

Microbiology Specimen Collection Guidelines

The proper collection and transport of patient specimens for culture is the most important step in the recovery of pathogenic organisms responsible for infectious disease. A poorly collected specimen may lead to failure to isolate the causative organism(s) and/or result in recovery and subsequent treatment of contaminating organisms. All procedures address culture for bacteria, fungus and mycobacteria (AFB) unless otherwise noted.

MATERIALS - PROCEDURE SPECIFIC

Blood culture bottles
Chlorhexidine gluconate preps
(if patient >2 months old)
Betadine solution
Alcohol wipes
Gauze
Intravenous catheters
Sterile collection containers
Anaerobic and aerobic specimen collection swabs
Sterile syringes and needleless canulas
Bone marrow needle assemblies

QUALITY CONTROL

Media and other materials used in the collection of specimens for culture or analysis are documented with manufacturers' certificate of performance or are verified internally for use.

BASIC INSTRUCTIONS FOR SPECIMEN COLLECTIONS

- 1 Collect the specimen from the actual site of infection. Avoid contamination from adjacent tissue or fluids.
- 2 Collect the specimen at the optimal time. For example, collect sputum for AFB culture in the early morning.
- 3 Collect a sufficient quantity of material.
- 4 Use appropriate collection devices such as sterile, leak-proof containers. Use appropriate transport media.
- 5 Whenever possible, collect the specimen prior to the administration of antibiotics.
- 6 Properly label the specimen with two patient identifiers (complete name and medical record number or date of birth) as well as the specific source of the specimen.
- 7 Minimize transport time and ensure that the transport environment is appropriate. For example, transport anaerobic cultures in anaerobic transport devices.
- 8 If skin decontamination is necessary, use Chloraprep to prepare the site. After cleansing the site, wait 30 seconds to allow the antiseptics to work before collecting the specimen.
- 9 Submission of a tissue biopsy is preferable to swabbing the area and submitting a swab for culture.

ABCESESSES

- 1 Decontaminate the surface with alcohol and tincture of iodine.
- 2 Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe. Open miliary abscesses with a sterile scalpel and collect expressed material with a needle and syringe.
- 3 Expel air from the syringe, remove the needle and cap the syringe with an appropriate syringe-capping device. Alternately, transfer 5-10 ml of the aspirated material into an anaerobic transport vial. Transport the specimen to the laboratory immediately.
- 4 Avoid the use of swabs. Swab specimens are of limited value due to the small amount of material taken and their tendency to dry easily.

ANAEROBIC STUDIES / COLLECTION

An aerobic culture is always done in conjunction with an anaerobic culture. Submit both an aerobic and anaerobic transport system.

Specimens from the following sites are **not** acceptable for anaerobic culture:

| | | |
|----------------------------|-------------------------------|-----------------------------------|
| Burns | Nasotracheal tube suctioning | Tonsillar swab |
| Bronchoscopic specimens | Nasopharyngeal | Urethral |
| Catheter tips | Nose | Urine (voided or cath) |
| Cerebral spinal fluid | Perineal swab | Vaginal (acceptable from ED only) |
| Cervical secretions | Post-partum endometrisis | |
| Cysts | Prostatic secretions | |
| Decubitis ulcers (surface) | Pus from superficial wounds | |
| Ear (surface) | Superficial ulcers (decubiti) | |
| Eye (external surface) | Sputum | |
| Gastric contents | Stool | |
| Gingival swab | Throat | |
| Intestinal contents | Tonsillar material | |

Equipment:

Anaerobic swab system (obtain from lab)
Appropriate dressing for wound

- 1 Remove swab from small inner tube, grasping the clear plastic plunger.
- 2 Procure culture, taking care to avoid contamination.
- 3 Immediately after collection, insert swab into smaller tube pushing it in as far as it will go. The plastic collar will be flush with the rubber stopper.
- 4 Apply dressing if necessary.
- 5 Label and transport to lab according to procedure.

BLOOD - AEROBIC AND ANAEROBIC (BACTERIA AND FUNGI)

Blood culture samples should be submitted in VersaTrek blood culture bottles. Two blood culture collections will usually be sufficient for most situations. More collections may be required for certain suspected diagnoses, including endocarditis, early typhoid or brucellosis. Blood for cultures should not be collected through an indwelling or intra-arterial catheter.

