport medium.
 3. Skin swabs from the catheter site are not acceptable specimens. If there is evidence of local tissue infection, an appropriately collected aspirate of the wound is preferable.

Cerebrospinal Fluid (CSF) Cultures:

A. Specimen collection

– **NOTE:** This is a medical procedure that is **performed by a physician** guided by appropriate precautions.

Lumbar puncture

- a. Disinfect the puncture site with antiseptic solution or alcohol.
- b. Insert a needle with stylet at the L3-L4, L4-L5, or L5-S1 interspace. When the subarachnoid space is reached, remove the stylet; spinal fluid will appear in the needle hub.
- c. Measure the hydrostatic pressure with a manometer.
- d. Slowly drain the CSF into the sterile leakproof tubes.
- e. Submit the most turbid tube to microbiology. Otherwise no. 2 is the preferred tube.
- f. Submit an appropriate amount commensurate with the tests required to make the diagnosis, using a guideline of a minimum of 2 ml of fluid for each culture request: routine, fungal, and acid-fast bacillus (AFB).

Ommaya reservoir fluid or ventricular shunt fluid

- a. Clean the reservoir site with antiseptic solution or alcohol prior to removal of fluid.
- b. Remove fluid via the reservoir unit, and place it in a sterile tube.

NOTE: Collect prior to antimicrobial therapy for highest sensitivity.

B. Specimen transport

1. Submit to laboratory as soon as possible and alert laboratory that specimen is in transit.
2. Do not refrigerate.
3. Label specimens with demographic information and time, date, and site of collection, e.g., ventricular shunt, lumbar puncture.
4. Complete requisition with demographic and specimen collection information. Record the **patient diagnosis**.

C. Rejection criteria

1. Call physician to prioritize requests if there is insufficient volume.
- **NOTE:** Fungal and AFB cultures of the CSF are infrequently indicated in acute community-acquired meningitis. *Cryptococcus neoformans* is most rapidly diagnosed by serologic methods or cultures of other sites; fungal CSF culture is discouraged. However, the fungi that cause CSF disease grow well on the media inoculated for routine culture. *M. tuberculosis* is best diagnosed by PCR.
2. Specimens in leaky containers must be processed, but alert the physician of the possibility of contamination.
 3. Unlabeled specimens will have to be labeled by the physician who collected the fluid specimen and appropriate paperwork completed.

Stool Cultures

Stool for Salmonella and Shigella

Stool for Campylobacter

Stool for Yersinia

A. Specimen collection

1. Have patient obtain stool specimen.

- a. Patient should pass the stool into a clean, dry pan or special container mounted on the toilet for this purpose.
- b. Transfer the amount of diarrheal stool or fecal specimen that displaces to the line of the Modified Cary-Blair medium commercial transport vials.

— **NOTE:** Do not fill commercial transport vials above indicator line

— **NOTE:** Do not use toilet paper to collect stool, because it may be impregnated with barium salts, which are inhibitory to some fecal pathogens. The specimen should not be mixed with urine, but semisolid to solid feces can be scooped out of urine, if necessary.

2. Submit duodenal, colostomy, or ileostomy contents in Cary-Blair transport vial.

B. Timing and transport

1. Submit specimen during the acute stage of infection (usually 5 to 7 days), because pathogens decrease in number with time.
2. Submit and culture fresh stool within 30 min of collection to allow for isolation of *Shigella* spp., which are extremely fragile.
3. Store and transport stool in transport medium at 4°C and submit within 24 h for best recovery of pathogens.
4. Generally submit two stools per patient from different days to diagnose bacterial causes of gastroenteritis.

C. Labeling

1. Note on laboratory order form or computer entry screen if the stool is bloody.
2. Label specimen and accompanying requisition with patient name, hospital medical record number, room number (location), physician, culture site, and date and time of collection, AND patient diagnosis.
3. Document when specimen is received; if it is liquid, formed, or solid; or if it contains mucus.

D. Rejection criteria

1. Reject stools not in transport medium.
2. If specimen in transport medium is delayed for more than 3 days at 4°C or is delayed for more than 24 h at 25°C, request recollection since yield will be compromised.
3. Reject routine fecal cultures received from patients hospitalized for >3 days.
4. Too much specimen submitted in vial.
5. If transport vial indicator has turned yellow, reject specimen, since *Shigella* organisms are killed at low pH.
6. Specimen on dry swabs.
7. Stools with barium.
8. Reject multiple specimens received on the same day.
9. Reject fecal specimen if it has been frozen.
10. Reject fecal specimen in transport media other than *modified* Cary-Blair medium.

***Clostridium difficile* Toxin A and B**

A. Collect stool specimens in SEALED leakproof container, and transport and store without preservative at 2 to 8°C until processing (within 24 h of collection).

B. Also acceptable are lumen contents and surgical or autopsy samples of the large bowel.

C. Rejection criteria

– **NOTE:** The SHEA recommends that testing not be performed on asymptomatic patients, even for test of cure.

1. Specimens received on swabs.
2. Reject stools that are not liquid or soft.
3. Request repeat collection if there is not enough specimen for test; 10 to 20 ml of watery, diarrheal stool is preferred, or a minimum of 3 ml or 3 g is required.
4. Meconium may interfere with the assay.
5. Reject specimens from infants less than 1 year of age (SHEA guidelines). Include comment on report indicating lack of specificity of test for this population.

– **NOTE:** Infants have been shown to be asymptomatic carriers, with colonization rates as high as 50%.

6. Limit testing of stools from cystic fibrosis patients, because these patients have been shown to have colonization rates as high as 32%.
7. Patients with positive tests should *not* have repeat testing for cure, unless they again become symptomatic after completion of therapy.

Genital Cultures

A. Specimen collection

Female specimens

Amniotic fluid

- (1) Aspirate fluid by catheter at cesarean section or at amniocentesis.
- (2) Order body fluid culture.

Bartholin cyst

- (1) Decontaminate the skin with povidone-iodine, 3% chloroxylenol, or other surgical disinfectant, and aspirate material from the duct(s).
- (2) Order aerobic genital culture and anaerobic abscess culture

Cervical

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

Culdocentesis

- (1) After cleaning the vaginal wall with surgical disinfectant, such as povidone-iodine or 3% chloroxylenol, perform transvaginal puncture of the cul-de-sac to aspirate fluid.
- (2) Order aerobic genital culture and anaerobic abscess cultures.

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 aerobic genital culture and anaerobic abscess cultures.

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 aerobic genital culture and anaerobic abscess cultures.

Skene's glands

- (1) Decontaminate the skin with surgical disinfectant, and aspirate material from the gland(s).
- (2) Order *N. gonorrhoeae* culture.

Vagina

- (1) Collect fluid from the vagina with sterile pipette or Dacron swab.

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Vulva

- (1) Collect only if pain, erythema, or edema is present.
- (2) Clean the surface of the lesion with 0.85% NaCl.
 - (a) Sample exudate or area of erythema with swab for yeast culture.

Male specimens

Epididymis or testicular fluid

- (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.
 - (a) Routine aerobic genital culture for bacteria.
 - (b) *Mycobacterium tuberculosis*, generally occurring after involvement of the prostate or seminal vesicles.
 - (c) *N. gonorrhoeae* culture.

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) Order a series of urine cultures, with appropriate labeling, and if indicated, add *N. gonorrhoeae* culture or *Trichomonas vaginalis* wet mount. For swab specimens, order *N. gonorrhoeae* culture and wound culture.

Male or female cultures

Rectal cultures:

Use Amies CHARCOAL swabs or directly inoculate Modified Thayer-Martin plates.

