

Specific Guidelines for Specimen Collection

Abscess (anaerobic culture)

1. Decontaminate the surface with 70-95% ALC and Chlorhexidine Gluconate (ChloraPrep).
2. Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe. Open miliary abscesses with a sterile scalpel and collect the expressed material with a sterile needle and syringe.
3. Expel air from the syringe, remove the needle and cap the syringe with a plastic stopper.
4. Alternatively, transfer 5-10 mL of the aspirated material to an anaerobic transport vial.
5. Transport the specimen to the laboratory immediately.
6. Swabs are of limited value due to the small amount of material, possible inadequacy of the sample, and their tendency to dry easily. Swabs are not acceptable for mycobacterial or fungal cultures.

Blood Cultures

Collect 2-3 cultures from separately prepared sites prior to starting therapy.

1. Wash hands.
2. Wear gloves.
3. Find vein for venipuncture.
4. Scrub venipuncture site for 30 seconds with ChloraPrep Frepp (Chlorhexidine).
5. Using a butterfly needle set and adaptor, draw 10mls into an aerobic blood culture vial (mark graduations on side of vial) and 10mls into an anaerobic blood culture vial.

Note: If pediatric patient, draw 1-5mls using the aerobic pediatric vial.

Note: Prior to inoculating blood, flip off plastic caps on top of the blood culture vials and wipe rubber septum with alcohol wipes.

6. Repeat steps 4-6 for second or third set of cultures.
7. Label each vial with the patient name, date and time drawn and site drawn from. Preprinted labels can be used, but do not cover barcode label on the vial.

Central venous catheter (CVC) cultures:

Draw one set of blood cultures from the CVC (M.D. may order cultures from each lumen of the CVC) and one set from a peripheral site.

1. Clean CVC cap with alcohol swab for 3 seconds.
2. Draw 20ml blood from the CVC lumen(s). Do not draw “waste” blood or flush the lumen when drawing cultures, the heparin/saline will not affect the cultures.
3. Prepare culture vials as described above.
4. Inoculate 10mls into both an aerobic and anaerobic blood culture vial.
5. Label as indicated above.

Blood Culture vial Identification

BLOOD CULTURE VIAL	CAP COLOR (FLIP TOP/METAL)
Bactec Plus Aerobic/F	Gray/Blue
Bactec Lytic/10 anaerobic/F*	Purple/Purple
Bactec Peds Plus/F	Pink/Silver

*The Bactec Lytic/10 Anaerobic/F lyses the red blood cells when inoculated. Do not be alarmed if the contents turn a black cherry color.

Body Fluid (excluding CSF, Urine and Blood)

Physicians collect sterile body fluids. Complete a body fluid order form, order appropriate tests and promptly deliver to the laboratory for testing. If delivery/analysis is delayed beyond 2 hours, specimen must be refrigerated.

Bone Marrow

Specimens collected by Pathologist or Oncologist. Transfer 3-5 mL to a sterile tube containing SPS for bacterial culture or directly into a mycolytic blood culture vial. Transport immediately at ambient temperature.

Bordetella PCR

Refer to Nasopharyngeal swabs

Bronchial Brush/Washing/Lavage

1. This technique is best done by an experienced individual. Descriptions of the methodology are readily available in the literature.
2. Transport in a sterile container at 2-8°C for cultures, or frozen for molecular tests.

Bullae, Cellulitis, Vesicles

A. Bullae, Vesicles

1. Cleanse the skin as for blood cultures.
2. Aspirate the fluid/purulent material using a sterile needle and syringe.
3. If an aspirate is obtained, place in appropriate viral or bacterial transport.
4. If no material is obtained, unroof vesicle or bullous lesion and use a swab to collect cells from the base of the lesion. Place in appropriate viral or bacterial transport media.

B. Cellulitis

Swabs and leading-edge aspirates with or without injection of saline fail to yield etiologic agents in the majority of cases. If an unusual organism is suspected, a leading-edge (advancing margin) punch biopsy is the recommended specimen of choice.

