Microbiology Collection Guidelines

Anaerobic culture

Transfer aspirated material to an anaerobic transport vial or anaerobic culture transport swab (gel in bottom). Large volumes of purulent material may be transported in a sterile, closed screw-cap tube or container. Place tissue samples, biopsy samples, or curettings into an anaerobic transport device or anaerobic culture transport swab (gel in bottom). Large pieces of tissue can be transported in a wide-mouthed anaerobic transport device or in a sterile tube or jar.

Abscess

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 minute. 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

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.

Decubiti and other surface ulcers

Submit specimens form punch biopsy, or aspirated material obtained by needle and syringe after thorough and proper disinfection of the surface area, or small curettings of material from deep tissue at the wound margin.

Pulmonary specimens

Collect lung tissue, transtracheal aspirate, percutaneuous aspirate, transcutaneous aspirate, and bronchial brushings via double-lumen catheter.

Female genital tract specimens

Disinfect the cervical opening by swabbing it with povidone-iodine. Sample the upper genital tract by using a double-lumen collector and self-contained transport system. Specimens collected by laproscopy, culdocentesis, or surgery are appropriate for anaerobic culture.

Urinary tract

Obtain material via suprapubic bladder tap.

Arthropod identification

Specimen should be submitted in a clean, leak-proof, tightly sealed container.

Blood culture

Bottle Preparation

- Inspect each blood culture bottle before use to ensure the integrity of the bottle and that the sensor on the bottom is intact. The sensor is normally a uniform grayish-green color and a yellow color indicates contamination of the broth.
- Ensure that the bottles have been stored appropriately at room temperature (15-30°C) and protected from direct sunlight.
- An expiration date is printed on each bottle's label. Do not use the culture bottles beyond the last day of the month indicated.
- Check the bottles for damage, leakage, or deterioration.
- Discard any bottle found to be damaged, stored improperly, or with a yellow sensor. Report bottles found in poor condition to the Microbiology personnel or the Laboratory Manager.
- Remove the plastic flip-top over the cap. Sterilize the exposed rubber stopper with a sterile 70% alcohol pad. Allow to air dry.

Site Preparation

- Clean the venipuncture area with a Chloraprep swab. Pinch the wings on the applicator to break the ampule and release the antiseptic. Do not touch the sponge. Wet the sponge by repeatedly pressing the sponge against the venipuncture site until liquid is visible on the skin. Use repeated back and forth strokes for approximately 30 seconds. Allow the area to completely air dry for approximately 30 seconds. Do not blot or wipe away. Do not touch the venipuncture site with unsterile fingers. If you find it necessary to palpate the site before drawing the blood culture, cleanse the finger used to palpate with an alcohol prep and then Chloraprep swab.

Venipuncture

- Using a syringe or collection device, aseptically draw an appropriate amount of blood for the appropriate bottles being collected.
- Aerobic and anaerobic bottles require 5-10 mls per bottle (10 mls being preferable). Pediatric bottles require 2-4 mls per bottle (4 mls being preferable). Recommended blood to broth ratio is 1:5 to 1:10. As the volume of blood drawn increases, the yield of a positive blood culture increases. Optimally, a total of 20 mls of blood should be drawn from adults (10 mls per bottle).
- Do not overfill the bottles, as this may cause false positive readings.
- Inoculate the blood culture bottles <u>first</u> to avoid contamination, and then fill additional blood collection tubes.
- After drawing the appropriate bottles for the patient, carefully apply the patient's label to the bottle as to not cover the bottle's barcode or lot number.

Timing of Blood Cultures

- Although drawing blood cultures before or during the fever spike is optimal for recovery, <u>volume</u> is more important than timing in the detection of agents of septicemia.

- When acute sepsis or another condition (osteomyelitis, meningitis, pneumonia or pyelonephritis) requires immediate institution of antimicrobial agent therapy, draw blood cultures before starting therapy.
- For fever of unknown origin, subacute bacterial endocarditis, or continuous bacteremia or fungemia, a maximum of three blood cultures is recommended.