- (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.

Throat cultures:

Use Amies CHARCOAL swabs or directly inoculate Modified Thayer-Martin plates.

- (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.

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Urethral discharge:

Use Amies CHARCOAL swabs or directly inoculate Modified Thayer-Martin plates.

- (1) Express exudate onto swab from distal urethra.
- (2) If there is no exudate, collect 1 h after urination. Wipe area clean, insert a urethro-genital 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*.

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

- a. Disinfect skin with surgical disinfectant.
- b. Aspirate the lesion with needle and syringe
- c. Order aerobic and anaerobic abscess cultures.

B. Specimen transport

1. Transport medium
 - a. Submit specimen swab in Amies transport tube. If culturing for GC, use Amies CHARCOAL transport media.
2. 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 and patient DIAGNOSIS.

C. Rejection criteria

1. Do not accept vaginal swabs from women in childbearing years for "routine genital culture."
2. Reject specimens not received in transport medium, since the agents of genital infections lose viability easily.

Ocular Cultures

_ **NOTE:** Most eye specimens are collected by an ophthalmologist. These specimens are inoculated onto culture media at the bedside. The conjunctiva is constantly contaminated by various bacteria from the environment and ocular adnexa. Therefore, specimens from the conjunctiva serve as a control when compared with specimens collected by more aggressive or invasive techniques.

- A. Fresh culture plates are provided as needed to the clinical areas routinely collecting ocular cultures.
- B. Obtain viral and chlamydial samples before topical anesthetics are instilled.
 - 1. Obtain samples for chlamydial cultures with calcium alginate swabs.
 - 2. For viral cultures, use Dacron or cotton swabs with non-wood shafts.

C. **Collection by anatomic site**

_ **NOTE:** *Specimen collection must be performed by a qualified physician.*

Conjunctiva (bacterial conjunctivitis) and lid margin (if staphylococcal blepharoconjunctivitis is suspected)

- a Obtain the specimen with a sterile, premoistened cotton or calcium alginate swab.
- b Roll the calcium alginate or cotton swab over the conjunctiva before topical medications are applied.
- c Culture both eyes with separate swabs.
- d Immediately inoculate the material at the bedside onto BAP and CHOC.
- e Inoculate the swab from the right conjunctiva in horizontal streaks, and inoculate the swab from the left conjunctiva in vertical streaks, each on one half of the same agar plate.
- f Inoculate specimens from the right and left lid margins, if collected, by making an R and an L to represent the respective sites on another agar plate.
- g Obtain conjunctival scrapings for a smear preparation as follows.
 - i Instill 1 or 2 drops of proparacaine hydrochloride.
 - ii Using a Kimura spatula, gently scrape across the lower right tarsal conjunctiva.
 - iii Smear the material in a circular area 1 cm in diameter on a clean glass slide.
 - iv Prepare at least two slides.
 - v Immerse the slides in 95% methyl alcohol or 100% methanol for 5 min.
 - vi Repeat steps on the left conjunctiva.

Bacterial keratitis

- a Instill 1 or 2 drops of proparacaine hydrochloride.
- b Obtain conjunctival samples as described above, and then obtain corneal scrapings from the advancing edge of the ulcer by scraping multiple areas of ulceration and suppuration with a sterile Kimura spatula, using short, firm strokes in one direction. (Keep the eyelid open, and be careful not to touch the eyelashes.)
- c Obtain approximately three to five scrapings per cornea.
- d Inoculate each set of scrapings onto BAP and CHOC, using a C formation for each scraping.
- e Prepare smears by applying the scrapings in a gentle circular motion over a clean glass slide or by compressing material between two clean glass slides and pulling the slides apart.

Bacterial endophthalmitis

- a Collect an aspirate of the vitreous fluid or perform a paracentesis of the anterior chamber using a needle aspiration technique to collect intraocular fluid.
- b Collect specimens for conjunctival cultures along with the fluid to determine the significance of indigenous microbiota.

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- c If a small volume of fluid is collected, inoculate cultures at the bedside by inoculating 1 or 2 drops of fluid onto culture media.
- d Alternatively, submit in anaerobic transport tube or capped syringe after removing needle and replacing the needle with a Luer-Lok.

Preseptal cellulitis

- a Cleanse the skin with alcohol and tincture of iodine or iodophor.
- b In the absence of an open wound, the physician makes a stab incision in either the upper or lower lid with a no. 11 Baird-Parker blade.
- c If there is an open wound, collect the purulent material with a syringe and needle.
- d Inoculate media, and prepare slides as described above for conjunctivitis.
- e Inject some material into an anaerobic transport vial, and process specimens for anaerobes as described in section 4 of this handbook.

Orbital cellulitis

- a Obtain aspirate or biopsy sample of the wound, and process as described above for preseptal cellulitis. Additionally, inoculate fungal media or submit to the laboratory for inoculation.
- b Collect blood cultures.

Dacryoadenitis

- a Collect a specimen of the purulent discharge by using a swab, as described above for conjunctivitis. Inoculate media.
- b Do not perform a needle aspiration of the lacrimal gland.

Dacryocystitis

- a Obtain conjunctival cultures.
- b Press the lacrimal sac to remove exudate material for culture and smear, or collect exudate in a needle and syringe.
- c Place aspirated material in a transport vial, and transport to the laboratory.

Canaliculitis

- a Compress the inner aspect of the eyelid to express pus.
- b Follow procedure outlined above for conjunctivitis.
- c Inoculate media, and prepare slides as described above for conjunctivitis.
- d Additionally, inoculate fungal media or submit to the laboratory for inoculation.
- e Inject some material into an anaerobic transport vial, and process specimens for anaerobes.

B. Rejection criteria

1. Request that a swabbed specimen of the conjunctiva accompany any specimen collected by invasive technique.
2. Even in cases of suspected unilateral conjunctivitis, indicate that bilateral bacterial cultures are mandatory.
3. When inoculated plates are delayed in transit, notify the physician that the culture may be compromised or contaminated.

Detailed Collection Procedures for Clinical Microbiology

<u>Clinical condition and specimen</u>	<u>Expected organism(s)</u>	<u>Primary isolation media</u>	<u>Probable contaminants</u>	<u>Additional comments</u>
Bacterial conjunctivitis	<i>Haemophilus influenzae</i> <i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i> <i>Neisseria gonorrhoeae</i> <i>Streptococcus pyogenes</i> <i>Moraxella</i> spp	BAP CHOC	<i>P. acnes</i> <i>Peptostreptococcus</i> spp. Coagulase-negative staphylococci	<i>P. aeruginosa</i> or <i>Enterobacteriaceae</i> may be cause in immunocompromised or hospitalized patients. Perform anaerobic or fungal cultures if organisms are suspected. Smears: Gram stain
Bacterial keratitis (corneal scrapings)	<i>Pseudomonas aeruginosa</i> <i>S. pneumoniae</i> <i>Moraxella</i> spp. Viridans group streptococcus <i>S. aureus</i> Rapidly growing mycobacteria Contact lens associated <i>Bacillus</i> spp. <i>Serratia</i> spp.	BAP CHOC Fungal media, e.g., potato flake agar Lowenstein-Jensen or other mycobacterial agar and broth media	Coagulase-negative staphylococci Diphtheroids <i>P. acnes</i> Viridans group streptococci	Other causes <i>Enterobacteriaceae</i> <i>N. gonorrhoeae</i> <i>N. meningitidis</i> <i>H. influenzae</i> <i>Acanthamoeba</i> <i>Candida albicans</i> <i>Fusarium</i> spp. Smears of corneal scrapings should accompany cultures
Bacterial endophthalmitis	Postsurgical: <i>S. aureus</i> Coagulase-negative staphylococci <i>S. pneumoniae</i> <i>Streptococcus</i> spp. <i>P. aeruginosa</i> <i>Propionibacterium acnes</i> (postcataract) Traumatic: <i>Bacillus</i> spp. <i>Clostridium</i> spp. Immunosuppression and i.v. drug abusers <i>S. aureus</i> <i>H. influenzae</i> <i>S. pneumoniae</i> <i>Neisseria meningitidis</i> <i>Bacillus</i> spp. <i>Mycobacterium</i> spp.	BAP CHOC Fungal media, e.g., potato flake agar Supplemented blood agar (anaerobic) Lowenstein-Jensen Liquid anaerobic media, e.g., THIO	Coagulase-negative staphylococci Diphtheroids	Conjunctival cultures may be collected simultaneously to determine significance. Smears should accompany cultures of fluid. Postcataract surgery can result in chronic infection, occurring months to years after surgery. Any fungal or bacterial microorganism can be involved.