Catheters (bacteria only)

1. Short catheters (2-3 inches)
 - a. Decontaminate the skin at the catheter site.
 - b. Aseptically remove the catheter. Cut the catheter at the skin interface point using sterile technique. Place the catheter segment in a sterile, wide-mouth container.
 - c. Transport immediately at ambient temperature.
2. Long catheters (8-24 inches)
 - a. Decontaminate the skin at the catheter site.
 - b. Aseptically remove the catheter. Submit two segments for analysis. Cut a 2-inch segment of the catheter that was within the blood vessel, using sterile technique. Place the segment in a sterile, wide-mouth container. Cut a second 2-inch segment of the catheter from the skin interface. Place the segment in a sterile, wide-mouth container. Label the containers appropriately.
 - c. Transport immediately at ambient temperature.

Cerebral Spinal Fluid

1. Physicians should wear gowns, masks and gloves to collect the specimen. Because an open tube is held to collect the fluid, other personnel should stand away or wear masks in order to avoid respiratory contamination.
2. Decontaminate the skin with 1-2% TOI or Chlorhexidine, followed by 70-90% ALC using an increasingly outward circular movement.
3. Drape sterile linen over the skin surrounding the puncture site.
4. Insert the needle. Collect the fluid into three sterile leak-proof tubes. Collect an adequate volume of fluid as recommended below.
 - a. bacterial culture > 1 mL
 - b. fungal culture 8 – 10 mL
 - c. molecular > 1 mL
 - d. mycobacterial culture 8 – 10 mL
 - e. viral culture > 2 mL
5. Cap the tubes tightly. Submit the second or third tube for culture to reduce the possibility of contamination due to skin flora.
6. Complete a CSF order form, order appropriate tests and promptly deliver to the laboratory for testing.
7. Transport immediately with form. If delivery/analysis is delayed beyond 2 hours, specimen must be refrigerated.

Cervix (Endocervix)

1. Place the patient in the lithotomy position.
2. Prepare the speculum, avoiding the use of a lubricant other than warm water.
3. Insert the speculum and visualize the cervical os.
4. Remove excess mucus with a cotton ball.
5. Gonococcal cultures – refer to **Gonorrhea**.
 - a. Chlamydia – refer to specific test type.
 - b. Cervical cultures for other reasons:
 1. Insert a dacron swab in the distal portion of the cervical os, rotate gently, and allow to remain for 10 to 30 seconds.
 2. Remove swab and place in transport medium.
 3. Transport at ambient temperature or 2-8°C for viral cultures.
6. Vaginal cultures, in general, do not often produce meaningful results and are not recommended, except for group B streptococcal screen.

Chlamydia

Refer to Gonorrhea

Cutaneous (fungus only)

1. Hair
 - a. Scrape the scalp with a blunt scalpel.
 - b. Place specimen in a dry sterile container.
 - c. Transport at ambient temperature.
 - d. The following specimens are also acceptable:
 - 1.) Hair stubs
 - 2.) Contents of plugged follicles
 - 3.) Skin scales
 - 4.) Hair plucked from the scalp with forceps

Cut hair is **NOT** an acceptable specimen.

2. Nails
 - a. Cleanse the nail with 70-95% ALC.
 - b. Remove the outermost layer by scraping with a scalpel.
 - c. Place specimen in a dry, sterile container.
 - d. Transport at ambient temperature.
 - e. The following specimens are also acceptable:
 - 1.) Clippings from any discolored or brittle parts of nail
 - 2.) Deeper scrapings and debris under the edges of the nail
3. Skin
 - a. Cleanse the skin with 70-95% ALC.
 - b. Collect epidermal scales with a scalpel, at the active border of the lesion.
 - c. Place specimen in a dry sterile container.
 - d. Transport at ambient temperature.

Ear

1. External ear cultures are processed as superficial wounds.
2. Middle ear fluid will be process as sterile body fluid. If the diagnosis is otitis media, the specimen of choice is middle ear fluid collected by tympanocentesis.

Eye

1. Cleanse the skin around the eye with a mild antiseptic.
2. Purulent conjunctivitis:
 - a. Collect purulent material with a regular dacron swab.
 - b. Place the swab into transport media and transport at ambient temperature or 2-8°C for viral cultures.
3. Corneal infections:
 - a. Swab the conjunctiva as described above.
 - b. Collect multiple corneal scrapings and inoculate directly onto bacterial agar media (chocolate agar and sheep blood agar) or viral transport media.
 - c. Transport at ambient temperature or 2-8°C for vial cultures.
4. Intraocular fluid:
 - a. Collect fluid by surgical needle aspiration.
 - b. Transport bacterial cultures at ambient temperature, viral cultures at 2-8°C, or frozen for molecular tests.