Chlamydia trachomatis/ Neisseria gonorrhoeae

Endocervical swab (unisex)

- Remove excess mucus from the cervical os and surrounding mucosa using the cleaning swab (white shaft swab in the package with red printing). Discard this swab. To remove excess mucus from the cervical os, a large-tipped swab (not provided) may be used.
- Insert the specimen collection swab (blue shaft swab in the package with the green printing) into the endocervical canal.Gently rotate the swab clockwise for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
- Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
- Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
- Carefully break the swab shaft against the side of the tube at the score line and discard the top portion of the swab shaft; use care to avoid splashing of contents.
- Re-cap the swab specimen transport tube tightly.

Male urethral swabs

- The patient should not have urinated for at least 1 hour prior to sample collection.
- Insert the specimen collection swab (blue shaft swab in the package with the green printing) 2 to 4 cm into the urethra.
- Gently rotate the swab clockwise for 2 to 3 seconds in the urethra to ensure adequate sampling.
- Withdraw the swab carefully.
- Remove the cap from the swab specimen transport tube and immediately place the specimen collection swab into the transport tube.
- Carefully break the swab shaft against the side of the tube at the score line and discard the top portion of the swab shaft; use care to avoid splashing of contents.
- Re-cap the swab specimen transport tube tightly.

Urine Collection

- The patient should not have urinated for at least 1 hour prior to specimen collection.
- Direct patient to provide a first-catch urine (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 rRNA

target dilution that may reduce test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.
- 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 specimen transport tube label.
- Re-cap the urine specimen transport tube tightly.

Vaginal Swab Collection

- Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. If the soft tip is touched, the swab is laid down, or the swab is dropped, use a new Aptima Vaginal Swab Specimen Collection Kit.
- Hold the swab, placing your thumb and forefinger in the middle of the swab shaft covering the score line. Do not hold the swab shaft below the score line.
- Carefully insert the swab into the vagina about 2 inches (5 cm) past the introitus and gently rotate the swab for 10 to 30 seconds. Make sure the swab touches the walls of the vagina so that moisture is absorbed by the swab and then withdraw the swab without touching the skin.
- While holding the swab in the same hand, unscrew the cap from the tube. Do not spill the contents of the tube. If the contents of the tube are spilled, use a new Aptima Vaginal Swab Specimen Collection Kit.
- Immediately place the swab into the transport tube so that the score line is at the top of the tube.
- Carefully break the swab shaft at the score line against the side of the tube.
- Immediately discard the top portion of the swab shaft.
- Tightly screw the cap onto the tube.

Clostridium difficile

Transfer liquid or soft stool (but not urine) into a dry, clean container. Avoid mixing toilet paper, water, or soap with the sample. Label the container.

Crystal analysis

Physician should disinfect skin before aspirating specimen with needle aspiration (arthrocentesis). Fluid should be sent to lab immediately at room temperature in a sterile, screw cap container or tube or syringe as long as needle is not attached. Synovial fluids should not be sent in tubes with anticoagulant other than EDTA or sodium heparin.

Bile should be submitted in sterile, screw cap container or tube.

Enteric Parasite Panel

(*Cryptosporidium* spp., *Giardia* spp., *Entamoeba histolytica*)

Unpreserved specimens
Transfer the stool specimens to a dry, clean container. Avoid contamination with water or urine. Avoid mixing toilet paper, water or soap with the specimen.

10% formalin-fixed specimens
Transfer the stool specimen into the fixative device according to the manufacturer's instructions. Avoid contamination with water or urine. Avoid mixing toilet paper, water or soap with the specimen.