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<u>Clinical condition and specimen</u>	<u>Expected organism(s)</u>	<u>Primary isolation media</u>	<u>Probable contaminants</u>	<u>Additional comments</u>
Preseptal cellulitis (aspirate)	<i>S. aureus</i> <i>S. pyogenes</i> <i>H. influenzae</i> (6–30 mo) Other streptococci	BAP CHOC Supplemented blood agar for anaerobes THIO (or other anaerobic blood medium)		Do Gram-stained smears. Trauma: <i>Clostridium</i> spp. and mixed anaerobes. Possible other etiologies: <i>P. aeruginosa</i> Other gram-negative bacilli
Orbital cellulitis (aspirate or biopsy specimen)	<i>S. aureus</i> <i>S. pneumoniae</i> <i>P. aeruginosa</i> <i>H. influenzae</i> (under 5 yr) <i>S. pyogenes</i> Gram-negative bacilli	BAP CHOC Supplemented blood agar for anaerobes THIO or other anaerobic medium Fungal media, e.g., potato flake agar	Coagulase-negative staphylococci Diphtheroids	Mixed aerobic and anaerobic infections may occur in trauma cases. Obtain blood culture specimens simultaneously. Do smears along with cultures
Miscellaneous Dacryoadenitis	<i>S. aureus</i> <i>S. pneumoniae</i> <i>S. pyogenes</i>	BAP CHOC	Coagulase-negative staphylococci Diphtheroids (<i>P. acnes</i>) Viridans group streptococci	Gram stains may help determine significance.
Dacryocystitis	<i>S. pneumoniae</i> <i>S. aureus</i> <i>S. pyogenes</i> <i>H. influenzae</i>	BAP CHOC Supplemented blood agar plate for anaerobes THIO or other anaerobic broth	Coagulase-negative staphylococci Diphtheroids Viridans group streptococci	Obtain smears along with cultures. With fistulae, contamination may be difficult to determine.
Canaliculitis	<i>Actinomyces israelii</i> <i>Propionibacterium propionicum</i> <i>Moraxella</i> spp. Diphtheroids Viridans group streptococci	BAP CHOC Supplemented blood agar for anaerobes Fungal media, e.g., potato flake agar THIO or other anaerobic broth	Coagulase-negative staphylococci Diphtheroids <i>P. acnes</i>	Do Gram stains to help determine significance of isolates and/or presence of <i>Actinomyces</i> spp.

a Abbreviations: i.v., intravenous; DFA, direct fluorescent-antibody assay.

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Respiratory cultures:

Table 3.11.1–1 Appropriate specimens for diagnosis of bacterial and yeast upper and lower respiratory diseases

<u>Disease(s) or condition</u>	<u>Common agent(s)</u>	<u>Specimen(s)</u>
Candidiasis (oral thrush)	<i>Candida albicans</i>	Swab of buccal mucosa, tongue, or oropharynx
Cystic fibrosis	<i>Pseudomonas aeruginosa</i> <i>S. aureus</i> <i>Burkholderia cepacia</i> and others	Deep throat Lower respiratory ^a
Diphtheria	<i>C. diphtheriae</i>	Nasopharyngeal swab
Epiglottitis	<i>H. influenzae</i>	Blood culture
Esophagitis	<i>C. albicans</i>	Biopsy sample
Gonococcal pharyngitis	<i>Neisseria gonorrhoeae</i>	Oropharyngeal swab
Laryngitis, bronchiolitis	<i>Mycoplasma pneumoniae</i>	Lower respiratory ^a
Lemierre's disease	<i>Fusobacterium necrophorum</i>	Blood culture
Meningococcal carriage	<i>N. meningitidis</i>	Oropharyngeal swab
Otitis externa	<i>P. aeruginosa</i>	Ear canal swab
Otitis media	<i>S. pneumoniae</i> <i>H. influenzae</i> <i>M. catarrhalis</i> <i>Alloiococcus otitis</i>	Tympanocentesis fluid
Pertussis	<i>B. pertussis</i>	Nasal wash, nasal aspirate, nasopharyngeal swab ^b
Pleural effusion	<i>S. aureus</i> <i>S. pyogenes</i> <i>S. pneumoniae</i>	Pleural fluid
Pneumonia, bronchitis	Many agents <i>M. pneumoniae</i> <i>Legionella spp.</i>	Lower respiratory ^a
Pneumonia, plague	<i>Yersinia pestis</i>	Oropharyngeal swab Lower respiratory
Pneumonia, tularemia	<i>Francisella tularensis</i>	Lower respiratory
Sinusitis (acute)	<i>S. pneumoniae</i> <i>H. influenzae</i> <i>M. catarrhalis</i>	Maxillary sinus puncture and aspiration Rigid endoscopy, <i>NOT</i> Nasal washes or drainage
Staphylococcal carriage	<i>S. aureus</i>	Nasal swab
Streptococcal pharyngitis	<i>S. pyogenes</i>	Nasopharyngeal swab
Vincent's angina	<i>Borrelia vincentii</i> (spirochetes) Anaerobes (fusiform rods)	Oropharyngeal swab

^a Lower respiratory includes sputa, endotracheal aspirates, lung biopsy samples, lung aspirates, and bronchoscopic specimens. Procedures for detection of respiratory viruses (section 10), *Mycobacterium tuberculosis* (section 7), aerobic *Actinomyces* (section 6), and fungi, such as *Histoplasma capsulatum* and *Coccidioides immitis* (section 8), should be included, depending on the symptoms and duration of illness.

^b Specimen is also useful to diagnose diseases caused by respiratory viruses (see section 10).

Lower Respiratory Collection:

A. Specimen collection

Expectorated sputum

- a Do *not* have the patient rinse mouth and gargle with nonsterile water prior to sputum collection, since this can introduce contaminating microbiota.

– **NOTE:** Commensal mycobacteria from tap water can contaminate mycobacterial cultures but are rarely an issue for routine bacteriology culturing. For specialized cultures (e.g., mycobacteria and legionellae), supply sterile saline or water to gargle prior to collection.

- b Instruct the patient not to expectorate saliva or postnasal discharge into the container.
- c Collect specimen resulting from deep cough in a leakproof cup or suitable other collection assembly.

Induced sputum

– **NOTE:** “The utility of induced sputum for detecting pathogens other than *Pneumocystis carinii* or *Mycobacterium tuberculosis* is poorly established”.

