Microbiology Specimen Collection Guidelines

The quality of a result is directly related to the quality of the specimen being cultured. The best results are obtained when the following guidelines are maintained.

- Follow universal precaution guidelines. Treat all specimens as potential biohazards.
- Collect specimen before administering antimicrobial agents when possible.
- Collect specimen with as little contamination from indigenous flora.
- Utilize appropriate collection devices, sterile equipment, and aseptic technique to collect specimens. Various types of transport media are provided depending on the type of culture required.
- All swabs are to be kept moist in a transport medium after the specimen is collected.
- Clearly label the specimen container with:
  - patient's name
  - date
  - time of collection.
- Collect an adequate amount of specimen. Inadequate amounts of specimen may yield false negative results.
- Identify the specimen source and/or specific site correctly so that proper culture media will be selected during processing in the Laboratory.
- Transport all specimens to the Laboratory promptly. This ensures the survival and isolation of fastidious organisms and prevents overgrowth by more hardy bacteria. It also shortens the duration of specimen contact with some local anesthetics used in collection procedures that may have antibacterial activity.

Standard Specimen Collection Procedures:
Physicians or specialists with advanced training and skills should collect specimens requiring extreme invasive technique. Specimens not listed below or any other questions or requests should be directed to the Microbiology Laboratory. Microbiology should be informed in advance if there are any special requests that might require special handling.

Transport systems for microbiology specimens.

<table>
<thead>
<tr>
<th>Cultures</th>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic</td>
<td>A plastic tube containing a sterile swab and anaerobic transport medium.</td>
</tr>
<tr>
<td>Aerobic</td>
<td>A plastic tube containing a sterile swab and transport medium.</td>
</tr>
<tr>
<td>Nasopharyngeal Male urethral</td>
<td>A plastic tube containing transport medium and a swab with a flexible wire shaft and small tip. Make sure glass ampoule is broken and swab is moistened by transport medium.</td>
</tr>
<tr>
<td>Urine</td>
<td>Sterile screw capped cups. If biopsy specimen is small, add a small amount of sterile non-bacteriostatic saline to the cup. Never place the biopsy specimen in formalin or wrap in gauze.</td>
</tr>
<tr>
<td>Sputum</td>
<td>Sterile tubes</td>
</tr>
<tr>
<td>Stool</td>
<td>Sterile tubes</td>
</tr>
<tr>
<td>Biopsies</td>
<td>Sterile tubes</td>
</tr>
<tr>
<td>Sterile fluids</td>
<td>Sterile tubes</td>
</tr>
<tr>
<td>Drainage Bronchial brush</td>
<td>Sterile tubes</td>
</tr>
<tr>
<td>Needle aspirate</td>
<td>Transfer to a sterile tube prior to transport to the Laboratory. If the specimen will be compromised by transferring it from the syringe, send in the syringe after needle removal and recapping with a sterile cap.</td>
</tr>
<tr>
<td>Virus</td>
<td>15 ml conical tube containing transport medium and specimens.</td>
</tr>
<tr>
<td>Chlamydia</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma</td>
<td></td>
</tr>
<tr>
<td>Stool</td>
<td>A clean, empty vial, one vial containing a parasite preservative and, for outpatients only, one vial containing bacterial transport medium.</td>
</tr>
</tbody>
</table>

Specimen Requirements and Collection Procedures:
BLOOD

1. **Number and timing:** Most cases of bacteremia are detected by using 2 to 3 separately collected blood cultures. More than 3 blood cultures yield little additional information. Conversely, a single blood culture may miss intermittent bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms. The following can be used as general guidelines:
   a) Acute sepsis. Collect 2 from separately prepared sites prior to starting therapy.
   b) Acute endocarditis. Obtain 3 blood cultures with 3 separate venipunctures over 1 to 2 hours.
   c) Subacute endocarditis. Obtain 3 blood cultures on day 1 (15 minutes or more apart). If all are negative 24 hours later, obtain 3 more.
   d) Fever of unknown origin. Obtain 2 separate blood cultures at least 1 hour apart. If these are negative, then 24 to 36 hours later obtain 2 more blood cultures 1 hour apart.