The optimal blood collection volume is 20 ml of blood. A smaller sample may be collected for infants and children.

- 1 Swab the tops of one aerobic and one anaerobic VersaTrek blood culture bottle with an alcohol swab. Allow the alcohol to dry while preparing the patient.
- 2 Cleanse the skin with Chloraprep using a circular outward motion. If patient is younger than 2 months, use betadine followed by a 70 percent alcohol pad.
- 3 Allow the collection site to air dry for 30 seconds. Do not palpate or blow on the collection site after it has been disinfected.
- 4 Apply a tourniquet proximal to the point of venous entry. The venipuncture site should not be palpated following disinfection unless sterile gloves are worn.
- 5 Use a sterile needle and syringe or butterfly for collection.
- 6 Collect blood. The volume of blood collected is critical. Inoculate the bottles without changing needles.
 - Inoculate the aerobic and the anaerobic bottles with 10 ml of blood each.
 - If less than 20 ml but >1 ml of blood is collected, divide the blood volume between the two bottles.
 - If less than 1 ml of blood is obtained, inoculate only the aerobic bottle. Do not inoculate the anaerobic bottle.
- 7 Send the bottles to the laboratory immediately. DO NOT REFRIGERATE.

BODY FLUIDS (EXCEPT URINE AND CSF)

The specimen should be obtained by aspiration and submitted to the lab in aerobic and/or anaerobic transport systems as appropriate.

- 1 Collect body fluids using aseptic technique.
- 2 If possible, submit at least 10 ml of fluid for culture. Submit fluid in a sterile transport cup.
- 3 If anaerobes are suspected and/or ordered, transport sample in anaerobic transport media.

- 4 If cell count glucose and protein are ordered, submit 1-2 ml fluid in a heparin tube (green top) and 10 ml in a sterile cup. For other tests, see the alphabetical test listing for test-specific sample requirements.
- 5 Transport specimens immediately to the laboratory. DO NOT REFRIGERATE.

BONE MARROW (BACTERIA AND FUNGUS)

- 1 Bone marrow collection is a surgical procedure. Physicians should wear gowns, masks and gloves for collection.
- 2 Prepare the skin in the same manner as for blood cultures.
- 3 Drape the surrounding skin with sterile drape.
- 4 Aspirate the marrow percutaneously using a sterile needle and syringe.
- 5 If sufficient quantity is collected, transfer the bone marrow to an aerobic blood culture bottle. If an AFB culture is also ordered, transfer part of the bone marrow to a heparinized tube.
- 6 Cap the tube tightly and transport immediately to the laboratory. DO NOT REFRIGERATE.

BRONCHIAL BRUSH/WASHING/LAVAGE

The use of bronchial brush/washing/lavage specimens for culture is not a routine procedure. The techniques for these procedures are best performed by a trained individual.

Transport 2-5 ml of the bronchial specimen to the laboratory. DO NOT REFRIGERATE.

BULLAE, CELLULITIS, PETECHIAE, VESICLES

- 1 Prepare the skin in the same manner as for blood cultures. Do not rupture bullae or vesicles.
- 2 Aspirate the purulent material using a sterile syringe and cannula.
- 3 If an aspirate is not obtained, inject sterile water or saline into the site and aspirate again. The optimum site is the lesion edge.
- 4 Petechiae require special considerations.
 - Perform a punch biopsy or scrape the skin with a sterile needle tip.
 - Place the biopsy or skin scrapings in a sterile container, moisten with a small amount of sterile saline (non-bacteriostatic) and transport immediately to the laboratory. DO NOT REFRIGERATE.

CATHETERS (EXCEPT URINARY CATHETERS)

Short Catheters

- 1 Decontaminate the skin at the catheter site.
- 2 Aseptically remove the catheter. Cut the catheter where the catheter interfaced with the skin using sterile technique.
- 3 Place the catheter segment in a sterile container and transport immediately to the laboratory.

Long Catheters

- 1 Decontaminate the skin at the catheter site.
- 2 Aseptically remove the catheter. Submit two sections for culture. Using sterile technique, cut a two-inch segment of the catheter that was within the lumen of the blood vessel. Cut a second 2-inch section of catheter from the area that was at the skin interface.
- 3 Appropriately label a sterile container and promptly submit to the laboratory.

