Label all microbiology specimens with the patient’s name, other patient identifier (medical record #, requisition #, etc.) and the source/site where the specimen was collected. Unless indicated in the individual test listing, hold specimens at Room Temperature (Ambient) until delivery to the laboratory.

**Aerobic Cultures**

**General Considerations**
- General terms like “wound”, “eye”, and “ear” are inappropriate for describing a specimen source. The name of the anatomic site is required.
- Distinguish between surface and deep or surgical wounds. Material from surface wounds are not cultured for anaerobes. Material from deep wounds are cultured for anaerobes.
- Attention to skin decontamination is critical. The representative specimen is taken from the advancing margin of the lesion and is not just pus or exudate. It is critical that lesion margins and abscess walls be firmly sampled with a swab.
- Specimens should be transported to the laboratory as soon as possible.
- Fluid specimens must be submitted in sterile tightly capped leak-proof containers, or tightly capped syringes with the needle removed.
- Swabs, synovial fluid, ear, eye, and catheter specimens are to be stored and transported at room temperature. Tissues and wound aspirates should be refrigerated.

**EAR**
A swab is not recommended for collecting specimens used in the diagnosis of otitis media infections. When a swab is used, external ear canal flora contaminates the specimen, making interpretation of clinically relevant growth difficult and misleading. The specimen of choice is tympanocentesis fluid. Clean the external canal with mild detergent. Using a syringe aspiration technique, the physician will obtain the fluid from the eardrum. Send the specimen in a sterile container, or send it in the syringe, with the needle removed and tightly capped. If the eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum.
**EYE (Ocular Specimens)**

Do not use the term “eye” in identifying a specimen. Specify what the specimen is; e.g. lid margin, conjunctiva, cornea, aqueous or vitreous sample. Specify left or right eye.

**Conjunctiva**

Moisten a cotton or calcium alginate swab with sterile saline (unless exudate is present) and scrub it over the inferior tarsal conjunctiva and fornix of the infected eye. An additional swab should be taken for gram staining. **NOTE:** Organisms are more readily detected in scrapings than from a swab.

**Conjunctiva scrapings**

Scrape the lower tarsal conjunctiva with a sterilized kimura spatula. Inoculate the appropriate media directly. (Contact lab to obtain media.) Prepare smears by applying the scraping in a circular manner to a clean glass slide or by compressing material between two glass slides and pulling the slides apart.

**Corneal scrapings**

Obtain conjunctival samples before corneal scrapings. Sometimes conjunctival cultures are helpful in assessing the possibility of contamination of corneal cultures. Using short, firm strokes in one direction, scrape multiple areas of ulceration and suppuration with a sterilized kimura spatula. (Keep the eyelid open, and be careful not to touch the eyelashes.) Inoculate each scraping directly to the appropriate media. (Multiple scrapings are recommended because the depth and extent of viable organisms may vary.) Prepare smears by applying the scrapings in a gentle circular motion over a clean glass slide or by compressing material between two clean glass slides and pulling the slides apart.

**Intraocular fluid**

Use a needle aspiration technique to collect intraocular fluid. Inoculate appropriate media directly, and/or immediately transport the samples to the laboratory in an anaerobic transport system or a capped syringe with air bubbles expelled.

Prepare smears by spreading a drop of material over the surface of a cleaned glass slide with a sterile kimura spatula or by compressing the material between two glass slides and pulling the slides apart.
FLUIDS

Cerebral Spinal Fluid (CSF)
The suggested sample volumes are 1, 2, and 2 ml for routine, fungal, and mycobacterial cultures, respectively.

Synovial fluid
Clean the needle puncture site with alcohol, and disinfect it with an iodine solution (1 to 2% tincture of iodine or a 10% solution of providone-iodine [1% free iodine]) to prevent introduction of infection. (If tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn.) The physician will aseptically perform percutaneous aspiration to obtain synovial fluid. Expel any air bubbles from the syringe, and immediately inject the specimen into an anaerobic transport system or send the specimen in the syringe, with the needle removed and tightly capped. Transport additional fluid or pus in a sterile screw-cap container.

