

Instructions for the Proper Collection and Handling of Culture Specimens

ANATOMIC AND SURGICAL PATHOLOGY SPECIMENS:

Selecting the proper specimen and collecting an adequate specimen for examination are essential. When the lesion is large or when there are several lesions, multiple specimens from different sites should be collected. Specimens from an abscess should include pus along with a portion of the wall of the abscess. Gross surgical specimens submitted for histopathologic examination are ideal for microbiological study since portions of the specimen may be carefully selected for analysis prior to placing the tissue in fixative. The tissue may be finely minced using a sterile disposable scalpel and a few drops of sterile saline.

BLOOD CULTURE COLLECTION:

A. Preparing the patient's arm:

1. Place the tourniquet on the patient's arm.
2. Palpate the vein.
3. Cleanse the venipuncture site with the isopropanol scrub by scrubbing vigorously for 60 seconds.
4. Clean the venipuncture site with 2% Chloraprep from the center of the site outward for 30 seconds.
5. Collect 2 mL to 20 mL of blood per set. A set of blood cultures is defined as two pediatric bottles or one aerobic bottle and one anaerobic bottle. Pediatric bottles should not be routinely collected on adults.

B. Preparation and inoculation of blood culture:

1. Remove the center tab from the top of the blood culture bottle.
2. Clean the rubber area of the top with 70% isopropyl alcohol.
3. Inoculate appropriate blood culture bottles based on provider's order:
 - Bactec Plus (ARDS) Aerobic/F 8-10 mL and Bactec Plus (ARDS) Anaerobic/F 8-10 mL
 - Bactec Standard Aerobic/F 8-10 mL and Bactec Standard Anaerobic 5-7 mL
 - Bactec Peds Plus 1-3 mL
4. Label bottles appropriately.
5. Load bottles into the BACTECT™.

BLOOD CULTURE WITH ANTIBIOTIC REMOVAL DEVICE (ARD):

Same procedure as regular blood culture using orange and silver label bottles.

EYE CULTURES:

Swabs for culture should be taken before topical anesthetics are applied and corneal scrapings should be taken after they are applied.

FECES:

Proper collection and preservation of feces is an important requirement for the isolation of microorganisms contributing to intestinal disease. The specimen must be transported immediately to the Microbiology Laboratory for proper handling, because a number of microorganisms (especially *Shigella* and *Salmonella*) will not survive changes in temperatures. If sterile swabs are used in obtaining the specimen, they should be passed beyond the anal sphincter, carefully rotated, and withdrawn. The swab is then returned to its transport container, labeled, and delivered to the laboratory for processing. Optimum timing and numbers of stool cultures has not been established when investigating the cause of a possible bacterial pathogen causing diarrhea. History may be helpful in noting the onset of symptoms in relation to time of ingestion of food and water. Some causes are related to bacterial invasion like *Salmonella*, *Shigella*, and *Campylobacter*. Others are toxin related like *E.coli 0157*, *C.difficile*, and some *Vibrio* species. Finally, notifying the laboratory of pertinent history and suspicion for unusual pathogens like *Yersinia* or *Aeromonas* is helpful.

The following are reasonable indications for doing a stool culture for bacterial pathogens. Generally no more than 1 specimen is accepted within a 24 hour period:

1. Patients who present with the onset of bloody diarrhea.
2. Immunocompromised patients with the acute onset of diarrhea.

3. Patients with comorbidities who would do poorly with untreated infectious diarrhea.
4. A food handler or cafeteria worker with symptomatic diarrhea or evaluating clearing of a pathogen.
5. Patients with inflammatory bowel disease and differentiation of that primary disease from bacterial pathogen would be important.

C. difficile is becoming the most common cause of infectious diarrhea, especially in relation to diarrhea that is associated with antibiotics, hospitalization, use of antiperistaltics or acid suppressors. The laboratory may accept no more than 1 stool specimen within a 72 hour period. Avoid contamination with urine or water from toilet. Formed stool specimens are not acceptable for *Clostridium difficile* PCR testing.