CSF (CEREBROSPINAL FLUID)

Collection of CSF is a surgical procedure. Physicians should wear gloves, masks and gowns for specimen collection. Due to open-tube collection technique for this procedure, other personnel should stand away or wear masks in order to prevent respiratory contamination.

A specimen is to be submitted in three separate sterile, screw-capped containers. The second sample aspirated is preferable for microbiology tests since there is less chance of possible skin contamination as compared to the first sample. The third sample will be used in the absence of any hematology test orders.

Laboratory personnel will select the most appropriate sample tubes for the tests ordered given that the sample tubes are labeled with the sequence in which they were collected.

- 1 Prepare the skin in the same manner as for blood culture.
- 2 Overlay the area surrounding the puncture site with sterile drapes.
- 3 Insert the needle. Collect the fluid into three leak-proof, sterile containers. Collect an adequate amount of fluid. Six to 10 ml of CSF is recommended if bacterial, fungal and mycobacterial cultures are to be performed.
- 4 Cap the tubes tightly. Submit to the laboratory immediately. DO NOT REFRIGERATE.

CERVIX (ENDOCERVIX) – FOR GONOCOCCAL CULTURES REFER TO GONORRHEA

- 1 Place the patient in the lithotomy position.
- 2 Prepare the speculum, avoiding use of lubricants other than warm water.
- 3 Insert the speculum and visualize the cervical os.
- 4 Remove excess mucous with a cotton ball.
- 5 Insert a dacron swab into the distal portion of the cervical os, rotate gently and allow to remain in place for 10-30 seconds.
- 6 Remove swab and place in transport medium.
- 7 Transport immediately to the laboratory.
DO NOT REFRIGERATE.

CHLAMYDIA

Chlamydia testing is available by three methods: culture, direct fluorescent antibody (DFA) or molecular probe. The culture method can be used for all specimen sites and is the only acceptable method for diagnosis of chlamydia in children. In cases of suspected sexual abuse in either children or adults, the only legally accepted results are from culture when chlamydia or GC (*Neisseria gonorrhoeae*) is ordered. No other testing methodology is legally binding.

CHLAMYDIA CULTURE COLLECTION

- 1 Utilizing a sterile swab, obtain a suitable specimen.
- 2 Place the swab in transport media (M4 media - tube with pink liquid).
- 3 Label the transport media vial.
- 4 Transport the vial to the laboratory (may be refrigerated).

CHLAMYDIA DFA COLLECTION

DFA method for chlamydia utilizes an antibody to detect chlamydia organisms on a slide. This method can be used for urethral, cervical, rectal, conjunctival or nasopharyngeal samples.

Urethral Samples (male):

Patient should not have urinated one hour prior to sampling.

- 1 Insert small dacron swab (mini-tip) 2-4 cm into urethra.
- 2 Rotate swab and withdraw.

Cervical Samples (cytobrush):

- 1 Wipe exocervix with cotton or dacron swabs to remove all excess mucus. Dispose of swab.
- 2 Gently insert cytobrush into endocervical canal past squamocolumnar junction.
- 3 Leave in place 2-3 seconds.
- 4 Rotate cytobrush one full turn (360 degrees) and then withdraw, taking care to not touch vaginal surfaces.

Cervical Samples (swab):

The large or small swab should be used to sample pregnant patients or patients with a small cervical os.

- 1 Wipe exocervix with cotton or dacron swab to remove excess mucus. Dispose of swab.
- 2 Insert large or small dacron swab into the endocervical canal until most of dacron tip is not visible.
- 3 Rotate swab 5-10 seconds inside endocervical canal.
- 4 Withdraw swab without touching any vaginal surfaces.

Rectal Samples:

Samples should be collected only from symptomatic patients.

- 1 Insert the large dacron swab about 3 cm into the anal canal.
- 2 Move swab from side to side to sample crypts.
- 3 Withdraw swab. If fecal contamination occurs, discard swab and obtain another specimen.

Conjunctival Swab:

Samples should only be collected from symptomatic patients.

- 1 Apply a topical proparacaine-based anesthetic to the eye or eyes (optional).
- 2 Using the smaller swab, thoroughly swab the inner surface of the lower, then the upper, eyelid. If samples are taken from both eyes, use the swab on the less-affected eye first to avoid further contamination of the eye.