2. **Volume:** The volume of blood is critical because the concentration of organisms in most cases of bacteremia is low. In infants and children, the concentration of organisms during bacteremia is higher than in adults, so less blood is required. In general:
   a) Infants: 0.5 to 1.5 ml of blood per venipuncture.
   b) Children: 1.0 to 10 ml of blood per venipuncture.
   c) Adults: 10 to 25 ml of blood per venipuncture.

3. **Type of blood culture:** Blood cultures may be drawn in a variety containers. Refer to the specific test for the type of container to use.
   a) Routine blood culture
   b) Treated blood culture
   c) Fungus blood culture
   d) Acid fast blood culture
   e) CMV blood culture

4. **Blood collection:**
   a) Clean venipuncture site with alcohol.
   b) Apply iodine solution in concentric circles working from the inside.
   c) Allow to dry for 30 seconds before performing the venipuncture.
   d) Do not touch the site with fingers or any non-sterile object.
CENTRAL NERVOUS SYSTEM SPECIMEN COLLECTION

1. Cerebrospinal Fluid
   a) Specimen is collected by a physician.
   b) CSF should be collected into sterile leak proof tubes. Three tubes are generally required for microbiology, hematology and chemistry testing. The second tube drawn will generally go to microbiology and the last tube drawn will generally go to hematology.
   c) Suggested volumes are 1, 2 and 3 ml for routine, fungal and mycobacterial cultures.

2. Brain abscess
   a) Specimen is collected by a physician.
   b) Most brain abscesses are caused by anaerobic bacteria. Specimen should be submitted in anaerobic transport or in a capped syringe.

CNS Collection Requirements: Tube #2 is preferred.

<table>
<thead>
<tr>
<th>Orangism</th>
<th>Vol. (ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>2</td>
<td>Rule out Cryptococcus sp., Coccidioides immitis.</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>2</td>
<td>M. tuberculosis, M. avium-intracellulare</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>NA</td>
<td>Brain abscess or CNS biopsy specimens.</td>
</tr>
<tr>
<td>Parasites</td>
<td>NA</td>
<td>Brain abscess or CNS biopsy specimens for Ent. histolytica, Toxo. gondii, Naegleria sp., Acanthamoeba sp. CSF for Naegleria sp and Acanthamoeba sp.</td>
</tr>
<tr>
<td>Virus</td>
<td>1-2</td>
<td>Send to Laboratory on ice.</td>
</tr>
</tbody>
</table>
GASTROINTESTINAL TRACT SPECIMEN COLLECTION

1. Fecal specimens: Have patient obtain stool specimen by one of the following methods:
   a) Pass stool directly into a sterile, wide-mouth, leak proof container with a tight fitting lid.
   b) Pass stool into a clean, dry bedpan, and transfer into a sterile leak proof container with a tight fitting lid.
   c) Keep stool specimen cool. Do not incubate.
   d) Do not use toilet paper to collect stool. Toilet paper may contain substances, which are inhibitory for some fecal pathogens.
   e) Stool for ova and parasites should be placed in preservative immediately after collection.

OUTPATIENT STOOL COLLECTION GUIDELINES: After collection, patients should deliver specimens to the Laboratory as soon as possible. Please call the Laboratory if you have any questions BEFORE instructing the patient.