- a Using a wet toothbrush and *sterile* water or saline, brush the buccal mucosa, tongue, and gums for 5 to 10 min prior to the procedure. Do not use toothpaste.
- b Rinse the patient’s mouth thoroughly with *sterile* water or saline.
- c Using an ultrasonic nebulizer, have the patient inhale approximately 20 to 30 ml of 3% NaCl.
- d Collect induced sputum in a leakproof cup or suitable other collection assembly.

Tracheostomy and endotracheal aspirates

- a Aspirate the specimen into a sterile sputum trap (e.g., Luken trap) or leakproof cup.
- b Do not culture tracheostomy aspirate unless clinical pneumonia is present (fever and infiltrates). Tracheostomy is followed by colonization within 24 h of insertion, and results may not correlate with disease.

Bronchoscopy specimens—collected by a pulmonologist or other trained physician

- a Bronchoscopy specimens include bronchoalveolar lavage (BAL) samples, bronchial washings, protected specimen brushings (PSB), and transbronchial biopsy specimens.
- b Culture BAL samples and PSB quantitatively or semiquantitatively for bacterial pathogens.
- c Precautions
 - i To avoid excess blood in the recovered fluid, obtain bronchial wash and BAL specimens before brushing or biopsy specimens. Blood may alter the concentration of cellular and noncellular components.
 - ii Avoid suctioning through the working channel before retrieval of specimens to avoid contamination of the specimens.
 - iii Avoid the injection of topical anesthetic agents as much as possible, as the injection method may lead to contamination of the specimen. Aerosol application of anesthetic agents is preferred.
- d To obtain specimens, do the following.
 - i Bronchoalveolar washing or BAL sample

– **NOTE:** The difference between a BAL sample and a bronchial washing is not apparent from the appearance of the specimen. The BAL sample is from the distal respiratory bronchioles and alveoli. Bronchial washings sample the major airways, which is the same area sampled by an endotracheal aspirate.

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- ▶ Pass the bronchoscope transnasally or transorally in nonintubated patients or via the endotracheal tube in intubated patients.
- ▶ Inject sterile nonbacteriostatic 0.85% NaCl (generally 5- to 20-ml aliquots) from a syringe through a biopsy channel of the bronchoscope.
- ▶ Collect BAL sample by carefully wedging the tip of the bronchoscope into an airway lumen and instilling a large volume of sterile, nonbacteriostatic saline (greater than 140 ml). The sample returned contains secretions distal to the bronchioles and alveoli.
- ▶ Gently suction the recovered specimen into a sterile container before administering the next aliquot. (In general, 50 to 75% of the saline instilled is recovered in the lavage effluent.) Keep aliquots separate during collection.
- ▶ Discard the initial fluid as contaminated and submit the rest for culture and staining.

_ **NOTE:** In the laboratory, aliquots from the same site may be combined for microbiology cultures and smears, but aliquots from separate sites (for example, right upper lobe and right lower lobe) should be combined only after consultation with the physician of record.

ii Bronchial brush specimens

- ▶ Instill a brush to collect cellular material from the airway wall. This is the best specimen for viral culture and cytology studies.
- ▶ Only PSB are acceptable for bacterial culture. Obtain by inserting a telescoping double catheter plugged with polyethylene glycol at the distal end (to prevent contamination of the bronchial brush) through the biopsy channel of the bronchoscope.
- ▶ Place specimen in 1 ml of nonbacteriostatic saline.

iii Transbronchial biopsy samples

- ▶ Obtain the biopsy sample through the biopsy channel of the bronchoscope, and transport it in a sterile container with a small amount of nonbacteriostatic sterile saline.

Lung aspirates—collected by trained physician

- a Use a computed tomography scan to obtain lung aspirates by inserting a needle through the chest wall into a pulmonary infiltrate.
- b Aspirate material from the lesion.
- c If the lesion is large or if there are multiple lesions, collect multiple specimens from representative sites.
- d Transport specimen without needle in syringe capped with Luer-Lok or transfer to sterile tube or Vacutainer.

Lung biopsy samples—collected by trained physician

- a Obtain a 1- to 3-cm-square piece of tissue, if possible.
- b If the lesion is large or if there are multiple lesions, collect multiple specimens from representative sites.
- c Submit in a sterile container(s) without formalin.

Pleural fluid

—SEE Body Fluid Cultures

B. Specimen transport

1. Submission to the laboratory
 - a Collect specimens in leakproof cups or suitable other collection assembly (Luken trap) and label with source of material.
 - b Submit aspirates a sterile tube.
2. Label specimens with demographic information, date and time of collection, and site of collection.
3. List the diagnosis or ICD-9 code for proper evaluation of the cultures.
4. Order the appropriate tests.

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– **NOTE:** Anaerobic cultures should be limited to specimens that are not contaminated with upper respiratory microbiota (e.g., PSB and biopsy samples) and only when aspiration pneumonia or similar disease is being considered.

5. Store specimen at 2 to 8°C until cultures can be submitted or processed.

– **NOTE:** A delay in processing of more than 1 to 2 h may result in loss of recovery of fastidious pathogens, such as of *S. pneumoniae*, and overgrowth of oronasal microbiota.

C. Rejection criteria

1. Do not accept repeat cultures at intervals of less than every 48 h.
2. Reject the following specimens for diagnosis of lower respiratory tract disease.
 - a 24-h sputum collection
 - b Contaminated sputum and endotracheal specimens per Gram stain rejection criteria
 - c Saliva
 - d Induced sputum
 - e Nasal washes and aspirates or swabs of nares.
 - f Throat specimens, since they are not indicative of the infection of the lower airways (5)
 - g Specimens for anaerobic culture, except transtracheal aspirates, PSB, biopsy samples, pleural fluid, or other uncontaminated specimens
3. Culture bronchial brushings, if they are not collected with a protected catheter, only if the PSB is not available.
4. Bronchial washings are the secretions aspirated from the major airways and are less suitable for bacterial culture than BAL specimens collected from the bronchiolar and alveolar spaces. If both are received, culture only the BAL specimen quantitatively.
5. For specimens delayed in transit more than 2 h without refrigeration, indicate on the report that the delay in transit may compromise the culture results.

Respiratory specimens from patients with Cystic Fibrosis (CF)

A. Specimen collection

1. Collect the following specimens for culture of respiratory secretions from CF patients.
 - a Deep pharyngeal (also referred to as “cough or gagged” throat specimens)
 - i Place a plastic shaft, Dacron- or rayon-tipped swab in the back of the throat and induce coughing.
 - ii Remove the swab when coughed secretions have been collected.
 - _ **NOTE:** This technique is used in children, usually <10 years of age, who are unable to produce sputum. *Do not use* this technique in children or adults who are able to produce sputum.
 - b Sputum
 - c Endotracheal aspirates (on ventilated patients)
 - d Bronchoscopically obtained specimens, including bronchoalveolar lavage (BAL) specimens, protected specimen brushings, and transbronchial biopsy specimens. See quantitative culture method.
2. Use the same transport devices for these specimens obtained from CF patients that would be used for non-CF patients.
3. If the specimen cannot be transported and processed within 4 h, it may be held at 4°C for up to 24 h without affecting the recovery of the major pathogens of interest in CF patients.

B. In addition to specimen labeling which meets good laboratory practices, identify the specimen as being obtained from a “cystic fibrosis patient” to allow appropriate specimen processing and workup.