Nasopharyngeal Specimens:

Samples should be collected only from symptomatic patients. Collect specimens from the posterior nasopharynx by nasal swab or nasal aspirate using a standard collection method.

DFA Slide Preparation

Slides should be prepared immediately after specimen collection.

Cytobrush and Swabs:

- 1 Place the portion of the cytobrush or swab containing the specimen across the center of the well.
- 2 Rotate and twist the brush back and forth across the well.
- 3 Check coverage. The entire well should be covered.
- 4 Allow the specimen to completely air dry.
- 5 Lay the slide flat and flood with 0.5 ml methanol fixative and let entire quantity evaporate. To speed evaporation, tip slide after five minutes to drain excess fixative.
- 6 For best results, store and transport either at room temperature (20-30°C) or refrigerated (2-8°C) and stain within seven days after collection. If not stained within seven days, the fixed specimen should be stored at -20°C.

Nasal Aspirate:

- 1 Vortex specimen gently to break up mucus.
- 2 Place one drop of the vortexed specimen on a well. Cover entire well and stay within perimeter. Check for complete coverage.
- 3 Allow to completely air dry.
- 4 Use fixative and store as above.

*Chlamydia by Molecular Probe***Endocervical Swab Specimens:**

Use the Aptima® Gen Probe® Unisex Swab Specimen Collection Kit - purple package.

- 1 Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (large, white shaft swab in the package.) **Discard this swab.**
- 2 Insert the specimen collection swab (small, blue shaft swab) into the endocervical canal.
- 3 Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal.
- 4 Withdraw the swab carefully, avoiding any contact with the vaginal mucosa. Immediately place the swab in the transport tube.
- 5 Carefully break the swab shaft at the scoreline. Use care to avoid splashing of the contents and recap tightly.
- 6 This specimen is stable for 60 days at room temperature or refrigerated.

Male Urethral Swab Specimens:

Use the Aptima® Gen Probe® Unisex Swab Specimen Collection Kit - purple package.

- 1 The patient should not have urinated for at least one hour prior to specimen collection.
- 2 Insert the specimen collection swab (small, blue shaft swab) 2 to 4 cm into the urethra.
- 3 Gently rotate the swab clockwise for two to three seconds in the urethra to ensure adequate sampling.
- 4 Withdraw the swab carefully and place in the transport tube.
- 5 Carefully break the swab shaft at the scoreline. Use care to avoid splashing of the contents and recap tightly.
- 6 This specimen is stable for 60 days at room temperature or refrigerated.

Urine specimens:

Use the APTIMA Urine Specimen Collection Kit for Male and Female Urine Specimens - yellow label.

- 1 The patient should not have urinated for at least one hour prior to specimen collection.
- 2 Direct patient to provide a first-catch urine sample (approximately 20 to 30 ml of the initial urine stream) into a urine collection cup free of any preservatives. Collection of larger volumes of urine may result in specimen dilution that may reduce test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.
- 3 Remove the cap and transfer 2 ml of urine into the urine specimen transport tube using the disposable pipette provided. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine transport tube label.

ThinPrep Vials: Testing must be performed within 21 days of collection when the vials are stored between 2-8°C or within seven days of collection when stored at 15-30°C.

CUTANEOUS CULTURES (FUNGUS ONLY)**Hair**

- 1 Scrape the scalp with a blunt scalpel or a new toothbrush.
- 2 Place the specimen in a sterile cup and transport to the laboratory. DO NOT REFRIGERATE.
- 3 Other acceptable specimens include:
 - Hair stubs
 - Contents of plugged follicles
 - Skin scales
 - Hair plucked with forceps. CUT HAIR IS NOT AN ACCEPTABLE SPECIMEN.

Nails

- 1 Cleanse the nail with alcohol.
- 2 Remove the outermost layer by scraping with a scalpel.
- 3 Place the specimen in a dry, sterile container.
- 4 Transport to the laboratory. DO NOT REFRIGERATE.
- 5 Other acceptable specimens include:
 - Clippings from discolored or brittle parts of the nail.
 - Deeper scrapings and debris from under the edges of the nail.

Skin

- 1 Cleanse the skin with alcohol.
- 2 Collect epidermal scales with a scalpel or a new toothbrush. The best specimens are obtained at the borders of lesions.
- 3 Place specimen in a dry sterile container. Transport to the laboratory. DO NOT REFRIGERATE.