<table>
<thead>
<tr>
<th>Test</th>
<th>Collection Vial</th>
<th>Specimen Expires In</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine Culture</td>
<td>Alpha Tec ETM vial (red cap)</td>
<td>96 hours (DO NOT REFRIGERATE VIAL)</td>
</tr>
<tr>
<td>Ova &amp; Parasite</td>
<td>Alpha Tec Prototix vial (clear top)</td>
<td>96 hours at room temperature</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>clean vial</td>
<td>72 hours – refrigerated</td>
</tr>
<tr>
<td>Toxin (C. diff.)</td>
<td></td>
<td>2 weeks – frozen</td>
</tr>
<tr>
<td>Giardia Antigen</td>
<td>clean vial</td>
<td>48 hours - refrigerated</td>
</tr>
<tr>
<td></td>
<td>Alpha Tec Prototix vial (clear top)</td>
<td>preserved - up to 1 week at room temperature</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 days – frozen</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>clean vial</td>
<td>72 hours – refrigerated</td>
</tr>
<tr>
<td>Occult Blood (if cards not used)</td>
<td>clean vial</td>
<td>24 hours - refrigerated</td>
</tr>
<tr>
<td>WBC Stool</td>
<td>clean vial</td>
<td>2 hours – refrigerated; or - submit on a glass slide</td>
</tr>
</tbody>
</table>

2. Rectal swabs
   a) Pass the tip of a sterile swab approximately 1 inch beyond the anal sphincter.
   b) Carefully rotate the swab to sample the anal crypts and withdraw the swab. Place the swab in transport medium.
   c) If *Neisseria gonorrhoeae* is suspected, inoculate a Thayer Martin plate at the bedside and transport to the Laboratory immediately.

3. Gastric lavage
   a) Is submitted primarily for the detection of *Mycobacterium tuberculosis* in patients (most frequently children) unable to produce quality sputum. Should be performed after the patient wakes in the morning so that sputum swallowed during sleep is still in the stomach.
   b) The patient should fast prior to the procedure.
   c) Pass a well lubricated tube orally or nasally to the stomach of the patient, and perform the lavage.

4. Duodenal biopsies and washings
   a) Submitted primarily for the detection of *Giardia lamblia, Strongyloides stercoralis, Ascaris lumbricoides* and *Helicobacter pylori*.
   b) These specimens are obtained by endoscopic procedures.

5. Gastric biopsies and washings
   a) Submitted primarily for the detection of *Helicobacter pylori*.
   b) Obtained by endoscopic procedures.

6. Esophageal biopsies and washings
   a) Primarily used to detect *Candida species*, Cytomegalovirus and Herpes Simplex virus infections.
   b) Obtained by endoscopic procedures.

7. Pinworm: Use pinworm collection kit. Collect specimen when patient gets up in the morning before the patient bathes or defecates.
## Gastrointestinal Pathogens and Specimen Collection Procedure

<table>
<thead>
<tr>
<th>Organism</th>
<th>Specimen Type</th>
</tr>
</thead>
</table>
| **Bacteria**   | **Stools**: Rules out Salmonella, Shigella, and Campylobacter. Comment if other pathogens are suspected.  
|                | **Gastric biopsy**: Rule out *Helicobacter pylori*.                           |
|                | **Rectal swab**: Rules out enteric pathogens (especially *Shigella sp.*) and *Neisseria gonorrhoeae*. |
| **Fungi**      | **Gastric aspirate, gastric biopsy, esophageal brush, esophageal biopsy**      |
| **Mycobacteria** | **Gastric aspirate and feces.**                                             |
| **Parasites**  | **Preserved stool**: 3 stool specimens on consecutive or alternate days are recommended.  
|                | **Duodenal aspirates**: for detecting *Giardia lamblia, Strongyloides stercoralis* and *Ascaris lumbricoides*. |
|                | **Rectal biopsy**: for *Entamoeba histolytica* and *Balantidium coli*.        |
|                | **Small bowel biopsy**: for *Giardia lamblia, Cryptosporidium sp.* and *Microsporidium sp.* |
| **Pinworm**    | **Use pinworm collection kit. Collect specimen when patient gets up in the morning before patient bathes or defecates.** |
| **Virus**      | **Esophageal specimens**: for CMV and HSV.                                   |
|                | **Rectal Biopsy**: for HSV. Send to Laboratory immediately. If transport is delayed, place specimen on ice. Do not freeze. |
GENITAL TRACT SPECIMENS COLLECTION (FEMALE)

