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.

Safety Considerations

1. Follow STANDARD PRECAUTION guidelines. Treat all specimens as potentially hazardous.
2. Use appropriate barrier protection (gloves, gown) when collecting or handling specimens. If splashing is a possibility, protective eyewear, face masks and aprons may be necessary.
3. Do not contaminate the external surface of the collection container and/or the accompanying paperwork.
4. Minimize direct handling of the specimen in transit. Use plastic sealable bags with a separate pouch for paperwork.

General Guidelines for Specimen Collection

1. Collect specimen before administering antimicrobial agents when possible.
2. Collect specimens with as little contamination from indigenous flora as possible.
3. 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.
4. All swabs are to be kept moist in a transport medium after the specimen is collected.
5. Clearly label the specimen container with:
    a. Patient's name and another identifier (DOB, MRN etc.) verified with patient or armband
    b. Date
    c. Time of collection
    d. Specimen source
6. Collect an adequate amount of specimen. Inadequate amounts of specimen may yield false negative results.
7. Identify the specimen source and/or specific site correctly so that proper culture media will be selected during processing in the Laboratory.
8. If a specimen submitted for culture also has nucleic acid amplification tests ordered, first disinfect the processing area with a 10% bleach solution or DNA-Away. The specimen should be split prior to any manipulation and a separate aliquot set aside for molecular testing. If this is not possible, the specimen should be sent to the molecular testing section first.
9. 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.

Microbiology Specimen Collection Guidelines

Transport Systems for Microbiology Specimens

Cultures	System
Anaerobic	eSwab
Aerobic	eSwab
Nasopharyngeal Male urethral	eSwab mini-tip
Urine	BD Vacutainer Urine Collection Kit (gray top). Stable 48 hours at room temperature. Sterile screw-capped cup. Stable 24 hours if refrigerated.
Sputum Stools	Sterile screw-capped cup.
Biopsy	Sterile screw-capped cup. Add a small amount of sterile non-bacteriostatic saline to cup.
Sterile fluids Drainage Bronchial brush	Sterile tubes If the fluid will clot, add lithium heparin as an anticoagulant.
Needle aspirate	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.
Respiratory Virus (Molecular Testing)	3mL Universal Viral Transport Media Includes respiratory mycoplasma, chlamydia and Bordetella. Specimens should be delivered promptly to the laboratory. Due to the seasonal prevalence of some viruses, laboratory consultation may assist in the selection of appropriate testing.
GC – Chlamydia FEMALE	cobas PCR Female Sample Kit
GC – Chlamydia MALE	URINE – in a sterile, screw-capped cup (include initial stream of urine – not mid-stream) transfer urine to Roche cobas collection tube.
Stool	A clean, empty vial, one vial containing a parasite preservative (Protofix) and, for outpatients only, one vial containing bacterial transport medium (ETM).

Standard Specimen Collection Procedures

Providers 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.

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 culture sets 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:

Recommended Blood Culture Volume		Total Volume to be Drawn	Plus Aerobic /IF	Lytic/10 Anaerobic/IF
			Blood Volume	
Adult	Ideal Volume	20 mL	10 mL	10 mL
	Acceptable Volume	8-20 mL	4-10 mL	4-10 mL
	Difficult Collection	<7 mL	7 mL	

Recommended Blood Culture Volume by Patient Weight			Peds Plus/IF	Plus Aerobic /IF	Lytic/10 Anaerobic/IF	Total Volume to be Drawn
	Weight Range		Blood Volume			
	Pounds	Kilograms				
PEDIATRIC	<11 lbs	<5 kg	1 mL			1 mL
	11 – 22 lb	5-10 kg	2 mL			2 mL
	22.1 – 44 lb	10.1 – 20 kg	3 mL		3 mL	6 mL
	44.1 – 88 lb	20.1 – 40 kg		5 mL	5 mL	10 mL
	>88 lb	>40 kg		10 mL	10 mL	20 mL

3. Type of blood culture: Blood cultures may be drawn in a variety of containers. Refer to the specific test for the type of container to use.
   a. Routine blood culture – BD Bactec Plus Aerobic* & BD Bactec Anaerobic bottles (*BD Bactec Peds Plus is available for Pediatric patients)
   b. Fungus blood culture – Isolator* 10-mL Tube (alternatively, BD Bactec Aerobic bottle incubation can be extended to 28 days)
   c. Acid fast blood culture – Isolator* 10-mL Tube (*1.5-mL Isolator Tubes are available for pediatric patients)
   d. CMV blood culture- diagnosed by PCR
4. Blood collection:
   a. Locate a suitable vein before cleansing the skin.
   b. Using the PDI Chlorascrub Pad, apply to skin and using a back and forth motion, scrub the area for 15 seconds.
   c. Allow the area to dry for 30 seconds. Do not blow or touch the site after cleansing the skin.