C. Rejection criteria

1. Since bronchoscopic specimens are obtained at significant expense and some degree of risk to the patient, all attempts should be made to process these specimens, even if they are compromised. However, on the final reports, note that specimen quality may have been compromised.
2. Process no more than one specimen per month from nontransplant CF patients who are outpatients without physician or other caregiver consultation.
3. Process no more than two specimens per admission for nontransplant CF patients who are inpatients without physician or caregiver consultation.
4. If a swab is received labeled as sputum, contact caregiver prior to processing, to determine source of specimen. It is likely a deep pharyngeal specimen.
 - _ **NOTE:** The microbiota responsible for chronic lung disease in CF patients is very stable, with patients being infected with organisms such as *S. aureus* or *P. aeruginosa* for months to years.
5. No guidelines currently exist for frequency of culture from CF lung transplant recipients. Process specimens by request.
6. **Do not use culture rejection** criteria for sputum or endotracheal aspirates based on Gram stain quality, since they are of little value in CF patients. Specimens from CF patients will grow potential pathogens >90% of the time, although approximately 40% would be rejected based on the Gram stain evaluation.

Respiratory specimens for Legionella:

A. Specimen collection (see collection of lower respiratory specimens)

- _ **NOTE:** *L. pneumophila* survives in up to 3% salt solutions at temperatures below 30°C; in fact, small amounts of salt (0.1 to 0.5%) enhance survival (4). Saline is not toxic to the organism, as previously thought.

A. Respiratory secretions (sputum, bronchial and tracheal aspirates, bronchial washings)

1. Place expectorated specimen in a sterile screw-cap cup.
2. Specimens collected by bronchoscopy or aspiration may remain in the Luken trap for transport so long as the free ends of the tubing are securely joined together to prevent leakage of the specimen during transport.
3. Collect at least 3 ml of specimen for *Legionella* culture.

B. Bronchoalveolar lavage (BAL) fluid

Submit a minimum of 50 ml of fluid in a sterile container.

C. Sterile body fluids (pleural, pericardial, peritoneal)

1. Submit at least 5 ml in a sterile tube or in the syringe used for collection.
2. Remove the needle and cap the syringe with a Luer-Lok before transport.

D. Bronchial brushings

1. Cut the brush off at a point about 30 to 40 mm from the end.
2. Place the brush into a small tube containing no more than 0.5 ml of sterile saline or TSB.

E. Lung tissue

1. Place a piece of tissue approximating the size of a dime onto a gauze square moistened with sterile saline.
2. Place the gauze square and the tissue into a sterile specimen cup with a screw cap.

F. Other tissues and wound specimens (including prosthetic heart valves)

1. Perform culture for legionellae on such specimens, especially if routine bacterial cultures prove to be negative.

- _ **NOTE:** Legionellae survive in the specimen when stored in the refrigerator for long periods of time. However, they may also be present in a backup broth used for culture but will not grow or be detected in the broth. If the routine culture is negative, and the specimen is no longer available, subculture the broth to selective medium for legionellae. Postsurgical wound infections due to use of contaminated water for wound care have been reported.

2. Collect tissues as for lung tissues.
3. Collect external wound specimens on swabs after cleansing the site with sterile saline.

G. Blood

There are no reliable methods for recovering legionellae from blood. "Blind subculture" of "negative" standard blood culture bottles sometimes results in recovery of the organism. The sensitivity of this method is not sufficient for routine use.

B. Timing and transport

1. Submit samples in the acute phase of infection, preferably before the beginning of antimicrobial therapy.
2. Transport to laboratory quickly. While legionellae may survive extended transport, their isolation may be compromised by overgrowth of commensal bacteria in the specimens.
3. If specimens are being transported to a remote laboratory, place samples on wet ice for transport.
4. For extended transport times (>1 day), freeze samples (-70°C) and transport on dry ice.

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C. Rejection criteria

1. Respiratory secretions (sputum, etc.) submitted for routine bacterial culture are screened for adequacy by Gram stain; **do not apply** these criteria for specimens submitted for *Legionella* culture.
 - **NOTE:** Patients with Legionnaires' disease typically produce sputum which is thin and watery and may contain few WBCs. Additionally, only normal oral bacterial morphotypes may be seen in Gram stains because legionellae do not stain with Gram stain reagents in clinical specimens.
2. Reject BAL specimens with a volume of less than 30 ml. The procedure produces specimens which are very dilute and which must be concentrated by centrifugation before culture.
3. Do not perform "quantitative BAL cultures" for legionellae; their concentration in such specimens is always low.
4. Pleural fluid specimens of less than 5 ml should be cultured only after alerting the physician that such specimens are unreliable for the recovery of *Legionella*.
5. Reject "test-of-cure" cultures, since they should not be used to monitor a patient's response to therapy.

Otitis (Ear) specimens:

A. Specimen collection

External ear

- a Insert sterile swab into ear canal until resistance is met.
- b Rotate swab and allow fluid to collect on swab.

Tympanocentesis fluid

– **NOTE:** Because of the invasive nature of the collection process, these specimens are usually submitted primarily to diagnose middle ear infections only if previous therapy has failed.

- a Clean the external canal with mild detergent.
- b Using a syringe aspiration technique, the physician will obtain the fluid from the ear drum.
- c Send the specimen in a sterile container or in the syringe capped with a Luer-Lok and with the needle removed.
- d If the eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum.

B. Specimen transport

1. Submit to laboratory
 - a Submit swabs in tube of transport medium or in BD Culturette EZ.
 - b Submit aspirates in a sterile container or in the original syringe capped with a Luer-Lok to prevent leakage.
2. Label specimens with demographic information, date and time of collection, and site of collection.
3. List the diagnosis of otitis media, chronic otitis, or otitis externa.

Respiratory specimens for *Bordetella pertussis*

A. Specimen collection

1. Timing

- a Collect as soon as possible after symptoms develop.
- b Collect specimens up to 4 weeks after onset, provided that antimicrobial therapy has not been started.

— **NOTE:** The organism is rarely found by culture after the fourth week of illness, and the percentage of positive culture results decreases with time.

2. Collection of nasopharyngeal specimen

- **NOTE:** *B. pertussis* specifically to ciliated respiratory epithelial cells. Since the nasopharynx is lined with these cells, it is a far superior site for detection of the bacterium.

Nasopharyngeal swabs (refer to Fig. 3.11.6–1A)

— **NOTE:** Nasopharyngeal swabs *cannot* be used for PCR since both the alginate component and the aluminum shaft inhibit PCR-based assays.

- a Use a *calcium alginate* or *Dacron fiber tip* swab on a fine flexible wire. Bend the wire so that it mimics the curve of the nasal airway and gently pass the swab through the nostril to the posterior nasopharynx. Do not force the swab; resistance will be felt when the posterior nasopharynx is reached.
- b Rotate the swab and leave it in place for up to 30 s or until the patient coughs. Withdraw as quickly as possible.
- c Repeat procedure through the second nostril.
- d Submit both swabs for culture and DFA testing.

Nasal wash: syringe method (refer to Fig. 3.11.6–1B)

- a Use a 3- to 5-ml syringe with a 2-in 18-gauge tubing attachment. Fill the syringe with saline.
- b Instruct the patient not to swallow during the procedure.