Ears

- 1 Cleanse the external ear with mild detergent or antiseptic.
- 2 Collect material from the outer ear with a dacron swab or by gently scraping the ear with a scalpel.
- 3 Place the scrapings in a sterile cup. Moisten with a small amount of non-bacteriostatic sterile saline.
- 4 Transport to the laboratory. DO NOT REFRIGERATE.

ENDOMETRIUM

- 1 Place the patient in lithotomy position.
- 2 Insert the speculum and visualize the cervical os.
- 3 Place a narrow lumen catheter within the cervical os.
- 4 Insert the tip of a culture swab through the catheter and collect the endometrial specimen. This method prevents the touching of the cervical mucosa and reduces potential contamination.
- 5 Transport to the laboratory. DO NOT REFRIGERATE.

EYE

Purulent conjunctivitis

- 1 Cleanse the area around the eye with mild antiseptic.
- 2 Collect purulent material with a dacron swab.
- 3 Place the swab in transport media and transport to the laboratory. DO NOT REFRIGERATE.

Corneal Infections

- 1 Cleanse the area around the eye with mild antiseptic.
- 2 Swab the conjunctiva as described above.
- 3 Collect multiple corneal scrapings and inoculate directly in bacteria agar media. (chocolate, BHI and sheep blood agars.)
- 4 Transport to the laboratory. DO NOT REFRIGERATE.

Intraocular Fluid

- 1 Cleanse the area around the eye with mild antiseptic.
- 2 Collect fluid by surgical needle aspiration.
- 3 Transport to the laboratory. DO NOT REFRIGERATE.

GONORRHEA

Gonorrhea testing is available by two methods. The culture method can be used for all specimen sites and allows for susceptibility testing. Culture is the only legally acceptable method for diagnosis of gonorrhea in children. Molecular probe testing for gonorrhea and chlamydia can be used for female endocervical or male urethral specimens, urine specimens or ThinPrep specimens. See the “Chlamydia” section for collection details.

In cases of suspected sexual abuse in either children or adults, the only legally accepted results are from culture when GC (*Neisseria gonorrhoeae*) and/or chlamydia testing is ordered. No other testing methodology is legally binding.

Gonorrhea Culture Collection

Use only calcium alginate or dacron swabs for collection. Cotton is inhibitory to the gonococcal organisms.

Female Gonorrhea Culture Collection Procedure

- 1 Obtain a suitable specimen utilizing the methods for endocervical culture. Recovery rates are often enhanced by culture of both the endocervical and rectal areas.
- 2 Inoculate the specimen directly onto Modified Thayer-Martin and chocolate agar.
- 3 Label the agar plates.
- 4 Place the plates, along with a CO₂ generating tablet, in a plastic bag. Do not place the tablet on the plate itself.
- 5 Seal the bag and transport to the laboratory. DO NOT REFRIGERATE.

Male Gonorrhea Culture Collection Procedure

- 1 Insert the small tipped swab into the urethra 2-4 cm and rotate the swab three to five seconds to ensure adequate sampling.
- 2 Inoculate the specimen directly onto Modified Thayer-Martin and chocolate agar.
- 3 Label the agar plates.
- 4 Place the plates, along with a CO₂ generating tablet, in a plastic bag. Do not place the tablet on the plate itself.
- 5 Seal the bag and transport to the laboratory. DO NOT REFRIGERATE.

MOUTH

- 1 Rinse the mouth.
- 2 Scrape the mucosal surface of the gums or the teeth and place scrapings in sterile container. Moisten with non-bacteriostatic sterile saline.
- 3 Transport to the laboratory. DO NOT REFRIGERATE.

NASOPHARYNX

Nasopharyngeal secretions obtained by aspiration or washings are preferred over specimens collected on nasopharyngeal swabs. If it is necessary to collect by swab, the following procedure should be used:

Nasopharyngeal Swab

Equipment:

- Mini-tip culturette
- Light
- Tissues
- Sterile scissors and chlamydia media for chlamydia testing

- 1 Seat patient comfortably or lay child on back with head of bed elevated 20 degrees.
- 2 Using light to visualize nasal passages, gently cleanse passages if necessary or have adult patient blow their nose to clear passages.
- 3 Open mini-tip culturette; remove cap/swab from tube. The wire handle can be curved to the shape of the nasal passage. Avoid contaminating the tip. Moisten the tip with sterile water or saline.
- 4 Gently insert swab into patient's nostril and guide the swab backward and upward along the nasal septum until a distinct give of resistance indicates that the posterior pharynx has been reached. Take care not to perforate nasopharynx.
- 5 Remove the swab and return to the plastic tube for all tests except chlamydia culture.
 - For chlamydia culture, cut off culturette at vial rim with sterile scissors to place in culture media.
- 6 Label the specimen and transport to laboratory.