CEREBROSPINAL FLUID:

Lumbar puncture should be performed under conditions of strict asepsis since contamination of the specimen can occur readily and confuse the ID of the etiologic agent. The skin should be disinfected with providone iodine. Specimens should be collected in sterile containers which can be sealed with screw-cap in order to prevent leakage and loss or contamination of the contents. Prompt transport of the specimen to the laboratory is mandatory, since fastidious organisms such as *Haemophilus influenzae* and *Neisseria meningitidis* may not survive storage or variations in temperature.

FLUIDS OTHER THAN CSF:

The percutaneous aspiration of pleural, pericardial, peritoneal, and synovial fluids must be performed aseptically to avoid contamination of the specimen and to prevent the accidental introduction of microorganisms into these anatomical spaces. The specimen should immediately be injected into a sterile container. Since infection of these spaces may be due to anaerobes, it is recommended that fluid or pus be collected with a sterile syringe and needle so that any air bubbles present in the syringe be expelled. The syringe should be capped according to NMMC policies and transported immediately to the Microbiology Laboratory.

RESPIRATORY TRACT:

Requisitions for culture of specimens from the upper respiratory tract should specify the suspected etiological agent so that Microbiology personnel may take appropriate steps to ensure its isolation. Throat cultures should be obtained under direct visualization with a Dacron®, cotton, or calcium alginate swab by vigorously swabbing both tonsillar areas, the posterior pharynx and any areas of inflammation, ulceration, exudation, or capsule formation. The tongue should be depressed with a tongue blade or spoon to minimize contamination of the swab with oral secretions which may dilute, overgrow, or inhibit the growth of pharyngeal flora. A sterile disposable culture unit is available in the laboratory consisting of a plastic tube containing a sterile polyester-tipped swab. The unit is removed from its sterile envelope and the swab is used to collect the specimen. It is then returned to the tube, labeled and sent to laboratory. This tube will provide sufficient moisture for storage up to 72 hours at ambient temperature. Special requests for *Neisseria gonorrhoeae*, *Bordetella pertussis*, *Burkholderia cepacia* or *Corynebacterium diphtheria* should be made for proper collection and processing.

The collection for sputum for culture requires the cooperation of the patient and should include instructions to obtain material from a “deep cough” (tracheobronchial sputum) which is expectorated directly into a sputum kit. If the specimen is for pyogenic culture, 1 mL to 3 mL will be sufficient. The specimen will be evaluated to check the quality of the specimen. For TB and/or fungus culture, 5 mL to 10 mL is required and the specimen is not graded for suitability. The specimen is accepted as sputum if it contains <10 squamous epithelial cells per low power field. If it does not meet these criteria, the sputum is rejected and a new specimen is requested. If a specimen is rejected 3 times, the physician is notified that we have not obtained a suitable specimen. It is then the physician’s decision to decide whether or not to continue or cancel the request for a sputum culture.

The collection of suitable specimen for RSV Ag and Flu A and B Ag detection is a nasopharyngeal swab. The Floqswab by Copan, available from laboratory, is inserted into the nose back to the nasopharyngeal region, rotated and removed. Using same swab, repeat on other nostril. Swab may be placed in a container with 1 mL sterile saline or placed into sterile tube. Ensure proper labeling of specimen and transport to laboratory.

URINE:

Midstream, clean-catch urine kits are obtained from SPD. These kits contain the sterile urine containers. The directions on the kit should be followed to avoid contamination of the specimen. Ideally the periurethral area (tip of penis, labial folds, vulva) should be carefully cleansed with the glans penis or labial folds retracted with 2 separate washes with plain soap and water or a mild detergent and well rinsed with warm sterile water to remove the detergent.

The urethra is then flushed by passage of the first portion of the voiding, which is discarded. The subsequent urine, voided directly into a sterile container, is used for culturing and colony counting.

Since urine generally will support the growth of most of the urinary pathogens, it is absolutely essential for culture purposes that the urine be processed within 1 hour of collection or stored in the refrigerator at 4° C for not more than 24 hours until it can be cultured.