d. If the patient is one in who repeated palpations are needed to locate a vein, prepare the gloved fingers with a pad. Make sure that the gloves are not prepared until all materials are collected and you are ready to begin the venipuncture.
e. Disinfect the top of the bottle stopper on the BD Bactec bottles with a 70% isopropyl alcohol swab. Allow the alcohol to dry for 30-60 seconds. DO NOT USE IODINE TO DISINFECT BOTTLES
f. Use a vacutainer butterfly needle with hub to minimize chances of contamination.
g. Make certain that the needle does not touch anything before entering the skin. If you are unsuccessful in obtaining blood with the first puncture, be certain that you replace the needle and all other collection equipment with new ones before attempting a second puncture.
h. Draw the required amount of blood into each bottle filling the BD Bactec Plus Aerobic (aerobic – blue) bottle first, followed by the BD Bactec Anaerobic (anaerobic – purple) bottle. Butterfly needle/hub assembly or syringe/hub assembly is adapted to fit vacutainer tubes after blood cultures have been obtained.

Body Fluids, Sterile (excluding blood, CSF, Urine)

1. Disinfect the needle puncture site.
2. The physician will aseptically perform percutaneous aspiration to obtain pleural, pericardial, peritoneal or synovial fluids.
3. Expel any air bubbles from syringe, and immediately inject specimen into a sterile screw cap container.
4. Transport to the Laboratory immediately.
5. For large amounts of sterile fluid BD Bactec Plus Aerobic & BD Bactec Anaerobic bottles may also be used.

Central Nervous System

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.
3. CNS Collection Requirements:
   a. Tube #2 is preferred.

Organism	Volume (ml)	Comments
Bacteria	>1	

Fungi	2	Rule out *Cryptococcus sp.*, *Coccidioides immitis*.
Mycobacteria	2	*M. tuberculosis*, *M. avium-intracellulare*
Anaerobes	NA	Brain abscess or CNS biopsy specimens.
Parasites	NA	Brain abscess or CNS biopsy specimens for *Ent. histolytica*, *Toxo. gondii*, *Naegleria sp.*, *Acanthamoeba sp.* CSF for *Naegleria sp.* and *Acanthamoeba sp.*
Virus	1-2	Send to Laboratory on ice.

Gastrointestinal Tract

1. Fecal specimens:
    a. Have patient obtain stool specimen by one of the following methods:
        i. Pass stool directly into a sterile, wide-mouth, leak proof container with a tight fitting lid.
        ii. Pass stool into a clean, dry bedpan, and transfer into a sterile leak proof container with a tight fitting lid.
    b. Keep stool specimen cool. Do not incubate.
    c. Do not use toilet paper to collect stool. Toilet paper may contain substances, which are inhibitory for some fecal pathogens.
    d. Stool for ova and parasites should be placed in preservative immediately after collection.
    e. Patient Instructions for Stool Collection can be found at http://www.pathologylab.org/patient-collection-instructions.aspx
2. 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.
3. 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.
4. Gastric biopsies and washings:
    a. Submitted primarily for the detection of *Helicobacter pylori*.
    b. Obtained by endoscopic procedures.
5. Esophageal biopsies and washings:
    a. Primarily used to detect *Candida species*, Cytomegalovirus and Herpes Simplex virus infections.
    b. Obtained by endoscopic procedures.
6. Pinworm: Use pinworm collection kit. Collect specimen when patient gets up in the morning

before the patient bathes or defecates.

Genital Tract

Female

1. Amniotic fluid: Aspirate fluid by catheter, at caesarian section, or at amniocentesis.
2. Bartholin gland: Decontaminate skin with povidone-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 sterile gauze. Try to avoid bleeding.
   d. Press the base of the lesion until clear fluid is expressed. Using any of the following techniques:
      i. Aspirate vesicular fluid with a 26- to 27-gauge needle and place it in the appropriate transport system.
      ii. Unroof the vesicle and collect fluid with a sterile swab and place it in the appropriate transport system (for HSV detection).
      iii. 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.

Male

1. Epididymis: 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*.

a. 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. Using any of the following techniques:
      i. Aspirate vesicular fluid with a 26- to 27- gauge needle and place it in the appropriate transport system.
      ii. Un-roof the vesicle, and collect fluid with a sterile swab and place it in the appropriate transport system (for HSV detection).
      iii. 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: Used to diagnose acute and chronic prostatitis. For both diseases gram negative enteric organisms are the most frequently isolated pathogens.
   a. 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.

