

**SPECIMEN COLLECTION:  
GUIDELINES FOR BEST PRACTICES**

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Proper collection of specimen is important for recovery of pathogenic organisms responsible for disease. This guide was developed to provide healthcare personnel with best practice information regarding the collection of a laboratory specimen. Adhering with best practice enables the laboratory to produce results that can provide clinically relevant information to all involved in the care of the patient.

All diagnostic information from the Microbiology Laboratory is contingent upon the quality of the specimen received. Consequences of a poorly collected and/or poorly transported specimen include failure to isolate the causative microorganism and recovery of contaminants or normal flora, which can lead to improper treatment of the patient.

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Accuracy of diagnosis is essential for safe patient care. Providing for the safety of the healthcare worker may also hinge upon accurate diagnosis and rapid response. Ensuring that a microbiologic specimen yields clinically relevant information requires that the specimen be correctly collected, handled, and transported.

When obtaining a specimen to be used in diagnosing an infection of public health significance (e.g., SARS, Avian Influenza, Smallpox), additional information must also be available. Important resources include the following:

<http://www.cdc.gov>

<http://www.asm.org>

This guide is not meant to be a compendium of specimen collection procedures but instead, is designed to provide basic information that can be used to educate healthcare personnel and improve the safety of patients, healthcare personnel, and the community. We encourage readers to make this manual a living document and individualize it to their unique practice setting and continuously evaluate its content looking for improvements..

There are several guiding principles that should be considered and understood prior to obtaining/collecting any specimen.

1. Collect the material from the site in which the etiologic agent will most likely be found.
2. Collect the specimen at the optimum time (e.g., early morning sputum for acid-fast bacillus (AFB)).
3. Obtain cultures prior to administration of antibiotics whenever possible.
4. Collect adequate volume of material. Inadequate amounts of specimen may yield false negative results.
5. Collect specimen in a manner that minimizes or eliminates contamination from indigenous flora as possible to ensure that the sample will be representative of the infected site.
6. Use appropriate collection devices, transport media, and sterile, leak proof containers.
7. Use sterile equipment and aseptic technique to collect specimen to prevent introduction of microorganisms during invasive procedures.
8. Clearly label the specimen including specific information regarding site of collection (e.g., blood obtained via blue lumen of right subclavia central catheter) and complete the ordering process.
9. Identify the specimen source and/or specific site correctly so that proper processing methods and culture media will be selected by the laboratory personnel.
10. If the specimen is collected through intact skin, cleanse the skin first with 70% alcohol followed by an iodine solution (e.g. povidone-iodine) or chlorxidine/alcohol combination. If iodine is used, remove excess iodine after the specimen has been collected.
11. Provide clear instructions to patients if they are collecting their own specimen (e.g., clean catch urine, or stool) in order to obtain the best quality specimen and allay their fears.
12. Deliver the specimen promptly to the laboratory. Delay in transport may compromise the specimen.
13. As with all patient contact episodes, consistent attention must be given to hand hygiene and use of appropriate personal protective equipment.
14. Use appropriate safety devices to minimize risk of accidental needlestick, cut or puncture. It is advisable to make sure the user is knowledgeable about how the safety device works prior to its use.

**BLOOD CULTURE:**

Ensuring that blood cultures are obtained in a manner that prevents contamination is a cornerstone of an infection prevention and control process. In addition, the increasing use of blood cultures obtained through vascular/arterial devices necessitates meticulous technique and timely communication with the microbiology laboratory.

*Timing and Number:*

Acute Sepsis: Collect two or three sets of cultures from separately prepared sites prior to starting antimicrobial therapy. Each set consists of two bottles, one aerobic and one anaerobic or two aerobic.

Endocarditis:

Acute: Obtain three blood cultures from separate venipuncture sites over 1 – 2 hours, prior to beginning therapy. These cultures are often obtained 30 minutes apart in order to document persistent bacteremia.

Subacute: obtain three blood cultures on day 1 (15 minutes or more apart). If cultures are negative after 24 hours, obtain 3 more.

Pediatrics: collect one low volume pediatric bottle for all submissions.

*Volume of Blood:*

The volume of blood is **critical** because the concentration of organisms in most cases of bacteremia is low, especially if the patient is on antimicrobial therapy. In infants and children, the concentration of organisms during bacteremia is higher than in adults, so less volume of blood is required.

Adults: 10 ml of blood per culture bottle

Children and infants: 1 – 3 ml of blood per culture bottle. The volume necessary is dependent upon the weight of the child/infant so contact the microbiology department prior to obtaining the blood if assistance is needed in determining the correct amount of blood needed for that individual child/infant.

NOTE: In the event that 10 ml or less of blood is obtained from an adult, put it all into one aerobic blood culture bottle.

Collection:

Blood can be collected by venipuncture of peripheral veins or arteries. Collection from intravascular catheters is not recommended as they are intrinsically contaminated. If a line must be used, indicate the type of line or port through which the blood was obtained.