Extended Enteric Bacterial Panel

(*Salmonella* spp., *Campylobacter* spp., *Shigella* spp., Shiga toxin 1/ shiga toxin 2 genes, *Plesiomonas shigelloides*, *Vibrio* (*vulnificus, parahaemolyticus and cholerae*), Enterotoxigenic *E. coli* heat-labile enterotoxin (LT)/heat-stable enterotoxin (ST) genes, and *Yersinia enterocolitica*)

Unpreserved specimens
Transfer the liquid or soft stool specimen to a clean, dry container. Avoid contamination with water or urine. Label the container and transport to the laboratory at 2 to 25°C. Avoid mixing toilet paper, water or soap with the specimen.

Cary-Blair preserved specimens
Transfer the liquid or soft stool specimen to a 15ml transport device according to the manufacturer's instructions. Avoid contamination with water or urine. Avoid mixing toilet paper, water or soap with the specimen.

Fecal lactoferrin (Fecal WBC's)

Collect fecal specimens into a clean, airtight container with no preservatives.

Fluid/Exudate Culture

Pleural, peritoneal, pericardial, and synovial fluids
Collect by aspiration with a needle and syringe. Disinfect skin before aspirating specimen. Send specimen in sterile, screw-cap tube or anaerobic transporter immediately to laboratory. From 1 to 5 mL is adequate for isolation of most bacteria. If AFB or fungal testing is to be ordered also, at least 5mL should be submitted for each of these cultures. 10mL of fluid is recommended for the diagnosis of peritonitis.

CSF
Collect by aseptically inserting a needle into the subarachnoid space, usually at the level of the lumbar spine. Three or four tubes should be collected and immediately labeled with the patient's name. Tube three or four should be used for cell count and differential. The other tubes can be used for both microbiologic studies and chemical studies, including protein and glucose levels.
CSF specimens should never be refrigerated before processing.

Hemascreen (Fecal Immunochemical Test)

Patients that use the DEVEL-A-TAB Sampler will collect specimens at home over the course of two consecutive bowel movements. Using a ballpoint pen, write name, age, address, phone # and date of each bowel movement on card. Prior to defecation: Flush the toilet to clear bowl. For ease of collecting specimen, place tissue provided onto water in bowl. Lift front flap on test slide. After defecating into bowl, using applicator stick, stab stool in 4 different sites of the bowel movement and apply to circle # 1. Close flap. Discard stick in trash. DO NOT FLUSH APPLICATOR STICK. Repeat the same procedure for the next bowel movement and apply to circle #2. Place the card into the provided mailing envelope, seal by removing tape strip and fold as indicated. Mail or return to the laboratory.

IT IS AGAINST POSTAL REGULATIONS TO MAIL SPECIMEN IN A STANDARD PAPER ENVELOPE.

If not using the DEVEL-A-TAB Sampler for collection, collect stool specimens at home over the course of two consecutive bowel movements. Pass specimen directly into a wide mouth leak proof container with a tight fitting lid. Store specimens in refrigerator. Bring both specimens to laboratory.

Do not collect specimen during or until three days after a menstrual period, or while you have blood in urine or bleeding from hemorrhoids or dental work.

HSV 1 and 2 DNA amplification

Specimens obtained from vesicular lesions within the first three days after their appearance are the specimens of choice, but other lesion material from older lesions or swabs of genital secretions should be obtained if suspicion of HSV infection is high. Once crusting and healing have begun, the recovery rate of HSV drops sharply. The use of alcohol to cleanse the lesions may inactivate the virus and should therefore be avoided. Calcium alginate swabs are toxic to HSV and therefore should not be used.

The vesicle should be unroofed with a sterile needle or scalpel, and a sterile Dacron or rayon swab with a plastic shaft should be rotated firmly in the base of the lesion to allow epithelial cells to be collected onto the swab. Ideally, more than one lesion should be sampled. Similarly for ulcerative lesions, a swab should be firmly rotated in the base of one or more lesions. The swab(s) should be immediately inserted into viral transport medium such as M4 transport medium. The swab's shaft should be broken before the cap is replaced so that the shaft will not interfere with closure and leakage will be prevented. Place swab(s) in a viral transport medium. Samples should be stored refrigerated (2-8 C) after collection and during transportation to the laboratory.