Gonorrhea

1. Gonorrhea testing is available by several methods. An amplification method which detects *Neisseria gonorrhoeae* nucleic acid in urogenital specimens is the preferred diagnostic method. Amplification tests are available for *Chlamydia trachomatis* detection in combination with GC. Culture for *N. gonorrhoeae* is the method of choice in cases of treatment failure and sexual abuse and for non-genital sources.
2. Specimens for all of the above can be collected following the procedures below. Amplification tests require transport of swabs in the proprietary transport tube.
 - a. Females:
 - 1.) Place patient in the lithotomy position.
 - 2.) Insert speculum and visualize the cervical os.
 - 3.) Remove the excess mucus from cervical os and surrounding mucosa using the large swab provided in the kit. **Discard this swab.**
 - 4.) Insert second swab from kit, 1 to 1.5 cm into endocervical canal.
 - 5.) Rotate swab for 30 seconds in endocervical canal to ensure adequate sampling.
 - 6.) Withdraw swab carefully, avoiding any contact with vaginal mucosa.
 - b. Males:
 - 1.) Do not allow patient to urinate for at least 1 hour prior to collection.
 - 2.) If purulent discharge is present, collect discharge directly on swab.

- 3.) If no discharge is present, insert smaller swab 2-4 cm into urethra. Rotate gently to ensure contact with all urethral surfaces. Leave inserted for 2-3 seconds. Rotate gently while withdrawing swab.
 - c. Place swab into the amplification transport tube.
 - d. Break swab shaft to fit tube, if required.
 - e. Cap tube tightly.
 - f. Transport at 2-8°C. If transport is to be delayed beyond 24 hours, vortex specimen, remove swab, and store and transport tube at -20°C to -70°C.
 - g. Urine specimens from patients – transport frozen. See **Urine**.
 - h. For culture, inoculate sample as specified below.
3. For culture of *N. gonorrhoeae*, use Dacron swabs for specimen collection. Cotton fibers contain fatty acids which are inhibitory to the gonococcus. Avoid swabs with wooden sticks.
 - a. Inoculate culture directly onto a Modified Thayer-Martin agar plate. Direct plating is necessary because the gonococcus is extremely susceptible to drying. Use one plate for each culture obtained. Roll the swab in a Z pattern across the agar surface.
 - b. Label the plate with patient identification and specimen site.
 - c. Place the plate in a CO₂ generating plastic bag assembly. (If CO₂ generating assembly is not available, culture swabs can be transported using Amies media with charcoal.) Do not place the tablet on the plate itself. Seal the bag.
 - d. Transport immediately at ambient temperature. If there is any delay in transport, incubate at 37°C.
 - e. Cervical culture. Refer to **Cervix (Endocervix)**.
 - f. For male patients, also submit a slide of urethral material for Gram Stain. Gram stains of specimens from the endocervix are not as reliable.
 - g. Rectal culture:
 - 1.) Moisten a swab with sterile water and insert the swab into the anal canal just beyond the anal sphincter.
 - 2.) Allow 10-30 seconds for absorption of the organisms onto the swab.
 - 3.) Withdraw swab gently and inoculate plate as described above.
 - 4.) Stool is not an acceptable specimen for gonorrhoeal culture.
 - 5.) If disseminated gonococcal infection is suspected, culture blood and suspicious sites such as petechiae or joint fluid.

Nasopharyngeal Aspirates/Washings (virus only)

1. For aspirate, attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter. Turn on suction and adjust to suggested pressure.
2. Without applying suction, insert catheter into the nose, directed posteriorly and toward the opening of the external ear. **Note:** Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior nares and external opening of the ear.
3. Apply suction. Using a rotating movement, slowly withdraw the catheter.
4. Transport at 2-8°C or frozen for molecular tests.
5. For washings, suction 3-5 mL of sterile saline into a new sterile bulb.
6. Insert bulb into one nostril until nostril is occluded.
7. Instill saline into one nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
8. Empty bulb into suitable dry, sterile specimen container or add 3 mL or less to viral transport media.
9. Transport at 2-8°C.