1. **Amniotic fluid**: Aspirate fluid by catheter, at caesarian section, or at amniocentesis.
2. **Bartholin gland**: Decontaminate skin with providone-iodine. Aspirate material from duct(s).
3. **Cervix**: Do not use lubricant during procedure. Wipe cervix clean of vaginal secretion and mucus. Rotate a sterile swab, and obtain exudate from the endocervical glands. Do not use cotton swabs or swabs with wooden shafts for specimen collection. If no exudate is seen, insert a sterile swab into the endocervical canal and rotate the swab.
4. **Endometrium**: Collect endometrium specimens by transcervical aspiration through a telescoping catheter.
5. **Fallopian tubes**: Obtain aspirates or swab specimens during surgery.
6. **Urethra**:
   a) Collect specimen one hour or more after patient has urinated. Stimulate discharge by gently massaging urethra against the pubic symphysis through the vagina.
   b) Collect the discharge with a sterile swab if discharge cannot be obtained, wash external urethra with betadine soap and rinse with water.
   c) Insert a sterile min-tip swab 2 to 4 cm into the endourethra.
   d) Gently rotate the swab and leave it in place for one to two seconds.
   e) Withdraw the swab and place it in the appropriate transport system.
7. **Vagina**
   a) Use a speculum without lubricant.
   b) Collect secretions from the mucosa high in the vaginal canal with sterile swab.
   c) Withdraw the swab and place it in the appropriate transport system.
8. **Vulva**
   a) Clean the surface of the lesion with sterile saline. If there is a crust on the lesion remove it.
   b) Scrape the lesion until serous fluid emerges.
   c) Wipe away fluid and debris with a sterile gauze. Try to avoid bleeding.
   d) Press the base of the lesion until clear fluid is expressed.
   e) Aspirate vesicular fluid with a 26- to 27-gauge needle and place it in the appropriate transport system. OR
   f) Touch a slide to the fluid and cover the fluid on the slide with a coverslip (for *Treponema pallidum* detection). OR
   g) Unroof the vesicle and collect fluid with a sterile swab and place it in the appropriate transport system (for HSV detection). OR
   h) Scrape the base of an open vesicle with a sterile scalpel blade and then rub the base vigorously with a sterile swab (for HSV and *Haemophilus ducreyi* detection). Place swab in the appropriate transport system.

### Genital Tract Pathogens and Specimen Type

<table>
<thead>
<tr>
<th>Organism</th>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Cervical, urethral, anal, vaginal swabs.</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>Prostatic fluid, cervical, vaginal.</td>
</tr>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>Vaginal, prostatic fluid.</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td>Anal, vaginal or cervical.</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Epididymis aspirate, amniotic fluid, abscess.</td>
</tr>
<tr>
<td><strong>HSV</strong></td>
<td>Genital or perianal lesion.</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>Rectal, cervical, urethral, bubo or ulcer material.</td>
</tr>
<tr>
<td><strong>Haemophilus ducreyi</strong></td>
<td>Material from ulcers (genitalia and perianal) and from inguinal nodes.</td>
</tr>
</tbody>
</table>
GENITAL TRACT SPECIMEN COLLECTION (MALE)

1. **Epididymis**
   a) Used primarily to detect nonspecific bacterial and sexually transmitted epididymitis. Bacterial epididymitis is most commonly due to members of the family Enterobacteriaceae or pseudomonads and generally occurs in men over 35 years of age. Sexually transmitted epididymitis is most commonly due to *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.
   b) Use needle and syringe to collect material from epididymis.

2. **Penile lesion**
   a) Clean the surface of the lesion with sterile saline solution. If there is a crust on the lesion remove it.
   b) Scrape the lesion until serous fluid emerges.
   c) Wipe away fluid and debris with sterile gauze. Try to avoid bleeding.
   d) Press the base of the lesion until clear fluid is expressed.
   e) Aspirate vesicular fluid with a 26- to 27-gauge needle and place it in the appropriate transport system. OR
   f) Unroof the vesicle, and collect fluid with a sterile swab and place it in the appropriate transport system (for HSV detection). OR
   g) Scrape the base of an open vesicle with a sterile scalpel blade, and rub the base vigorously with a sterile swab (for HSV and *H. ducreyi* detection). Place swab in the appropriate transport system.