NASOPHARYNGEAL WASH

Nasopharyngeal wash is the only acceptable specimen for RSV rapid testing.

Equipment:

1 ml sterile saline
30 ml plastic medicine cup
1-oz tapered rubber bulb
Sterile container for transport to lab

- 1 Pour sterile saline into the plastic medicine cup and suction into the rubber bulb.
- 2 With the patient's head tipped back at 70 degrees, insert the bulb until it occludes the nostril.
- 3 With one complete squeeze and release, collect the nasopharyngeal wash in the bulb. Empty the contents of the bulb into a sterile container.
- 4 Label the specimen and transport to the laboratory.

Nose

Equipment:

Sterile culture tube with swab or mini-tip culturette

- 1 Collect anterior nares culture with a dacron swab. For small children, use a mini-tip swab.
- 2 Position patient in a sitting or supine position.
- 3 Insert sterile swab into interior nares, rotate once and remove. Immediately insert into culture sleeve.
- 4 Label and transport to the laboratory according to procedure.

PINWORM DETECTION

Diagnosis of pinworm infection of the rectal canal is made by demonstrating *Enterobius vermicularis* ova. The ova are best detected using clear (not frosted) tape or a commercially available "Swube" paddle with a flat, sticky surface.

Optimal recovery of pinworm ova is best achieved if the specimen is obtained early in the morning prior to bathing or using the toilet. The sticky side of a "Swube" paddle is applied to the perianal folds of a patient suspected of having pinworm infection. The paddle is then placed in a test tube for transport at room temperature. Alternatively, a 3- to 4-inch strip of clear (not frosted) tape, placed adhesive side down on a slide, is also acceptable.

Since the female worms migrate to the anus to deposit eggs on a sporadic basis, a series of four to six specimens may be necessary to demonstrate the infection.

Adult female worms may be seen on the surface of stool specimens. However, routine diagnosis by fecal examination is unreliable because eggs are not introduced into the fecal stream, but are instead laid on the surface of the fecal material as it passes through the rectum.

PROSTATE

- 1 Obtain prostatic fluid by digital massage through the rectum.
- 2 Collect the specimen in a sterile container.
- 3 Transport to the laboratory. DO NOT REFRIGERATE.

SKIN

See abscesses, bullae, cellulitis, petechiae, vesicles and wounds.

SPUTUM

Assure patient cooperation to collect an adequate acceptable specimen. The sputum should be produced from a deep cough from the respiratory tract. The optimal time of day for sputum collection is early morning. Collection of oral contents (spit or saliva) will be rejected by the laboratory.

Sputum for mycobacterial (AFB) or fungus cultures should be submitted on three consecutive mornings. If the specimen is for AFB, a minimum volume of 5 ml is needed. Do not pool multiple samples.

Equipment:

Sterile sputum specimen container
Box of tissues
Glass of water

- 1 Obtain a first morning specimen, if possible. Have the patient brush their teeth and gargle with water immediately before obtaining the sample. This will reduce the number of contaminating oropharyngeal bacteria. Mouthwashes containing antibacterial substances should be avoided.
- 2 Instruct the patient to expectorate into a sterile container. Do not ask the patient to spit into the sterile sputum container since this generally results in a saliva specimen instead of a sputum specimen. Cap container immediately.
- 3 If a patient is unable to produce a sputum specimen, induction may be necessary. Postural drainage, saline nebulization or chest percussion may be used.
- 4 Label and transport to the laboratory according to procedure.

STOOL/FECES

Specimens collected within five days of a barium enema are unsuitable for microbiology examinations. Stool cultures, *C. difficile* EIA and parasitology (O&P) exams will be performed on one stool per day. The collection of one specimen on three separate days is recommended for both culture and parasitology exams.