Superficial Wounds

Abscess: Unruptured
Do not swab. Decontaminate the skin overlying the abscess and aspirate the abscess contents with a syringe. After excision and draining, submit a portion of the abscess wall for culture. Submit the specimen in an anaerobic transport container.

Burn wounds
Debride the area and disinfect the wound. As exudates appear, sample it firmly with a swab. Submit the sample for aerobic culture only. Submit biopsy tissue as the specimen of choice. Surface specimens usually represent colonization.

Decubitus Ulcer
A swab is not the specimen of choice, as swabs tend to reflect surface colonization. Needle aspirates may result in underestimation of bacterial isolates. The specimen of choice is a tissue biopsy. For dry encrusted lesions culture is not recommended unless an exudate is present. A closed abscess is the specimen of choice. Collect exudates and a sample of the abscess wall.
**Open lesions and abscesses**
Remove as much of the superficial flora as possible by decontaminating the skin. Remove exudate and firmly sample the base or margin of the lesion with a swab. Submit the swab in aerobic transport medium. You can also culture a sample of the exudate aerobically. Do not request anaerobic cultures of material from open superficial lesions. Consult with the laboratory.

**Pustules or vesicles**
Select an intact pustule. Apply alcohol and allow it to dry. Unroof the pustule with a 23-gauge needle. Collect fluid and basal cells by rotating the swab vigorously in the pustule. If the pustule is large, an 18-gauge needle on a tuberculin syringe can be used to puncture it. If the lesion is older, the crust is removed and the moist base of the lesion sampled by swabbing with a premoistened sterile swab. Place the swab in collection tube containing Amies transport media.

**Anaerobic Cultures**

- The best specimen for anaerobic culture is obtained by using a needle and syringe.
- Tissue samples and biopsy samples are also very good specimens for anaerobic culture.
- When a swab must be used to collect a specimen, immediately place swab into the anaerobic transport tube. Special care must be taken to sample the active site of infection when a swab is used.
- Glass anaerobic transport tube. Fluid may be injected through the rubber stopper. For tissue; hold upright, uncap, insert specimen and recap. Send swab ONLY if not other sample is available.
- Transport specimens to the lab as soon as possible.
- Do not transport material for culture in the needle and syringe. Transfer aspirated material to an anaerobic transport vial. Large volumes of purulent material may be transported in a sterile screw-cap tube.

**Abscess**
Aspirate material with needle and syringe after the surface of intact tissue is disinfected with a providone-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. Insert needle into the rubber stopper of the glass anaerobic transport tube and inject material.
Alternatively, remove screw cap, place material into the tube while holding the tube upright, and immediately replace cap. Ensure that cap is tightly secured.

**Decubiti and other surface ulcers**
Aspirate material by needle and syringe after thorough disinfection of the surface area, or collect small curettings of material from deep tissue at wound margin. Immediately transfer material into an anaerobic transport tube. Insert needle into the rubber stopper and inject material. Alternatively, remove screw cap, place material into the tube while holding the tube upright, and immediately replace cap. Ensure that cap is tightly secured.

**Intrauterine devices**
Place the apparatus in a sterile container. Replace the cap of the container and tighten securely. Include note to culture for *Actinomyces*, if required.

**Pulmonary**
Collect lung tissue, transtracheal aspirate, percutaneous aspirate, transcutaneous aspirate, and bronchial brushing via double-lumen catheter. Remove screw cap, place material into the tube while holding the tube upright, and immediately replace cap. Ensure that cap is tightly secured.

**Sinus track or deep wound drainage**
Aspirate material after proper disinfection of the skin surface, or collect curettings of material from deep within the tract or wound. Insert needle into the rubber stopper and inject material. Alternatively, remove screw cap, place material into the tube while holding the tube upright, and immediately replace cap. Ensure that cap is tightly secured.