Nasopharyngeal Swabs

1. Seat the patient comfortably and tilt the head back.
2. Insert a nasal speculum.
3. Insert a nasopharyngeal swab (on a malleable wire) through the speculum into the nasopharyngeal area.
4. Rotate the swab gently and allow to remain for 20-30 seconds.
5. Remove the swab and place in a non-growth promoting transport media (such as the swab container, from which the original swab has been removed). Place swab in M4 media for viral cultures.
6. Transport at ambient temperature or 2-8°C for viral cultures.

Nose

1. Collect anterior nares culture with a swab. In small children, use a nasopharyngeal swab to facilitate collection.
2. Transport at ambient temperature.

Note: This is an inappropriate specimen for anything other than assessment of staphylococcal or streptococcal colonization.

Prostate

1. Cleanse the glans with soap and water.
2. Obtain prostate fluid by digital massage through the rectum.
3. Collect fluid using a sterile swab.
4. Transport at room temperature.
5. Alternatively, a urine specimen obtained immediately before and after massage may be submitted for culture.

Skin

Refer to Abscess; Bullae, Cellulitis, Vesicles; and Wounds.

Sputum

1. Assure patient cooperation to get an adequate specimen. Methodist Lab will determine the number of squamous epithelial cells present for specimen adequacy.
2. Instruct the patient as follows:
 - a. Rinse mouth with tap water to remove food particles and debris.
 - b. Have patient breath deeply and cough several times to receive deep specimen.
 - c. Patient should expectorate into dry, sterile container.
3. If patient is unable to produce sputum, induce using saline nebulization. Consult respiratory therapy for assistance.
4. Transport immediately at ambient temperature. Refrigerate if a delay of >1 hour is anticipated.

Stool, Feces

1. Collect specimen in a clean bedpan, commode specimen system, or use plastic wrap placed between the toilet seat and the bowl. Do not submit feces contaminated with urine or toilet water.
2. Transfer specimen into a clean, dry container.
3. Transport at ambient temperature within 2 hours of collection, 2-8°C for viral cultures, or frozen for *C. difficile*. Send to Methodist in the appropriate transport media.
4. Diarrhea that develops after 3 days hospitalization is likely due to *Clostridium difficile* toxin. Routine cultures and OVA & Parasite exams should not be performed on these patients.
5. Recommend that no more than 2 bacteriology specimens (cultures) be processed per patient without consultation. (2 separate bowel movements)

6. Ova & Parasite – 3 specimens collected over a 10 day period is optimum.

Notes:

- Only loose or diarrheal stools are recommended for routine bacterial and *C. difficile* cultures.
- If transport delay is unavoidable, place the specimen in an appropriate preservative or transport media, immediately after collection. For ova and parasite, use 10% formalin and modified PVA; for routine stool culture, use Cary-Blair transport media.
- If a stool specimen is not available, the following are suitable alternatives for culture:
 1. A swab of rectal mucus, or
 2. A rectal swab inserted one inch into the anal canal (not acceptable for Rotavirus/Adenovirus EIA).

See **Gonorrhea** section for rectal swabs.

Fecal Fat

Instruct the patient to eat a normal diet containing 50-150 g of fat per day for at least three days prior to beginning fecal collection. This diet should be maintained throughout the collection period. Ideally, no medications should be given immediately prior to and during the collection period. Exclude materials like castor oil and mineral oil from the diet.

Throat

1. Use a tongue depressor and a direct light source to ensure adequate visualization of the posterior pharynx.
2. Swab any area of exudate, ulceration or inflammation including the tonsils, using a culturette swab. Avoid touching the swab to the tongue or uvula.
3. Place the swab back in the transport tube.
4. Transport at room temperature or 2-8°C for viral cultures.

See Gonorrhea section for isolation from throat swabs.