Equipment:

- Bedpan or commode pan
- Appropriate transport container
- Parasitology (O&P): Protofix vial
- Culture: Enteric Plus vial (green cap)
- *C. difficile*, gram stain*
- Rotavirus: sterile screw top specimen cup

*NOTE: *Stool for gram stain must be received in lab within one hour of collection or stool must be placed in Protofix vial.*

- 1 Collect specimen in a commode pan or in plastic wrapped between the toilet seat and the bowl.
Specimen can be collected from an infant by use of a disposable diaper that is placed on the child inside out or the diaper can be lined with plastic wrap. DO NOT submit feces contaminated with urine or toilet water.
- 2 Transfer the specimen to the appropriate container for the test(s) ordered.
- 3 Select portions of the stool that appear bloody, slimy or watery and place in the appropriate cup or vial until the media and sample volume rises to the red line, mixing the specimen well with the spoon provided. Twist cap tightly.
- 4 If a stool specimen is not available, the following are acceptable alternatives for culture and rotavirus:
 - Swab of rectal mucous
 - Rectal swab inserted 1 inch into the anal canal
- 5 Label and transport to the laboratory according to procedure. Maintain all vials at room temperature.

THROAT

Assure patient cooperation by explaining that you will be collecting a throat culture by rubbing the back and sides of their throat with a swab. Obtain the support and assistance of the patient or the parent or legal guardian if the patient is a child.

Equipment:

Appropriate transport culturette
Tongue depressor

- 1 Seat the patient comfortably with head tilted back under a good light source.
- 2 Depress the tongue with a tongue depressor and visualize the uvular area.
- 3 Reach behind the uvula and swab:
 - Both tonsillar fauces
 - The posterior pharynx
 - Any ulceration, exudate, lesion or area of inflammation. It is important to avoid touching the tongue, lips or inside of the cheek with the culture swab.
- 4 Remove the swab and immediately reinsert into culturette casing containing transport media.
- 5 Label casing and transport to the laboratory according to procedure.

TISSUE

- 1 Tissue collection is an invasive procedure and requires surgery by a trained physician.
- 2 Collect tissue aseptically.
- 3 Place the specimen in a sterile container on sterile gauze moistened with non-bacteriostatic saline.
- 4 Transport to the laboratory. DO NOT REFRIGERATE.
- 5 Tissue submitted in formalin is unacceptable for culture.

TRANSTRACHEAL ASPIRATE

The use of bronchial brush/washing/lavage specimens for culture is not a routine procedure. The techniques for these procedures are best performed by a trained individual. No description of the methods is provided.

Ensure that specimen collection techniques provide acceptable culture and transport conditions for anaerobic organisms.

URETHRA

- 1 Insert the small-tipped swab into the urethra 2-4 cm and rotate the swab for three to five seconds to ensure adequate sampling.
- 2 Transport to the laboratory. DO NOT REFRIGERATE.

URINE

Acceptable specimens listed below include infant or pediatric urine collection bag, clean-catch voided sample, suprapubic aspiration and indwelling catheter samples.

Method: Infant or Pediatric – Urine Collection Bag

Equipment:

Pediatric urine collection bag
Soap and water
Towelettes, washcloth or sponge
Sterile water
Sterile 4 x 4 gauze
Sterile urine cup
Urine culture collection kit
Sterile gloves (non-latex)
Urine culture kit

Caretaker, parent or family member should:

- 1 Remove diaper.
- 2 Wash patient area with soap and water, removing all powder or ointment.
- 3 Rinse well with sterile water and dry area with sterile 4 x 4 gauze.
- 4 Apply sterile urine collection bag.
- 5 Reapply diaper, leaving bag unfolded and pulled through to the outside of diaper.
- 6 After patient voids, remove bag and pour into sterile urine cup. Aspirate into urine culture container if volume permits.

NOTE: *Patient and family education:*

- 1 Explain procedure to parents.
- 2 Explain need for aseptic urine specimen to parents.
- 3 Instruct parents to notify nurse as soon as patient voids.

Method: Clean-Catch Urine

Procedure for collection depends on the medical condition of the patient. Patient assistance may be required.