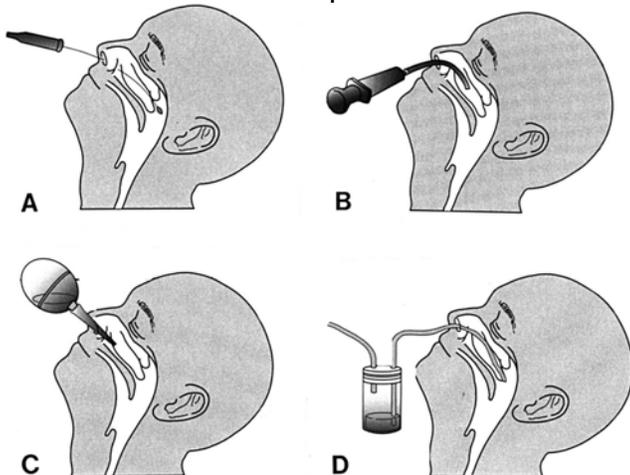


Figure 3.11.6–1 Collection of nasal pharyngeal swab(s) (A), nasal wash specimen(s) by syringe method (B), nasal wash specimen(s) by bulb method (C), and nasal aspirate specimen(s), assisted by vacuum (D). Diagrams courtesy of BD Diagnostic Systems, Sparks, Md., with permission.

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- c With the patient's head hyperextended (approximately 70° angle), quickly instill approximately 5 ml of sterile 0.85% NaCl into one nostril.
- d Immediately aspirate the saline solution back into the syringe, *or*
- e Tilt the head forward and allow the fluid to run out of the nares into a sterile container, *or*
- f Aspirate the fluid by inserting a rubber bulb syringe into each nostril.
- g Place the specimen in a sterile container.

Nasal wash: bulb method (refer to Fig. 3.11.6–1C)

- a Suction 3 to 5 ml of sterile 0.85% NaCl into a 1- to 2-oz tapered rubber bulb.
- b Instruct the patient not to swallow during the procedure.
- c With the patient's head hyperextended (approximately 70° angle), insert the bulb into one nostril until the nostril is occluded.
- d Quickly instill the sterile saline into the nostril with one squeeze of the bulb.
- e Immediately release the bulb to collect the nasal wash specimen.
- f Empty the bulb contents into a sterile container and transport.

Nasal aspirate: vacuum assisted (refer to Fig. 3.11.6–1D)

- a Connect a mucus trap (i.e., Luken's tube) to a suction pump and catheter, turn on suction, and adjust to suggested suction pressure (see chart below).
- b Insert the end of the catheter through the external nares to the posterior pharynx.
- c Apply suction while slowly withdrawing the catheter, allowing the catheter to remain in the nasopharynx no longer than 10 s.
- d After aspiration, flush material out of the catheter with a small volume (1 to 1.5 ml) of sterile saline.

Patient age	Catheter size (French)	Suction pressure (mm Hg)
adolescent / adult	14	120 – 150

– **NOTE:** Nasopharyngeal aspiration or wash yields sufficient material for numerous diagnostic procedures and gives an 11% higher yield on culture than nasopharyngeal swabs. Aspirate specimens are easily divided and saved, are suitable for all testing methodologies, and can be frozen for long periods (2 years at -70°C).

– **NOTE:** Nasopharyngeal specimen collection directions are taken in part with permission from BD Diagnostic Systems, Sparks, Md.

B. Specimen transport

- 1. Inoculate plates at bedside.

– **NOTE:** The best culture results are achieved when specimens are plated directly onto culture media at the bedside; unfortunately, for many this is not possible or practical to establish as a routine. Bedside plating results in a higher positive culture rate than that obtained with nasopharyngeal aspirates taken at the same time and cultured the same day in the laboratory.

- 2. Alternatively, submit in Amies transport medium

C. Rejection criteria

- 1. Throat specimens, nares swabs, and sputum are unacceptable specimens.
- 2. Do not perform cultures from specimens collected on rayon or cotton swabs, as they contain fatty acids that inhibit growth.

Urine culture specimens

A. Specimen collection

NOTE: All urine submitted for culture MUST be placed in the BD vacutainer “Urine C&S Preservative Plus Plastic Tube” (This is a gray top tube with a YELLOW label.)

Clean-voided midstream urine collection

a Preparation

i Females

_ **NOTE:** Collection of midstream urine specimens should be avoided during menses.

▶ While the labia are held apart with the aid of a pair of sponges, wash the vulva thoroughly from front to back with supplied towettes. Special attention should be paid to the urethral meatus.

▶ Then rinse the vulva.

ii Circumcised males: no preparation for midstream specimen.

iii Uncircumcised males: the process is similar to that described above for females.

▶ Retract the foreskin, and wash the glans penis thoroughly with two successive cotton pledgets or sponges soaked in soap, paying special attention to the urethral meatus.

▶ Rinse the glans with additional successive pledgets with sterile water or saline.

b Have the patient collect voided urine directly into a disposable leakproof container, instructing the patient to not halt and restart the urinary stream for a “midstream” collection but preferably move the container into the path of the already voiding urine.

_ **NOTE:** Never collect urine from a bedpan or urinal.

Catheter urine

a Using a needle and syringe, collect urine through the catheter port, after cleaning with alcohol. Alternatively, collect the sample directly into a Vacutainer tube without anticoagulant, using a Vacutainer holder and needle. *Do not send urine obtained from a catheter bag.*

b A straight catheter (in and out) is used by a physician or trained health care worker (HCW) to obtain urine directly from the bladder.

i This procedure must be carried out with aseptic technique, to avoid the risk of introducing microorganisms into the bladder.

ii Discard the initial 15 to 30 ml of urine and submit the next flow of urine for culture.

Ileal conduit

a Remove the external device.

b Cleanse the stoma with 70% alcohol followed by iodine.

c Remove the iodine with alcohol.

d Insert a double catheter into the cleansed stoma, to a depth beyond the fascial level, and collect the urine.

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Suprapubic needle aspiration:

--performed by a physician or trained HCW.

This method is the preferred method for infants, for patients for whom the interpretation of results of voided urine is difficult, and when anaerobic bacteria are suspected as the cause of infection.

- a Bladder should be full and palpable before aspiration.
- b Shave and disinfect the skin over the bladder.
- c Make a small lance wound through the epidermis above the symphysis pubis.
- d Aspirate using a needle and syringe. Submit in syringe or cup.

Prostatic massage is used primarily to diagnose acute or chronic prostatitis.

For both diseases, gram-negative enteric organisms are the most frequently isolated pathogens. *Neisseria gonorrhoeae* is found infrequently but is sometimes implicated in acute prostatitis. Fluid can also be used to demonstrate *Trichomonas* in males who act as vectors.

- a Perform a digital massage through the rectum.
- b Collect the specimen in a sterile tube or on a sterile swab.
- c Label clearly (e.g., "EPS" [for expressed prostatic secretions] or "VB3" [for voided bladder—third urine collection]) for proper culturing.

Cystoscopy

This is a bilateral ureteral catheterization to determine the site of infection in the urinary tract. This procedure is usually performed in specially designated areas such as operating rooms or specialty clinics.

- a Clean the urethral area (and vaginal vestibule in females) with soapy water, and rinse the area well with water.
- b Insert a cystoscope (obturator in place) into the bladder.
- c With sterile technique, collect approximately 5 to 10 ml of urine from open stopcock into a sterile container.
- d Label this sample "CB," for catheterized bladder urine, and refrigerate it. Then irrigate the bladder. (Use sterile nonbacteriostatic 0.85% NaCl to irrigate the bladder.)
- e After irrigation of the bladder and insertion of the ureteral catheters, collect irrigating fluid passing from the bladder through the ureteral catheters by holding the ends of both catheters over an opened sterile container.
- f Label this sample "WB," for washed bladder urine, and refrigerate it.
- g Pass the ureteral catheters to each midureter or renal pelvis without introducing additional irrigating fluid. Open both stopcocks of the cystoscope to empty the bladder.
- h Discard the first 5 to 10 ml of urine from each ureteral catheter.
- i Collect four consecutive paired cultures (5 to 10 ml each) directly into opened sterile containers.
- j Label these specimens "LK-1," "RK-1," "LK-2," and "RK-2" (LK for left kidney and RK for right kidney). Submit all samples for culture.