Influenza A and B

1. The kit includes swabs with a flocked tip for nasal specimen collection
2. Insert the swab into one nostril of the patient.
3. Rotate the swab two complete 360-degree turns; pressing firmly against the nasal mucosa to help ensure the swab contains both cells and mucus.
4. Withdraw the swab from the nasal cavity. The sample is now ready for processing using the system kit.

KOH Prep

Any scrapings, nails, hair, body fluids or exudates to be examined for fungal elements are acceptable. Skin, hair, and nails should be submitted in sterile, leak proof container and stored at room temperature. Body fluids, exudates should be submitted in sterile, leak proof container and stored at refrigerated temperature. Growth from a culture plate can also be used.

If collecting nails or skin scrapings, wipe with 70% alcohol before collection.
Hair: collect hairs with intact shaft
Nails: send clippings of affected area
Skin: scrape skin at leading edge of lesion.

Maldi-Tof identification

Submit plated isolate.

MRSA screen (nasal)

Insert Stuart's or Amies swab into one anterior naris. Apply slight pressure to the nostril and rotate to sample the inside surface. Insert the same swab into the other nares and repeat. Place swab into swab transport tube.

Ova, cysts and parasites

- It is recommended that no more than three specimens per patient for routine OCP be ordered without prior consultation with an individual who can explain the limited yield provided by additional specimens. For outpatients, specimens should be spaced a few days apart to assure recovery of parasitic elements that are passed intermittently. For hospitalized patients, specimens can be collected for a designated length of time to avoid prolonging the hospital stay.
- Specimens can be sent to lab in clean, leak proof, tightly sealed containers within one hour or stored at 2-8ºC for up to 24 hours. If sent in preservative, store at room temperature. Wait 7-10 days if patient has received antiparasitic compounds, barium, iron, Kaopectate, metronidazole, Milk of Magnesia, Pepto-bismol, antacids, bismuth, antidiarrheal medication, oily laxatives or tetracycline.
- The specimen is ideally passed into a bedpan but must not be contaminated with urine. Alternatively, a large plastic bag or "saran wrap" may be placed over the toilet seat opening and the specimen passed into

the bag. A thoroughly cleaned and dried milk carton cut so as to remove the upper and two thirds of the carton may also be used. It will be easier to collect the specimen if the water supply to the toilet is shut off and water drained from the bowl by flushing twice.

Routine aerobic culture

Abscess (also lesion, wound, pustule, ulcer)
Wipe area with sterile saline or 70% alcohol and swab along leading edge of wound with Stuart's or Amie's culture swabs.

Bone marrow and bone biopsies
Bone marrow aspirates are submitted in a sterile container containing an anticoagulant. The specimen must be delivered to the microbiology laboratory for immediate processing. Bone biopsies are submitted in a sterile container and delivered to laboratory for immediate processing.

Catheter tip
Disinfect skin before removal. Catheter tip is submitted for culture in sterile container.

Inner ear
Clean ear canal with mild soap solution before myringotomy (puncture of the eardrum). Aspirate material from behind drum with syringe if eardrum is intact. Send fluid in sterile container or syringe with needle removed as soon as possible to laboratory. Use a Stuart's or Amie's swab to collect material from ruptured eardrum.

Outer ear
Wipe away crust with sterile saline. Firmly rotate swab in outer canal. A Stuart's or Amie's swab should be used.

Conjunctiva
Sample both eyes with Stuart's or Amie's swab pre-moistened with sterile saline.

Corneal scrapings
Physician should instill local anesthetic before collection. Inoculate thiglycollate broth at bedside, if possible.

Foreign bodies (IUD, IV catheters, pins, prosthetic valves)
Physician should disinfect skin before removal. Once removed, place into a sterile container.