Equipment:

Urine culture kit
Urine specimen cup
Soap and water
Towelettes, washcloth or sponge
Clean bedpan, urinal or commode pan for patients who are unable to void directly into specimen cup

Females – Instruct Patient to:

- 1 If menstruating, insert a fresh tampon to halt flow.
- 2 Open the sterile specimen collection cup without touching the rim, inside of cup or inner surface of the cup lid.
- 3 Wash hands with soap and water; dry hands.
- 4 Separate the skin fold around the urinary opening with one hand and keep apart until finished collecting the sample.
- 5 Using a sterile, moist towelette (or cotton balls

soaked in soap and water), wash the urinary opening and surrounding tissue, front to back. Rinse with clear water.

- 6 Holding skin folds apart with your fingers, begin urinating into the toilet.
- 7 After the urine stream is well established, and without interrupting the urine flow, move the sterile container into the path of the stream to “catch” the urine.
- 8 Collect the urine until the container is approximately half full (or until flow of urine decreases substantially) and then finish voiding into toilet.

Males- Instruct Patient to:

- 1 Open the sterile specimen collection cup without touching the rim, inside of cup or inner surface of the cup lid.
- 2 Wash hands with soap and water; dry hands.
- 3 Retract the foreskin and thoroughly wash the end of the penis using a sterile, moist towelette or washcloth soaked in soapy water. Rinse with clear water.
- 4 Begin urinating into the toilet.
- 5 After the urine stream is well established and without interrupting the urine flow, move the sterile container into the path of the stream to “catch” the urine.
- 6 Collect the urine until the container is approximately half full (or until flow of urine decreases substantially) and then finish voiding into toilet.

General

- 1 Do not allow any part of the body to touch the rim or inside of the container.
- 2 Do not completely fill container. Testing can be performed on a small urine sample.
- 3 If the specimen is collected at home, label the container with your name, date and time of collection, and refrigerate immediately.

In all cases, the staff should:

- 1 Aspirate urine into urine culture tube system by submerging straw tip of transfer device to the bottom of urine container.
- 2 Place vacutainer tube in holder portion and pierce stopper.
- 3 Hold in position until urine stops flowing into tube.
- 4 Remove tube from device and dispose of device into a sharps container.
- 5 Shake vacutainer tube vigorously.
- 6 Label and transport according to procedure.

Never submit a specimen aspirated from a large container such as a urinal or bedpan, or a specimen brought from home that was not collected as a clean catch in the proper container.

If less than 3 cc is collected, put all of the specimen into a sterile specimen cup and notify the laboratory that a culture needs to be done on the specimen immediately.

Suprapubic Urine Aspiration

This is not a routine technique and is best performed by a trained individual. Ensure that specimen collection techniques provide acceptable culture and transport conditions for anaerobic organisms.

Indwelling Catheter Urine

Specimens obtained from the collection bag are not suitable for analysis. Foley tips are also not acceptable for culture.

Equipment:

Alcohol pad
Sterile syringe and needleless cannula
Urine culture kit

- 1 Clean the specimen port of the drainage tubing with an alcohol pad.
- 2 Using a sterile syringe, aspirate urine directly from the port.
- 3 Transfer the urine to a sterile urine culture tube.
- 4 Label and transport to the laboratory according to procedure.

WOUND

For closed wounds, refer to abscesses, bullae, cellulitis, petechiae and vesicles.

Open Wound Collection:

For collection from sinus tracts, the preferred specimens are aspirations or curettings (physician performed).

Equipment:

Swab Method:

- Sterile aerobic culture swab

Aspiration Method:

- Sterile container
- 35cc syringe with needleless cannula
- Sterile saline

Preparation:

Clean the sinus tract opening of the wound surface mechanically without using germicidal agents, flushing the wound well with sterile saline using syringe and cannula.

Method: Aspiration

- 1 Aspirate material obtained by needleless cannula after irrigating the wound again with sterile saline.
- 2 Do not touch bed or surrounding tissue with the needleless cannula.
- 3 Express culture material into a sterile container with a tight-fitting lid.
- 4 Dispose of the syringe into a sharps container.

Method: Swab

- 1 Attempt to culture the base or edges of the wound using swab and zigzag pattern. This step will reduce the chance of collecting normal flora.
- 2 Immediately reinsert swab into culturette tube, making sure the tip of the swab comes in contact with the moist sponge.

Labeling:

Label and transport to the laboratory according to procedure. Specimens received before 10:00 p.m. will have preliminary results the next day.

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