3. **Prostatic massage**
   a) Used to diagnose acute and chronic prostatitis. For both diseases gram negative enteric organisms are the most frequently isolated pathogens.
   b) Collect specimen in a sterile tube or on a sterile swab.

4. **Urethra**
   a) Collect specimen at least 2 hours after the patient has urinated.
   b) Insert a sterile mini-tip swab 2 to 4 cm into the endourethra. Gently rotate it, leave it in place for 1 to 2 seconds, and withdraw.

**Chlamydia trachomatis and Neisseria Gonorrhoeae Collection**

**Endocervical Swab Specimen** using Amplicor CT/GC PCR Assay Endocervical Specimen Collection and M4RT transport Media Kit:
1. Remove the cleaning swab from the packaging.
2. Using one swab, remove excess mucus from the cervical os. Discard the used swab.
3. Remove second swab from the packaging.
4. Insert the collection swab into the cervical canal and rotate for 15-30 seconds.
5. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
6. Immediately place the swab into the M4RT transport media. Break the shaft of the swab, leaving swab in transport media. Make sure the cap is tightly secured to the tube.
7. Label and date the tube. Place specimen in refrigerator.

**Male Urethral Swab Specimen** using Amplicor CT/GC PCR Assay Endocervical Specimen Collection and M4RT transport Media Kit:
1. Remove the one swab from the packaging.
2. Insert the swab 2-4 cm into the urethra and rotate for 3-5 seconds.
3. Withdraw the swab.
4. Immediately place the swab into the M4RT transport media. Break the shaft of the swab, leaving swab in transport media. Make sure the cap is tightly secured to the tube.
5. Label and date the tube. Place specimen in refrigerator.

**Swab storage and transport:**
Store and transport to the laboratory at 2-8°C within 4 days.

**Urine Specimen Collection:**
1. The patient should not have urinated for at least 1 hr prior to specimen collection.
2. Collect specimen in a sterile, plastic, preservative-free collection cup.
3. The patient should collect the first 20-60 ml of voided urine (NOT midstream).
4. Female urine may contain PCR inhibitors and is therefore not acceptable for this testing.

**Urine Storage and Transport:**
Store and transport urine specimens at 2-8°C within 4 days.
OCULAR SPECIMEN COLLECTION

General Considerations:
- Obtain viral and chlamydial samples before topical anesthetics are instilled.
- Do not use cotton or wooden shafted swabs to collect viral or chlamydial cultures.
- Send inoculated media and prepared smears to the Laboratory immediately.
- Do not use calcium alginate swabs for specimen collection for viral cultures.
- If *N. gonorrhoeae* is suspected, inoculate Thayer-Martin and chocolate plates.
- For anaerobic cultures, use anaerobic transport tube and inoculate media directly.
- *Acanthamoeba sp.* is the parasite associated with ocular infections.

1. Conjunctival specimens
   a) One or two drops of local anesthetic are generally instilled.
   b) Scrape the lower tarsal conjunctiva with a sterilized kimura spatula.
   c) Inoculate the appropriate media directly.
   d) 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.
   e) Alternately, use a sterile swab to sample the inferior tarsal conjunctiva (inside surface of eyelid) and the fornix of the eye. However, organisms are more readily detected in scrapings than from a swab.

2. Corneal scrapings
   a) Obtain conjunctival samples prior to corneal scrapings.
   b) One or two drops of topical anesthetic are generally instilled.
   c) Using short, firm strokes in one direction, scrape multiple areas of ulceration and suppuration with a sterilized kimura spatula. Take care to keep the eye open and not to touch the eyelashes.
   d) Inoculate each scraping directly to appropriate media. Multiple scrapings is recommended because the depth and extent of viable organisms may vary.
   e) 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.