Genital Tract Pathogens and Specimen Type

Organism	Specimen Type
Neisseria gonorrhoeae	Cervical, urethral, anal, vaginal swabs. Urine for PCR.
Bacteria	Prostatic fluid, cervical, vaginal.
Trichomonas vaginalis	Vaginal, prostatic fluid.
Fungi	Anal, vaginal or cervical.
Anaerobes	Epididymis aspirate, amniotic fluid, abscess.
HSV	Genital or peri-anal lesion.
Chlamydia trachomatis	Rectal, cervical, urethral, bubo or ulcer material.
Haemophilus ducreyi	Material from ulcers (genitalia and perianal) and from inguinal nodes.

Chlamydia trachomatis and Neisseria Gonorrhoeae Collection

Female Endocervical Swab Specimen using cobas® PCR Uni Swab Sample Packet containing PCR transport medium and swabs:

a. Remove the cleaning swab from the packaging.
b. Using one swab, remove excess mucus from the cervical os. Discard the used swab.
c. Remove second swab from the packaging.
d. Insert the collection swab into the cervical canal and rotate for 15-30 seconds.
e. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.

f. Immediately place the swab into the transport media. Break the shaft of the swab, leaving swab in transport media. Make sure the cap is tightly secured to the tube.
g. Label and date the tube. Place specimen in refrigerator.
h. Store and transport to the laboratory at 2-8°C within 7 days.

Male Urethral Swab Specimen using the cobas® PCR Uni Swab Sample Packet containing PCR transport medium and swabs:

1. **Urine is the preferred sample for males**
2. Remove the one swab from the packaging.
3. Insert the swab 2-4 cm into the urethra and rotate for 3-5 seconds.
4. Withdraw the swab.
5. Immediately place the swab into the transport media. Break the shaft of the swab, leaving swab in transport media. Make sure the cap is tightly secured to the tube.
6. Label and date the tube. Place specimen in refrigerator.
7. Store and transport to the laboratory at 2-8°C within 7 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. If possible, place urine into vial within the cobas® PCR Uni Swab Sample Packet
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.
5. Store and transport urine specimens at 2-8°C within 4 days.

Ocular

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 conjuctival samples prior to corneal scrapings.
b. One or two drops of topical anesthetic are generally instilled.
c. Using short, firm strokes in one direction and 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

Lower Respiratory - *Specimens consisting primarily of saliva are rejected.*

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.
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.

Microbiology Specimen Collection Guidelines

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.
8. Respiratory Pathogens and Specimen Type:

Organism	Volume (ml)	Specimen Type
Bacteria	NA	All specimen types
Fungi	3-5	*Sputum: Collect 3 first morning deep cough or induction specimens Lung biopsy* *Aspirates*

Anaerobes	1	Sinus aspirate, tympanocentesis fluid, transtracheal aspirate, lung aspirate, biopsies
Mycobacteria	5-10	Sputum: Collect 3 first morning deep cough or induction specimens Lung biopsy Bronchial Specimens *Aspirates*
Pneumocystis	2	Induced sputum, bronchoalveolar lavage fluid, or lung biopsy
Parasites	3-5	Sputum, Bronchial Specimens: for amoebae, helminth eggs, hooklets of *Echinococcus sp*., hookworm larvae, *Ascaris* and *Strongyloides*.

Tissues – Subcutaneous and Skin

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.
5. Subcutaneous and Skin Pathogens and Specimen Type:

Organism	**Specimen Type**
Bacteria	Syringe aspirates or biopsy specimens are preferable to swab specimens.

Anaerobes	Uncommon in burn, ulcer, nodules or superficial skin infections. Useful following bites or trauma.
Fungi	Useful in diagnosing dermatophytes, yeast, filamentous fungi and dimorphic fungi.
Mycobacteria	Useful in diagnosing *M. marinum*, *M. fortuitum*, and *M. chelonei*.

Tissue – Deep Wounds & Aspirates

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.
5. Deep Wound, and Tissue Pathogens and Specimen Type:

Organism	**Comments**
Bacteria	Biopsy specimens or aspirates are better than swabs.
Anaerobes	Useful in diagnosing actinomycosis.
Fungi	Useful in diagnosing *Pseudoallescheria boydii*, *Bipolaris sp.*, *Exophiala sp.*, and *Fusarium sp.*
Mycobacteria	Useful in diagnosing *M. tuberculosis*, *M. bovis* and *M. kansasii*

Urine

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 place 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 urine 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.
6. Urine Pathogens and Specimens Type:

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

Reviewed and Updated July 22, 2020 by Ryan Metzger, Microbiology Manager