GENITAL:

Males: When urethral discharge is sent, collecting first **early-morning** specimen prior to urination may be helpful. If discharge is not visibly apparent so as to be easily collected, then the tip of a narrow diameter cotton, rayon, or Dacron® swab on a plastic shaft may be inserted 3 cm to 4 cm into anterior urethra. The swab should be left in place for a few seconds to allow fibers to become saturated with exudate. Return swab to transport container, properly label specimen, and immediately send to laboratory for processing.

Females: The best site to obtain a culture is the cervix. The specimen should be collected by an experienced professional. A sterile bivalve speculum is moistened with warm water and inserted and the cervical mucus plug is removed with a cotton ball and forceps. A sterile culture swab is then inserted into the endocervical canal, moved from side to side, allowed to remain for a few seconds before removal and immediately returned to the plastic shaft and sent to laboratory for processing.

Cepheid collection kits are available for *Chlamydia*/GC DNA probe testing.

When indicated, viral culture PCRs are collected as outlined above. Contact Mail Off area of Pathology at 377-3066 for appropriate transport media/container.

Beta Streptococcus Screening-prenatal: Collect the sample from the endocervix and rectal per the CDC recommendations. Collect sample from endocervix using technique described within collection of genital culture, female. After sample is collected from endocervix, using the same swabs, insert into the rectum approximately 2.5 cm beyond the anal sphincter. Gently rotate the swabs and allow to sit for a minute to absorb sample onto the swabs. Remove the swabs, return to the plastic sheath, label appropriately and send the sample to the laboratory for processing. A urine sample should also be submitted for culture according to the CDC recommendations.

Group B Beta Streptococcus PCR – intrapartum: Collect using Copan swab. Collect from endocervix/rectal per CDC recommendations before any KY Jelly is used in examination. Return Copan swabs to proper sleeve, label, and send to laboratory for processing.

WOUNDS:

Material from a previously undrained wound abscess, if properly collected and transported to the laboratory, should contain the etiological agent of disease in most instances. An opened wound, ulcer, or sinus tract, however, frequently becomes contaminated with skin, mucosal or airborne microorganisms. In general, the use of a swab to collect material from these sites is of limited value, since the amount of material supplied for examination is not only very small but also likely to represent an inadequate specimen. Moreover, nothing is more worthless than a dry swab from a dry lesion. A way to prevent this is to use anaerobic transport tubes for transport of swab specimens. Specimens on swabs are protected from air but remain moist in a long column of solid medium under anaerobic atmosphere. Agar in the medium contained in tubes inhibits the diffusion of oxygen after a specimen on the swab is inserted. Reducing agents in the medium combine with free oxygen to maintain anaerobiosis. Salts and buffers provide a protective environment for microorganisms. Resazurin indicates the presence (pink to lavender) or absence (no color) of oxygen in the medium.

Instructions for use of anaerobic transport tubes and sterile anaerobic transport tubes are:

1. Using aseptic technique, obtain specimen with a swab.
2. Carefully loosen screw-cap and quickly insert swab into the tube to within approximately 5 mm from bottom of the tube.
3. Break off shaft of swab.
4. Replace and tighten cap.

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5. Label tube with appropriate patient information.
 6. Send to the Microbiology Laboratory

In chronic, localized lesions, the number of organisms present may be small, and a sterile needle and syringe should be used to collect a generous quantity of liquid material. Since anaerobes are commonly recovered from certain wounds, such material should be sent to the laboratory immediately.

SPECIMEN FOR FUNGUS CULTURE:

To obtain specimen of skin or nails, the affected site is carefully washed with 70% isopropanol and, after drying, the lesion is scraped with sterile scalpel and the material obtained is placed in a sterile, Petri dish or on a piece of white paper carefully folded in a packet to prevent loss of the specimen. Hairs from infected areas are clipped or plucked and sent to the laboratory in a similar manner.

In the subcutaneous mycoses, a variety of materials may be submitted, including pus, exudate from draining lesions, material aspirated with syringe and needle from unopened abscesses or sinus tracts, or biopsied tissue. These should be placed in a sterile tube or Petri dishes and submitted promptly to the laboratory.