3. Intraocular fluid
   a) Prepare smears by spreading a drop of material over the surface of a cleaned glass slide.
   b) Use a needle aspiration technique to collect intraocular fluid.
   c) Inoculate appropriate media directly, and/or immediately transport the samples to the Laboratory in a capped syringe.
RESPIRATORY SPECIMEN COLLECTION

LOWER RESPIRATORY
1. Expectorated sputum
   a) Have the patient rinse mouth and gargle with water prior to sputum collection.
   b) Instruct the patient not to expectorate saliva or post nasal discharge into the container.
   c) Collect specimen resulting from deep coughing in a sterile screw cap container.
   d) For routine bacteria a single specimen is sufficient. For acid fast bacilli and fungi three first
      morning specimens on consecutive days is recommended.
   e) Specimens consisting primarily of saliva are rejected.
2. Induced sputum
   a) Using a wet toothbrush, brush the buccal mucosa, tongue, and gums prior to the procedure.
   b) Rinse the patient's mouth thoroughly with water.
   c) Using an ultrasonic nebulizer, have the patient inhale approximately 20 to 30 ml of 3 to 10% 0.85%
      NaCl.
   d) Collect the induced sputum in a sterile screw cap container.
3. Tracheostomy and endotracheal aspirations
   a) Aspirate the specimen into a sterile sputum trap.
4. Bronchial specimens
   a) Bronchoscopy specimens include bronchoalveolar lavage, bronchial washing, bronchial brushing,
      and transbronchial biopsy specimens.
   b) Bronchial wash and bronchoalveolar lavage specimens are generally obtained before brushing or
      biopsy specimens to avoid excess blood in the recovered fluid. The bronchial brushes should be
      place in the special bronchial brush transport tubes supplied by the Microbiology Laboratory.
   c) Quantitative bacteriology cultures can be performed on the bronchial washing and bronchial brush
      specimens.
5. Lung aspirations or biopsies
   a) These specimens are obtained by inserting a needle through the chest wall into the pulmonary
      infiltrate.
   b) If the lesion is large or if there are multiple lesions, collect multiple specimens from representative
      sites.

UPPER RESPIRATORY
1. Throat (pharyngeal specimens)
   a) Do not obtain throat sample if epiglottis is inflamed, as sampling may cause serious respiratory
      obstruction.
   b) Depress tongue gently with tongue depressor.
   c) Extend sterile swab between the tonsillar pillars and behind the uvula. Avoid touching the cheeks,
      tongue, uvula or lips.
   d) To obtain sample, sweep the swab back and forth across the posterior pharynx, tonsillar areas and in
      particular any inflamed or ulcerated areas.
2. Nasal
   a) Insert a sterile swab into the nose until resistance is met at the level of the turbinates (approximately
      1 inch into the nose). Rotate the swab against the nasal mucosa.
   b) Repeat the process on the other side.
3. Nasopharyngeal suction
   a) Suction material from the nasopharynx, and collect it in a sterile container.
4. Nasopharyngeal swabs
   a) Carefully insert a flexible-wire calcium alginate-tipped swab through the nose into the posterior
      nasopharynx and rotate the swab. Keep the swab near the septum and the floor of the nose.
5. Nasopharyngeal washings
   a) Submitted primarily for viral studies.
   b) Instruct the patient not to swallow during the procedure.
   c) With the patient's head hyperextended instill 2 to 5 ml of sterile saline into each nostril.
   d) Aspirate the fluid by inserting a rubber bulb syringe into each nostril.
   e) Place the wash in a sterile container or in viral transport medium if viral culture is desired.
6. Sinus
   a) The only appropriate specimen is material directly aspirated from a sinus cavity.
   b) Using syringe aspiration technique, a specially trained physician will obtain material from 
      maxillary, frontal or other sinuses.
   c) Send the specimen in a capped syringe.
7. Middle ear
   a) Submitted primarily to diagnose middle ear infections only if previous therapy has failed.
   b) The physician will obtain the fluid from behind the eardrum by a syringe aspiration.
   c) Send the specimen in a sterile container or send it in the syringe.
   d) If eardrum is ruptured, collect exudate by inserting sterile swab through an auditory speculum.