**Urinary Tract**
Obtain material via suprapubic bladder tap. Immediately transfer material into an anaerobic transport tube. Insert needle into the rubber stopper of the Glass Anaerobic transport tube and inject material.
## Acceptable Specimens for Anaerobic Culture

<table>
<thead>
<tr>
<th>Site</th>
<th>Acceptable Specimens</th>
<th>Unacceptable Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck</td>
<td><strong>Abscess aspirate</strong> obtained by needle and syringe after surface decontamination</td>
<td>Throat or nasopharyngeal swabs</td>
</tr>
<tr>
<td></td>
<td><strong>Biopsy material</strong> surgically obtained</td>
<td>Gingival swabs</td>
</tr>
<tr>
<td></td>
<td>Anaerobic swab surgically obtained when aspiration is not feasible</td>
<td>Superficial material collected with swabs</td>
</tr>
<tr>
<td>Lungs</td>
<td>Transtracheal aspirate</td>
<td>Expectorated sputum</td>
</tr>
<tr>
<td></td>
<td>Material from percutaneous lung puncture</td>
<td>Induced sputum</td>
</tr>
<tr>
<td></td>
<td>Biopsy material surgically obtained</td>
<td>Endotracheal aspirate</td>
</tr>
<tr>
<td></td>
<td>Bronchoscopic specimen obtained by protected brush</td>
<td>Bronchoscopic specimens not specially collected</td>
</tr>
<tr>
<td></td>
<td>Thoracotomy specimen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic swab surgically obtained</td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Abscess aspirate obtained by needle and syringe</td>
<td>Aerobic swabs</td>
</tr>
<tr>
<td></td>
<td>Biopsy material surgically obtained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic swab surgically obtained</td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>Peritoneal fluid obtained by needle and syringe</td>
<td>Aerobic swabs</td>
</tr>
<tr>
<td></td>
<td>Abscess aspirate obtained by needle and syringe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bile</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopsy material surgically obtained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic swab surgically obtained</td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>Suprapubic aspirate</td>
<td>Voided urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catheterized urine</td>
</tr>
<tr>
<td>Female genital tract</td>
<td>Culdoscopy specimens</td>
<td>Vaginal or cervical swabs</td>
</tr>
<tr>
<td></td>
<td>Endometrial aspirate obtained by suction or protected collector</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abscess aspirate obtained by needle and syringe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopsy material surgically obtained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic swabs surgically obtained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUD for <em>Actinomyces</em> species or</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Eubacterium nodatum</strong></td>
<td></td>
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<td>-----------------------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| **Bone and joint**                | Aspirate obtained by needle and syringe  
Biopsy material surgically obtained  
Anaerobic swap surgically obtained | Superficial material collected with swabs  
Superficial material collected from skin surfaces or edges of wound |
| **Soft tissue**                   | Aspirate obtained by needle and syringe  
Biopsy material surgically obtained  
Aspirate from sinus tract obtained by needle and small plastic catheter  
Deep aspirate of open-wound obtained through decontaminated skin  
Deep aspirate of surface ulcer obtained through decontaminated skin | Superficial material collected from skin surfaces or edges of wound |

**Fungal Culture**

- Collect specimens aseptically, place in sterile containers, and deliver the containers to the laboratory as soon as possible. Viability may decrease with prolonged specimen storage.
- Swabs are not encouraged; however, specimens from certain body sites, such as the ear canal, nasopharynx, throat, vagina, and cervix, are not readily collected by other means. Swabs for collection of material from open wounds or draining lesions are frequently contaminated with environmental microorganisms.
- Transport specimens in sterile, humidified, leak proof containers. Only dermatological specimens, i.e. nail clippings, skin scrapings, hair, should be transported in a dry container. Do not use transport medium unless the specimen can be easily and completely retrieved from the medium. Although fungi can be recovered at times from specimens submitted in anaerobic transport media, such media should be avoided.
- The effect of refrigeration on fungal specimens has not been well studied, but if transport is to be delayed for more than several hours, store the specimens under refrigeration with the following exceptions: store blood and CSF at 30 to 37°C; store dermatological specimens at 15 to 30°C.