Urethral

Refer to Gonorrhoea

Urine

1. Instructions for female patients to collect midstream urine for bacterial culture:
 - a. Remove undergarments.
 - b. Wash hands thoroughly with soap and water, rinse them, and dry them on a disposable paper towel or shake off excess water.
 - c. Spread labia, with one hand, and keep them continuously apart.
 - d. Take the open sterile cup in the other hand without touching the rim or inner surface of the cup or lid.
 - e. Void 20 to 25 mL into the toilet and catch a portion of the rest of the urine in the container without stopping the stream. Do not touch the legs, vulva or clothing with the cup.
 - f. Place the lid on the cup.
2. Instructions for male patients to collect midstream urine for bacterial culture:
 - a. Wash hands.
 - b. Retract the foreskin completely.
 - c. Void 20 to 25 mL into the toilet and catch a portion of the remaining urine in the cup without stopping the stream. Do not touch the cup with the penis.
 - d. Place the lid on the cup.
3. First-void urine for nucleic acid amplification (LCx® tests for Chlamydia/Gonorrhoea):
 - a. Patient must have urinated during the previous 2 hours.
 - b. Collect the first 10 to 15 mL of the urine stream in a clean, empty plastic cup.
 - c. Place the lid on the cup.
4. Suprapubic aspiration:
 - a. This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in the literature.
 - b. These specimens are acceptable for anaerobic culture and should be submitted in an anaerobic environment if an anaerobic culture is requested.
5. Indwelling catheter urine:
 - a. Do not collect urine from the drainage bag because of growth of bacteria outside the catheter may have occurred at this site.
 - b. Clean the catheter with an alcohol pad.
 - c. Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
 - d. Transfer the urine to a sterile specimen container.

- e. Urine catheter tip cultures are not acceptable.
- 6. Specimen handling:
 - a. Label the container immediately and refrigerate at 2-8°C within 10 minutes of collection.
 - b. Freeze urine for LCx® tests.

Urine: 24 Hour Collection

Collection Instructions to the Patient

1. Avoid alcoholic beverages for at least 24 hours before starting to collect urine and during the collection period. Do not discontinue medications unless instructed to do so by your physician.
2. Do not exceed your normal intake of liquids or change your dietary habits during the day before and the day of your collection unless your physician gives you specific instructions to do otherwise.
3. On the day of collection, discard the first morning void and begin the collection after this void.
4. Collect all urine for the next 24 hours. The morning urine void on the second morning is the final urine added to the collection.
5. Keep the urine refrigerated.

Vaginal

Vaginal cultures, in general, do not often produce meaningful results and will only be cultured for group B streptococcus.

Wounds

1. For closed wounds, refer to **Abscess and Bullae, Cellulitis, Vesicles**.
2. For open wounds:
 - a. Clean the sinus tract opening of the wound surface mechanically, without using a germicidal agent, to remove as much of the superficial flora as possible.
 - b. Attempt to culture the base or edges of the wound to avoid collecting “normal flora” organisms.
 - c. The following are preferred specimens for sinus tracts:
 - 1.) Aspiration material obtained by needle or catheterization.
 - 2.) Curettings from the lining of the sinus tract.
 - d. Specimen swabbings of sinus tracts are acceptable **only** if the above cannot be obtained.

- e. Do not submit cultures of superficial lesions for anaerobic culture. Biopsy of advancing margin of wound is the preferred specimen for anaerobes, mycobacteria and fungi.

Viral Transport Media (M4)

Some samples can be submitted, without utilizing a transport media, with a reasonable expectation of virus viability. Specimens in this category include, sterile fluids such as cerebrospinal fluid, pleural fluid, blood submitted in EDTA, urine, as well as some non-sterile specimens such as nasopharyngeal washings, sputum, bronchoalveolar lavage and feces. Whenever there is a question of stability, the specimen should be placed in a suitable virus transport media such as M4. Refer to specific test in the alphabetical test list of this User's Guide for more information.

1. Tissue and biopsy material can be placed directly into the M4 media. Each sample need not be more than 1-2 cm in diameter.
2. Abscess material, bullae, pustules, vesicles, lesions and skin scrapings can be collected on the swab and placed directly into M4. If the material has been aspirated, place no more than 3 mL (equal to the amount of transport media) in the vial of M4.
3. CSF should be submitted in a sterile container or no more than 3 mL added to the M4 tube.
4. Urine should be submitted in a sterile container or no more than 3 mL added to the M4 tube.
5. Bronchoalveolar washings, nasopharyngeal washings, sputums, and other sterile body fluids can be submitted in sterile containers or no more than 3 mL placed in the M4 tube.
6. Stool should be submitted in a sterile container, or a small aliquot the size of a walnut can be placed in the M4 tube.
7. Blood should be submitted in an EDTA tube. Do not extract the buffy coat.