<table>
<thead>
<tr>
<th>Respiratory Pathogens and Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
</tr>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>Fungi</td>
</tr>
<tr>
<td>Anaerobes</td>
</tr>
<tr>
<td>Mycobacteria</td>
</tr>
<tr>
<td>Pneumocystis</td>
</tr>
<tr>
<td>Parasites</td>
</tr>
</tbody>
</table>
STERILE BODY FLUIDS: (excluding CSF, urine, blood)

a) Disinfect the needle puncture site.
b) The physician will aseptically perform percutaneous aspiration to obtain pleural, pericardial, peritoneal or synovial fluids.
c) Expel any air bubbles from syringe, and immediately inject specimen into a sterile screw cap container.
d) Transport to the Laboratory immediately.
SUBCUTANEOUS TISSUE AND SKIN SPECIMEN COLLECTION

1. **Burn specimens:** The surfaces of burn wounds will become colonized by the patient's microbial flora or by environmental organisms. When the organism load is large, infection of underlying tissue may occur and bacteremia may result. Cultures of the surface alone are misleading. Therefore, biopsies of deeper tissue are often indicated. Additionally, organisms may not be distributed evenly in the burn wound, so sampling of different areas of the burn is recommended.
   a) Disinfect the surface of the burn. Allow the disinfectant to dry prior to collecting the specimen.
   b) Collect a punch biopsy sample for quantitative culture.

2. **Superficial wound, bacterial:** Syringe aspiration is preferable to swab collection.
   a) Disinfect the surface of the wound and allow the disinfectant to dry prior to collecting the specimen.
   b) Using a sterile needle and syringe, a physician will aspirate the deepest portion of the lesion. If a vesicle is present, collect both fluid and cells from the base of the lesion.
   c) If the initial aspiration fails to obtain material, inject sterile, nonbacteriostatic saline subcutaneously.
   d) Repeat the aspiration attempt.

3. **Superficial lesions, fungal**
   a) Clean the surface with sterile water.
   b) Using a scalpel blade, scrape the periphery of the lesion border. Samples from scalp lesions should include hair. If there is nail involvement, obtain scrapings of debris or material beneath the nail plate. Transport in a sterile container.

4. **Ulcers and nodules**
   a) Disinfect ulcer or nodule.
   b) Remove overlying debris.
   c) Curette the base of the nodule or lesion.
   d) If exudate is present, collect it with a syringe or sterile swab.

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<table>
<thead>
<tr>
<th>Organism</th>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Syringe aspirates or biopsy specimens are preferable to swab specimens.</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Uncommon in burn, ulcer, nodules or superficial skin infections. Useful following bites or trauma.</td>
</tr>
<tr>
<td>Fungi</td>
<td>Useful in diagnosing dermatophytes, yeast, filamentous fungi and dimorphic fungi.</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Useful in diagnosing <em>M. marinum</em>, <em>M. fortuitum</em>, and <em>M. chelonei</em>.</td>
</tr>
</tbody>
</table>
DEEP WOUNDS, ASPIRATES AND TISSUE SPECIMEN COLLECTION

1. Bite wounds
   a) Aspirate pus from the wound, or obtain at the time of incision, drainage, and debridement of infected wound.
   b) Do not culture fresh bite wounds, as infectious agents will likely not be recovered.

2. Bone
   a) Obtain bone specimen at surgery.
   b) Submit in sterile container without formalin.

3. Deep wounds, abscesses or sinus tracts
   a) Disinfect the surface of the wound or abscess.
   b) Aspirate the deepest portion of the lesion or sinus tract, avoiding contamination by the wound surface.
   c) If collection is done at surgery, a portion of the abscess wall should also be sent for culture.