**Body fluids (pleural, synovial, and peritoneal)**

Collect specimens aseptically, and place them in sterile containers. Heparin or SPS anticoagulant should be added if sample might clot. Submit as much fluid as possible.
**Eye**
Optimally, corneal scrapings should be inoculated directly onto Sabouraud Dextrose Agar at the time of specimen collection. Alternatively, transport intraocular fluid in a sterile tube or capped syringe at room temperature.

**Hair**
No cleaning of scalp is needed.
Select the infected areas and with forceps, epilate at least 10 hairs.
For hairs broken off at the scalp level, use a scalpel or a blade knife.
Place hairs in a sterile cup labeled with the patient’s information.

**Nail**
Clean the nail with 70% alcohol.
For a specimen of the dorsal plate, scrape the outer surface and discard the scrapings. Then scrape the deeper portion for the specimen. Remove a portion of debris from under the nail with a scalpel. Collect the whole nail or nail clippings.
Place all material in a sterile cup labeled with the patient’s data.

**Pus, exudates, and drainage**
Cleanse skin with alcohol.
Using a sterile needle and syringe, aspirate material from undrained abscesses.
Place the material in a sterile container.
Collect specimen on a swab only if volume is insufficient for aspiration by needle.

**Respiratory specimens other than sputum**
Specimens include tracheal aspirates, lung biopsy material, and bronchoscopy specimens and are collected aseptically by physicians and sent to the laboratory for examination and processing as soon as possible.
Collect in sterile leak proof container.

**Skin and interspaces**
Wipe lesions and interspaces between the toes with alcohol sponge or sterile water.
Scrape the entire lesion(s) and both sides of interspaces with a sterile scalpel.
Place scrapings in a sterile cup labeled with the patient’s data.

**Sputum (tracheal lavage, bronchial lavage, and aerosol collection)**
Sputum should be fresh. Collect it in the early morning.
Have patient remove dentures and rinse mouth.
Sputum should be the result of a deep cough (not saliva) or should be induced by an aqueous aerosol. Collect 5 to 10 ml in sterile container.

**Urine**
The urine specimen most suitable for making a diagnosis of mycoses of the urinary tract is a catheterized specimen. Collect a clean-catch midstream specimen when aspiration or cystoscopy cannot be done. Collect early-morning specimens aseptically in sterile containers.

**Parasitology**
- To insure the recovery of parasitic organisms that are passed intermittently and in fluctuating numbers, the examination of a minimum of three specimens collected over a 7-10 day period is recommended. Three specimens spaced 2-3 days apart are optimal.
- Infections with *Entamoeba histolytica* or *Giardia lamblia* may require the examination of up to six specimens spaced 2-3 days apart before the organism is detected.

**Stool Collection**
Freshly passed stool collected in a clean dry container. Collect all fecal specimens prior to administration of antibiotics or antidiarrheal agents. Avoid use of mineral oil, bismuth or barium prior to fecal collection. Avoid contamination with urine or water from toilet. Fill SAF Transport tube up to fill line. Clearly label containers with patient name, date and specimen consistency. (Specimen consistency includes formed, soft, loose, or liquid). Transport the specimens to the lab as soon as possible. SAF preserved specimens should be transported at room temperature.

**Respiratory Culture**

**Sputum Collection**
If both Culture and Cytology are requested, two separate specimens must be collected.

Early morning specimens are highly recommended and should be collected shortly after waking. Label the specimen container and instruct the patient to keep the sterile container at their bedside so it can be easily reached in the morning.
Instruct the patient to cough deeply to bring sputum up from the chest. The patient should be told that it should feel as though the specimen came from below the throat (lung area). Saliva or postnasal drip specimens are unsuitable for testing. The patient is instructed to not add additional specimen to the container if continued coughing brings up more sputum. Instruct the patient to refrigerate the specimen and return it to the office or laboratory the same day.