B. Timing of specimen collection

1. Obtain early-morning specimens whenever possible. Allowing urine to remain in the bladder overnight or for at least 4 h will decrease the number of false-negative results.
2. Do not force fluids in order to have the patient void urine. Excessive fluid intake will dilute the urine and may decrease the colony count to $\leq 10^5$ CFU/ml.

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C. Specimen transport

1. Collect and transport in BD vacutainer "Urine C&S Preservative Plus Plastic Tube" (This is a gray top tube with a YELLOW label.)
 - a. Place at least 3 ml of urine into the transport tube to avoid an inhibiting or diluting effect on the microorganisms.

D. Specimen labeling and request submission

1. Label the urine container with demographic information of the patient and the time of collection. Ensure that the collection method is communicated to the laboratory.
2. **NOTE:** Fungal cultures of urine usually are requests to detect the presence of yeasts. Notify the physician that yeast cultures are included as part of the routine urine culture and yeasts will be cultured and reported if found.

E. Rejection criteria:

1. Urine in containers other than the BD Urine C&S Preservative tube.
2. Request a repeat specimen or obtain the information when the collection time and method of collection have not been provided.
3. Reject 24-h urine collections.
4. **Reject urine specimens obtained with the same collection method within 48 h of receipt of first specimen. Call this a duplicate specimen.**
5. For infants, a voided specimen provides misleading information; a catheterized specimen should be collected. Institute a policy to discourage submission of voided or bagged specimens for culture.
6. Reject Foley catheter tips as unacceptable for culture; they are unsuitable for the diagnosis of urinary infection (3).
7. Reject urine from the bag of a catheterized patient.
8. Reject specimens that arrive in leaky containers.
9. **Except for suprapubic bladder aspirates, reject specimen requests for anaerobic culture.**

Wound and Soft Tissue Cultures

A. General considerations

1. *Preferably collect specimen prior to initiation of therapy and only from wounds that are clinically infected or deteriorating or that fail to heal over a long period.*
2. Cleanse skin or mucosal surfaces.
 - a For closed wounds and aspirates, disinfect with 2% chlorhexidine or 70% alcohol followed by an iodine solution (1 to 2% tincture of iodine or a 10% solution of povidone-iodine [1% free iodine]). Remove iodine with alcohol prior to specimen collection.
 - b For open wounds, debride, if appropriate, and thoroughly rinse with sterile saline prior to collection.
3. Sample viable infected tissue, rather than superficial debris.
4. Avoid swab collection if aspirates or biopsy samples can be obtained.
5. Containers
 - a Anaerobe transport vial for small tissues
 - b Sterile cup for large tissues with nonbacteriostatic saline on a gauze pad to keep moist
 - c Syringes with safety devices to protect from needle exposure
 - i Expel the air from the syringe, remove the needle after activating the safety apparatus, and cap the syringe with a sterile Luer-Lok.
 - ii Alternatively, place the aspirated contents in a sterile blood collection tube without anticoagulant (e.g., Vacutainer or similar type).
 - d Broth culture medium in small sterile snap-top microcentrifuge tubes for fine-needle aspirates (FNA). These tubes are ideal for this type of specimen, because the specimen is easily visible and can be minced with a sterile glass rod in the laboratory, if necessary.
 - e Swabs (*ideally, submit two, one for Gram stain and one for culture*) in an Amies gel swab transport system.

B. Specimen collection after proper disinfection

Closed abscesses

- a Aspirate infected material with needle and syringe.
- b If the initial aspiration fails to obtain material, inject sterile, nonbacteriostatic saline subcutaneously. Repeat the aspiration attempt.
- c Remove needle and submit with Luer-Lok on the syringe or place contents in a sterile blood collection tube without anticoagulant.

FNA

- a Insert the needle into the tissue, using various directions, if possible.
- b If the volume of aspirate is large, remove the needle and submit with Luer-Lok on the syringe.
- c If the volume is small, aspirate the specimen into the sterile locking microcentrifuge tube containing broth by drawing up and down to release the specimen from the syringe.

_ **NOTE:** Always use a safety device on the needle. Do not submit needle to the laboratory.

Open wounds

- a Cleanse the superficial area thoroughly with sterile saline, changing sponges with each application. Remove all superficial exudates.
- b Remove overlying debris with scalpel and swabs or sponges.
- c Collect biopsy or curette sample *from base or advancing margin of lesion*.

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Pus

- a Aspirate the deepest portion of the lesion or exudate with a syringe and needle.
- b Collect a biopsy sample of the advancing margin or base of the infected lesion after excision and drainage.
- c For bite wounds, aspirate pus from the wound, or obtain it at the time of incision, drainage, or debridement of infected wound. (Do not culture fresh bite wounds, as there is generally not yet evidence of infection. These wounds will harbor the resident respiratory microbiota introduced from the bite, but cultures cannot predict if they will cause infection.)
- d Submit as for closed abscesses.

Tissues and biopsy samples

- a Collect sufficient tissue, avoiding necrotic areas. Collect 3- to 4-mm biopsy samples.
 - b Place small pieces of tissue in anaerobic transport vial; place larger pieces of tissue in a sterile container.
2. Collect swabs **only** when tissue or aspirate cannot be obtained.
- a Limit swab sampling to wounds that are clinically infected or those that are chronic and not healing.
 - b Remove superficial debris by thorough irrigation and cleansing with nonbacteriostatic sterile saline. If wound is relatively dry, collect with two cotton-tipped swabs moistened with sterile saline.
 - c Gently roll swab over the surface of the wound approximately five times, focusing on area where there is evidence of pus or inflamed tissue.
 - d If anaerobic and aerobic culture is indicated, transfer swabs immediately to an anaerobic transport tube or submit in CultureSwab EZ II system. For aerobic culture, submit in aerobic transport tube or CultureSwab EZ II system.

NOTE: Organisms may not be distributed evenly in a burn wound, so sampling different areas of the burn is recommended. Blood cultures should be used to monitor patient status.

B. Label specimen and requisition.

1. List demographic information on the patient.
2. Describe the type of specimen (deep tissue, superficial tissue, decubitus, catheter site, boil, abscess, cellulitis, aspirate, pus, drainage, surgical incision site, etc.)
3. State anatomic location (arm, leg, etc.)
4. Record collection time and date.
5. List diagnosis or ICD9 code, including cause and clinical signs of infection.
6. List antimicrobial therapy prior to specimen collection.
7. Choose tests requested, including anaerobic culture, if appropriate.

NOTE: To avoid the overuse of full fungal cultures that require incubation periods of greater than 1 week, the laboratory can offer a fungal culture with a shorter (2- to 4-day) incubation period. Such cultures are useful and cost-effective for the diagnosis of nosocomial, foreign-body, and postoperative infections, where the likely pathogen is either bacteria, *Candida* species, or *Aspergillus* species. *Candida* or *Aspergillus* species will grow on routine bacterial culture media within 1 week; however a selective fungal medium may be indicated for cultures expected to contain mixed microbiota. Full fungal cultures should be reserved for diagnosis of chronic infections, particularly those caused by dematiaceous and biphasic molds, and should be performed only from specimens not submitted on swabs.