Bartholin cyst
Disinfect skin before collection. Aspirate fluid and send to lab in sterile, leak-proof container. Send in anaerobic transport swab (gel in bottom) or other anaerobic transport system if anaerobic culture is requested.

Cervix
Remove mucus before collection of specimen. Do not use lubricant on speculum. Swab deeply into endocervical canal using Amie's or Stuart's swab.

Cul-de-sac
Submit aspirate in sterile, leak proof container. If anaerobic culture is ordered send in anaerobic swab (gel in bottom) or other anaerobic transport system.

Endometrium
Collect surgical biopsy or transcervical aspirate via sheathed catheter. If anaerobic culture is ordered send in anaerobic swab (gel in bottom) or other anaerobic transport system.

Tissue
Disinfect skin before collection. Send specimen in sterile, screw-cap container. Do not allow specimen to dry out. Moisten specimen with sterile, distilled water if not bloody.

Urethra
Remove exudate from urethral opening. Using Amie's or Stuart's swab collect discharge by massaging urethra against pubic symphysis or insert flexible swab 2-4 cm into urethra and rotate swab for 2 seconds.

Vagina
Remove exudates before collecting specimen. Swab secretions and mucous membrane of vagina using Amie's or Stuart's swab.

Prostate, expressed prostate fluid
Clean glans with towellette provided. Collect secretions on Stuart's or Amie's swab or in sterile container or tube.

Urethra (male)
Insert flexible swab 2-4cm into urethra and rotate for 2 seconds or collect discharge. Use Stuart's or Amie's swab.

Endotracheal or tracheostomy suction aspirates
Collect in a sterile, leak proof container.

Bronchial washings (BW), bronchoalveolar lavage (BAL), and bronchial brush samples (BB)
These specimens are collected by a physician during a bronchoscopy procedure. Submit bronchial washings and broncheoalveolar lavage specimens in a sterile, leakproof container. BAL results in collection of 50 ml or more of saline from a larger lung volume. Bronchial brush specimens, which contain approximately 0.01 to 0.001 ml of secretions, should be placed in 1 ml of sterile

nonbacteriostatic saline after collection. These specimens should be delivered to lab immediately.

Skin scrapings for scabies

Skin scrapings are either submitted from the physician's office or performed by a trained medical technologist. Proper PPE must be worn during examination of the infected area and collection. It is critical to do a thorough examination of the patient's skin with magnifying lens. Although 80% of mites are found in the webbing between the fingers on the hands, and on the folds or wrists, they can also be found on the shoulders, back, abdomen, elbows, buttocks, axillae, under the breasts, behind the knees, and on the thighs. The mites burrow into the skin, but never below the outer layer of the epidermis, the stratum corneum. Look for burrows, which will appear as serpentine, redline marking tunnels in the skin up to several centimeters long and unexcoriated papules (unscratched bumps) that suggest site of active mites. These tunnels may be made more visible by rubbing a felt tip pen (green or blue) over the area of the burrow and immediately wiping with an alcohol wipe gently to remove excess ink. The remaining ink will penetrate the stratum corneum (outer layer of skin) and stain the tunnel that will appear as a zigzag line. The mites will not be easily demonstrated in excoriated, scabbed, or infected skin. The sample should be taken from unexcoriated burrows, or intact papules (unscratched bumps).

The skin should be scraped vigorously at a minimum of six different sites. The skin should be scraped hard enough where there are some erythrocytes on the slide, but no visible bleeding.

An alternative method to obtaining a specimen for the identification of scabies is before bathing; closely trim the patients' fingernails. Place nail clippings into a clean, sealable container and process immediately. The process is consistent with the skin scraping.

Sputum culture

Expectorate sputa

Have patient rinse or gargle with water before collection. Patient should be instructed to provide a deep-coughed specimen. The material should be expelled into a sterile container, with an attempt to minimize contamination with saliva.

Induced sputa

Aerosol-induced specimens are collected by allowing the patient to breathe aerosolized droplets of solution containing 15% NaCl and 10% glycerin until a strong cough reflex is initiated. The material should be expelled into a sterile container, with an attempt to minimize contamination with saliva.