**Throat Swab Collection**
Assemble a sterile tongue depressor and a double swab culture collection device. Ask the adult patient to sit very straight in the chair. If it is a child 3 years or older, the child can be held by the parent, having the child sit very straight in their lap. When it is time to collect the sample, ask the patient to say “AH”. If the child is cultured, you may have the parent hold their head still to prevent an improper collection or the child gagging. Those patients with a “gag reflex”, it is helpful to have them either close their eyes or focus on the ceiling. In one sweep, gently depress the tongue with the tongue depressor. Guide the swab over the depressor. Be certain to touch only the tongue depressor at this point. Move the swab into the back of the throat (posterior pharynx). The area behind the uvula and between the tonsillar pillars is swabbed with a gentle back and forward sweeping motion. Be sure to sample any red areas or white spots in this area. Return the swab to the holder making sure that the swab is in the media provided. Label culture swab with patient name, date, and collector’s initials.

**Enteric Pathogen Culture**

**Stool Collection**

**Adults**
First, pass urine into the toilet. Move the toilet seat to the up position. Place sheets of plastic wrap (like Saran wrap®) over the bowl, leaving a small dip in the center. Place the toilet seat in the down position. Pass stool onto the plastic wrap. A “Commode Hat” can also be used in place of the plastic wrap. Remove the container cap and use the attached collection device to move stool sample into the C&S tube. Fill container to the fill line. Selectively collect stool containing mucus or blood, if present. You may also collect stools in clean containers (cottage cheese contain, margarine container, etc.) without disinfectant or detergent residue. Specimens should not be collected from bedpans, as they may contain residual disinfectant or other contaminants. Mix contents with collection device, replace cap, and tighten. Wash and dry your hands before and after collection. Store at room temperature and transport to laboratory as soon as possible (within 18 hours).
**Infants and Small Children**

Fasten plastic kitchen wrap to the inside of the diaper using childproof safety pins. As soon as possible following the bowel movement, remove stool from the plastic liner and transfer it to the C&S collection tube. Fill transport tube to fill line, if possible. Stool collected in diapers is not acceptable for culture. Replace cap and tighten. Wash and dry your hands. Store at room temperature and transport to laboratory as soon as possible (within 18 hours).

**Comments**

- Includes detection of Salmonella, Shigella, Shiga-like toxin-producing E. coli, Campylobacter, Aeromonas spp. and Plesiomonas spp. Yersinia spp. and/or Vibrio spp. screening available as a separate order.
- The patient should be instructed not to take any antacids, oily laxatives, or anti-diarrheal medications unless prescribed by the physician.
- **DO NOT MIX URINE OR WATER WITH THE STOOL SPECIMEN.**
- Do not use toilet paper to obtain stool specimen. Chemicals found in toilet paper may inhibit bacterial growth.
- Fecal specimens for the diagnosis of acute infectious diarrheas should be collected in the early stage of illness and prior to treatment with antimicrobials.
- A stool specimen rather than a rectal swab is preferred.
- Do not submit stool in diaper (see collection procedure).
- Stool specimen must be placed in C&S (Cary Blair) Transport media as soon as possible in order to ensure optimal detection of bacterial pathogens. Store at room temperature and transport to the laboratory within 18 hours.
- Recommend 2 samples collected on consecutive days. Submission of additional samples does not significantly increase yields.
- Enteric pathogen cultures have been shown to have very small yields on patients who have been hospitalized for more than 3 days. Consider ordering Clostridium difficile toxin assay instead of culture for those patients.

**Urine Collection**

**Female**

Note: Collection of midstream urine specimens are not recommended during menses. First morning specimens are preferred. While holding the labia apart, with the aid of a pair of sponges, wipe the vulva from front to back with two successive cotton gauzes or sponges soaked in soap (optional). Rinse with two
sponges and sterile water or saline. After several mL have passed, collect a midstream sample in a sterile container without stopping the flow of urine.

**Male**

First morning specimens are preferred. While holding the foreskin retracted, wipe with two sponges/gauze soaked in saline and begin voiding. After several mL have passed, collect a midstream portion in a sterile container without stopping the flow of urine. Samples are collected in sterile cups or tubes and refrigerated immediately after collection. If used, urine transport tubes are filled to the fill mark on the side of the tube. Samples submitted in urine transport tubes are stable for 48 hours at room temperature.

**Reflex Testing:** Cultures exhibiting pathogenic growth may have further testing performed at additional charges. Further testing includes, but is not limited to, gram stains, organism identifications and susceptibilities.