C. Deliver aspirates and tissues to the laboratory within 30 min for best recovery.

1. Keep tissues moist to preserve organism viability.

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2. Do not refrigerate or incubate before or during transport. If there is a delay, keep sample at room temperature, because at lower temperature there is likely to be more dissolved oxygen, which could be detrimental to anaerobes.

D. Rejection criteria

1. Do not accept specimens for microbiological analysis in container with formalin.
2. If numerous squamous epithelial cells are present on the Gram stain, especially from swab specimens, request a recollection if there is evidence of infection.
3. Discourage submission of specimens to determine *if* an infection is present.
4. Reject swabs that have been delayed in transit more than 1 h if they are not in some transport system (either CultureSwab EZ II system or one with preservative).
5. For multiple requests (acid-fast bacilli, fungal, bacterial, and viral) but little specimen, contact the physician to determine which assays are most important and reject the others as “Quantity not sufficient.”

Anaerobic cultures:

A. Specimen collection

1. The best specimen for anaerobic culture is obtained by using a needle and syringe.
2. Tissue samples and biopsy samples are also very good specimens for anaerobic culture.
3. The least desirable specimen is collected by swab. Generally the specimen volume when collected by a swab is small, reducing the probability of isolating organisms, and many organisms adhere to the fibers of the swab, which reduces the opportunity of recovering organisms (1, 2, 4, 5). If collecting a specimen by swab is unavoidable, then collect as much specimen as possible, use a commercially available anaerobe swab system (see Table 4.2–1), and use special care to sample the active site of infection to prevent contamination.

B. Specimen transport

1. Transport time depends on the volume and nature of the specimen. Large volumes of purulent material and large pieces of tissue maintain the viability of anaerobes for many hours. Swabs (when necessary) and small volumes of aspirated material, biopsy samples, or curettings should be transported in an anaerobic transport device (Table 4.2–2). Suggested transport times relative to specimen volumes and methods of collection are listed in Table 4.2–3.
2. Avoid extremes of heat or cold. *If delays are unavoidable, hold the specimen at room temperature until processing.*
3. Do not transport material for culture in the needle and syringe. Needle transport is very unsafe because there is always the risk of a needle stick injury, and syringe transport poses a risk because the specimen may be expelled during transport, creating a threat to personnel and the environment (1). Transfer aspirated material to an anaerobic transport vial. Large volumes of purulent material may be transported in a sterile screw-cap tube.
4. Place tissue samples, biopsy samples, or curettings into an anaerobic transport device or a sterile tube or petri dish. Place all of this into a sealable plastic bag (Becton Dickinson [BD], Oxoid, Mitsubishi) that generates an anaerobic atmosphere. Large pieces of tissue can be transported in a widemouthed anaerobic transport device or in a sterile tube or jar.
5. If specimens must be collected by swab, transport swabs in a tube containing anaerobic transport medium (see Table 4.2–2).

C. Collection methods

Abscess

- a Aspirate material with needle and syringe after the surface of intact tissue is disinfected with a povidone-iodine wash that remains on the surface for at least 1 min. When needle use is contraindicated, aspirate material through a flexible plastic catheter or directly into the syringe with no needle.

Sinus tract or deep-wound drainage

- a Aspirate material with a small flexible plastic catheter and syringe after proper disinfection of the skin surface, or collect curettings of material from deep within the tract or wound.
2. Decubiti and other surface ulcers
 - a Results on specimens from decubiti and other surface ulcers can be very misleading unless special precautions are utilized. Analysis should be performed only on specimens from punch biopsy, on aspirated material obtained by needle and syringe after thorough and proper disinfection of the surface area, or on small curettings of material from deep

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tissue at the wound margin. **Swabs from decubiti and other surface ulcers are never appropriate for anaerobic culture.**

Pulmonary specimens

- a Collect lung tissue, transtracheal aspirate, percutaneous aspirate, transcutaneous aspirate, and bronchial brushings via double-lumen catheter. The use of shielded catheters to obtain specimens from pulmonary sources is essential to obtain proper specimens; otherwise the laboratory will be working up and identifying normal respiratory microbiota and providing useless information to the physician. Bronchial washings and other respiratory specimens not obtained via double-lumen catheters are not appropriate for anaerobic culture.

Female genital tract specimens

- a Disinfect the cervical opening by swabbing it with povidone-iodine.
- b Sample the upper genital tract by using a double-lumen collector and self contained transport system. The Pipelle system obtains cellular material from the uterine wall by suction, and the AccuCulShure uses a double lumen collector that reduces the potential of contamination (1, 2). Specimens collected by laparoscopy, culdocentesis, or surgery are appropriate for anaerobic culture.
- c Culture intrauterine devices anaerobically for *Actinomyces* species or *Eubacterium nodatum*.

Urinary tract

- a Obtain material via suprapubic bladder tap.

Other situations

- a In some cases, when aspiration or biopsy is not feasible (e.g., animal bite wounds), an anaerobic swab may be used for anaerobic culture. Anaerobic swabs are the least desirable specimen for a number of reasons, including small volume of specimen, greater chance of contamination with normal microbiota, excessive dryness, bacterial adherence to cotton fibers, and poor Gram stain quality. Studies have shown poor recovery of anaerobic organisms from some swab transport systems beyond 24 h (3, 4, 5). If a swab must be used, a swab using polyurethane adsorbing material instead of cotton, with two swabs (one for culture and the other for smear), may provide a useful alternative. An aspirate or biopsy sample or even a very small sliver of tissue may often be a better specimen than a swab for anaerobic culture.

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Acceptable specimens for anaerobic culture

<u>Site</u>	<u>Acceptable specimens</u>	<u>Unacceptable specimens</u>
Head and neck	Abscess aspirate obtained by needle and syringe after surface decontamination Biopsy material surgically obtained Anaerobic swab surgically obtained when aspiration is not feasible.	Throat or nasopharyngeal swabs Gingival swabs Superficial material collected with swabs
Lungs	Transtracheal aspirate Material from percutaneous lung puncture Biopsy material surgically obtained Bronchoscopic specimen obtained by protected brush Thoracotomy specimen Anaerobic swab surgically obtained	Expectorated sputum Induced sputum Endotracheal aspirate Bronchoscopic specimens not specially collected
Central nervous system	Abscess aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swabs surgically obtained	Aerobic swabs
Abdomen	Peritoneal fluid obtained by needle and syringe Abscess aspirate obtained by needle and syringe Bile Biopsy material surgically obtained Anaerobic swab surgically obtained	Aerobic swabs
Urinary Tract	Suprapubic aspirate	Voided urine Catheterized urine
Female genital tract	Culdoscopy specimens Endometrial aspirate obtained by suction or protected collector Abscess aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swabs surgically obtained IUD ^a for <i>Actinomyces</i> species or <i>Eubacterium nodatum</i>	Vaginal or cervical swabs
Bone and joint	Aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swabs surgically obtained	Superficial material collected with swabs
Soft tissue	Aspirate obtained by needle and syringe Biopsy material surgically obtained Aspirate from sinus tract obtained by needle and small plastic catheter Deep aspirate of open-wound margin obtained through decontaminated skin Deep aspirate of surface ulcer obtained through decontaminated skin	Superficial material collected from skin surface or edges of wound.
Stomach and small bowel	Only for workup of blind-loop or malabsorption syndrome	
Large bowel	Only for culture or toxin assay for suspected involvement of <i>Clostridium difficile</i> or <i>Clostridium botulinum</i> , <i>Anaerobiospirillum succiniciproducens</i> , and other etiologic agents	

^a IUD, intrauterine device