4. Punch skin biopsies
   a) Disinfect the skin surface.
   b) Collect 3 to 4 mm sample with dermal punch.
   c) Submit in sterile container without formalin.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Biopsy specimens or aspirates are better than swabs.</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>Useful in diagnosing actinomycosis.</td>
</tr>
<tr>
<td>Fungi</td>
<td>Useful in diagnosing <em>Pseudoallescheria boydii</em>, <em>Bipolaris sp.</em>, <em>Exophiala sp.</em>, and <em>Fusarium sp.</em></td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Useful in diagnosing <em>M. tuberculosis</em>, <em>M. bovis</em> and <em>M. kansasii</em></td>
</tr>
</tbody>
</table>
URINE SPECIMEN COLLECTION

General considerations
- Never collect urine from a bedpan or urinal.
- Thoroughly clean the urethral opening (and vaginal vestibule in females) prior to collection procedures to ensure that the specimen obtained is not contaminated with colonizing microorganisms in this area.
- Use soap rather than disinfectants for cleaning the urethral area. If disinfectants are introduced into the urine during collection, they can inhibit the growth of microorganisms.
- Transport the specimen to the laboratory such that it will be plated within two (2) hours of collection. Urines from clinics outside the main hospital campus should be placed in tubes with preservative. These specimens can be held for eight (8) hours. Alternatively, urines can be refrigerated for 24 hours before plating.
- Use sterile tubes or cups to collect and transport the urine.

1. **Clean catch urine specimen collection (female)**
   a) The person obtaining the specimen should wash hands with soap and water, rinse and dry. If the patient is collecting the specimen, she should be given detailed instructions.
   b) Cleanse the urethral opening and vaginal vestibule area with soapy water or clean gauze pads soaked with liquid soap.
   c) Rinse the area well with water or wet gauze pads.
   d) Hold labia apart during voiding.
   e) Allow a few milliliters to pass.
   f) Collect the midstream portion of urine in a sterile container.

2. **Clean catch specimen collection (male)**
   a) The person obtaining the specimen should wash their hands with soap and water, rinse and dry. If the patient is collecting the specimen, he should be given detailed instructions.
   b) Cleanse the penis, retract the foreskin (if not circumcised), and wash with soapy water.
   c) Rinse the area well with water.
   d) Keeping foreskin retracted, allow a few milliliters of urine to pass.
   e) Collect the midstream portion of urine in a sterile container.

3. **Ileal conduit urine collection**
   a) Remove the external urinary appliance and discard the urine within the appliance.
   b) Gently swab and clean the stoma opening with a 70% alcohol pad and then with an iodine solution. Remove excess iodine with an alcohol pad.
   c) Using sterile technique, insert a double catheter into the stoma.
   d) Catheterize the ileal conduit to a depth beyond the fascial level.
   e) Collect the urine drained into a sterile container.

4. **Indwelling catheter urine collection**
   a) Clean the catheter collection port with a 70% alcohol swab.
   b) Using sterile technique, puncture the collection port with a needle attached to a syringe.
   c) Aspirate the urine and place it into a sterile container.

5. **Straight catheter urine collection**
   a) Clean the patient's urethral opening (and in females the vaginal vestibule) with soap, and carefully rinse the area with water.
   b) Using sterile technique, pass a catheter into the bladder.
   c) Collect the initial 15 to 30 ml of urine and discard it.
   d) Collect a sample from the mid- or later flow of urine in a sterile container.

### Urine Pathogens and Specimens Type

<table>
<thead>
<tr>
<th>Organism</th>
<th>Vol. (ml)</th>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>0.5-1</td>
<td>After proper cleansing of patient, collect midstream void.</td>
</tr>
<tr>
<td>Fungi</td>
<td>0.5-1</td>
<td>1st morning void is recommended. Do not collect 24 hour specimen.</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>&gt;20</td>
<td>1st morning three consecutive voided urine specimens are recommended. Do not collect 24 hour specimen.</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>1</td>
<td>Use suprapubic aspirate.</td>
</tr>
<tr>
<td>Virus</td>
<td>10-50</td>
<td>1st morning void is recommended. Transport to the Laboratory immediately. Useful for adenovirus, mumps, and CMV.</td>
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