Strep screen (rapid strep)

When swabbing the throat, be careful not to the touch the tongue, sides or top of mouth with the swab. Rub the swab on the back of the throat, on the tonsils and

any other area where there is redness, inflammation or pus. Bloody specimens can create an interfering background and can cause an invalid result.

Use rayon-tip or dacron-tip swabs with plastic shafts. Do not use calcium alginate, cotton-tip or wooden shafted swabs. Swab specimens should be processed as soon as possible after collection. However, swabs can be stored in 1ml or less liquid media, such as modified Stuart's Transport Media, for up to 8 hours room temperature or 72 hours refrigerated. Do not use charcoal agar or semi-solid transport media. If collected off-site, Bactiswab with liquid Stuart's media should be used.

A second swab for throat culture should always be collected when a rapid strep is ordered. A Stuart's or Amie's culture swab should be used. If the rapid strep is negative, the rapid strep order code will reflex to a throat culture. This swab will be used for the throat culture to confirm the negative rapid strep result.

Urine Culture

Clean-voided midstream or clean-catch midstream

Females: Clean area with towelette provided. Hold labia apart and begin voiding into commode. After several ml have passed, collect midstream into sterile, screw-cap container.

Males: Clean glans with towelette provided. Retract foreskin and begin urinating into commode. Once several ml have passed, collect midstream into sterile, screw-cap container.

Straight catheter (in and out)

Clean urethral area with soap and water and rinse with water. Insert catheter into bladder and allow 15 ml to pass then collect remainder into sterile, screw-cap container.

Vaginal strep (GBS)

- Swabbing both the lower vagina and rectum (through the anal sphincter) increases the culture yield substantially compared to swabbing the cervix or the vagina without also swabbing the rectum. Although a small number of studies have examined the ability of perianal or vaginal-perianal cultures to detect GBS colonization, the available data on their performance compared with vaginal-rectal cultures are limited.
- Transport the specimen to the laboratory in a non-nutritive transport medium (e.g. Amies or Stuart) within 48 hours.
- If vaginal-rectal swabs are collected separately from the same patient, both swabs can be placed in the same transport container.
- Label specimens clearly for GBS testing.

Vaginal Panel

(Bacterial vaginosis marker, *Candida* spp.(*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*) *Candida glabrata, Candida krusei, Trichomonas vaginalis)*

- Collect swab prior to pelvic, speculum, or bimanual exam. No lubricant is used for the sample technique. Gently slide the swab 2 inches (5 cm) into the vagina. If the swab does not slide easily, gently rotate the swab as you push. If it is still difficult, do not attempt to continue.
- Rotate the swab for 10 to 15 seconds. Withdraw the swab without touching the skin outside the vagina.

Note: If a speculum will be inserted prior to collecting the Vaginal Panel swab:

- Do **not** collect specimen at the posterior fornix.
- Lukewarm water may be used to warm and lubricate the speculum.
- If lubricant must be used, lubricant should be used sparingly (1.8mm) and applied only to the exterior sides of the speculum blades, avoiding contact with the tip of the speculum.
- Avoid contact between the swab and the speculum or lubricant.
- Insert the BD MAX Vaginal Panel collection swab to contact the vaginal sidewall, 2 inches (5 cm) within the vagina, rotate gently for 10-15 seconds; withdraw the swab without touching the speculum.

<u>Specimen Transport/Preparation</u>

- Fully insert the swab into the UVE Sample Buffer Tube so that the tip is at the bottom.
- Carefully break the shaft at the score mark. Be careful to avoid splashing.
- Tightly re-cap the tube.
- Label tube with patient information, date, and time collected. Be careful not to obscure barcodes on the tube.

Worm identification

Submit any fresh specimen, or any liquid or loose stool specimen that has not been refrigerated or frozen. Specimen should be collected in leak-proof container.