Technique is important to prevent contamination of the blood resulting in inaccurate results. The following represent basic tips to help ensure the highest quality results.

- Perform hand hygiene, explain the procedure to the patient prior to collection of all specimen, and utilize all appropriate safety equipment.
- Locate the venipuncture site prior to skin disinfection.
- Disinfect the venipuncture site and the stoppers of the bottles prior to blood collection.
- Use chlorhexidine /alcohol combination (e.g., Chloraprep™) for skin disinfection for optimal results.
- Disinfect the top of the blood culture bottle(s) with 70% isopropyl or ethyl alcohol.
- Scrub the site using a chlorhexidine/alcohol swab or wand.
- Allow the disinfectant to dry. (DO NOT palpate the vein after disinfecting the skin prior to inserting the needle.)
- Draw blood using a sterile safety syringe and needle, or safety butterfly designed to attach to a vacutainer holder and dispense the appropriate amount of blood into the bottles.

NOTE: The blood culture bottles can be used with the vacutainer adapter, but it may not deliver a controlled draw. Care must be taken to dispense the appropriate amount of blood into the culture bottle.

- After venipuncture and inoculation of bottles, engage safety device on needle or butterfly and immediately dispose of collection materials in a sharps container. Wipe residual chlorhexidine/alcohol from skin with alcohol to prevent irritation of the skin.
- Indicate site of draw, date and time of draw, and initials of person drawing blood.
- If blood has been obtained through an indwelling intravascular device, provide specific information including lumen and location of the device.
- Transport blood cultures to the Laboratory IMMEDIATELY. Do not refrigerate. Delay in transport may compromise the specimen and recovery of organisms.

<b>POLICY/PROCEDURE</b>		
Subject:		
<b>BLOOD CULTURE, COLLECTION FROM AN ADULT PATIENT</b>		
Author:		
<b>INFECTION CONTROL</b>		

**PURPOSE:** To provide guidelines for obtaining blood samples for culture in a manner that minimizes contamination and maximizes clinically relevant results.

**POLICY STATEMENTS:**

1. Critical elements involved in obtaining blood cultures includes adequate skin disinfection, adequate blood volume (i.e., at least 10 cc per bottle for adults), and hand hygiene practice performed by the collecting healthcare worker.
2. Blood cultures are to be drawn 30-60 minutes apart unless ordered otherwise by the clinician.
3. If one culture is to be obtained from a central vascular access device (CVAD) and another via a peripheral venipuncture, those cultures can be obtained one right after the next unless otherwise ordered by the clinician.
4. If a peripheral blood culture is ordered to be obtained after removal of a CVAD, blood for that culture should be obtained 30- 60 minutes after removal of the CVAD.
5. Blood drawn via arterial lines is never an acceptable specimen for blood culture in the adult patient. Blood drawn via an umbilical artery catheter (UAC) is acceptable practice in the neonate.
6. Peripherally drawn blood cultures represent the optimal specimen as those obtained via CVAD are more likely to be contaminated by organisms residing in the device itself or device components (i.e., tubing, end caps).
7. Blood specimen collection may be drawn from CVADs by Registered Nurses or physicians only.
8. It is not within the scope of LPN practice to aspirate any CVAD for blood specimens.
9. Appropriate safety devices and personal protective equipment must be used when obtaining any blood specimen. Examples of such safety devices include safety needle/butterfly, blood transfer device, and gloves. Additional safety devices are selected and used based upon anticipated activities of the individual obtaining the specimen.
10. If the patient has a multi-lumen device (i.e., triple lumen central venous catheter), it is not necessary to draw cultures from each of the lumens unless specified by the ordering clinician.

**PROCEDURES:**

***Peripheral blood culture- Adult***

11. Gather supplies:
  - a. Exam gloves
  - b. Blood culture bottles
  - c. Tourniquet
  - d. Chlorhexidine/alcohol combination for skin preparation
  - e. Alcohol swabs for disinfecting top of blood culture bottle(s)
  - f. Syringes- sized to allow 10 cc of blood per culture bottle to be filled
  - g. Safety needle
  - h. Blood transfer device (one for each blood culture bottle to be filled)
  - i. Adhesive bandage or gauze for venipuncture site dressing
  - j. Labels and transport bags for blood culture bottles
12. Verify clinician order.
13. Perform hand hygiene.
14. Verify patient's identity and explain procedure to patient.
15. Apply tourniquet and identify vein. Release tourniquet if delay is expected prior to performing venipuncture and reapply immediately prior to venipuncture.
16. Remove hard plastic top from blood culture bottles and swab rubber top with alcohol swab. Leave alcohol swab on top of bottle to prevent inadvertent recontamination of the top.
17. Disinfect skin at proposed venipuncture site using chlorhexidine/alcohol combination. Scrub anticipated area at the proposed venipuncture site using vigorous motion for approximately 30 seconds so area the disinfected area is at least 5 cm x 5cm
18. Don gloves.
19. Do not touch prepared area of skin as this causes recontamination of the disinfected site.
20. Using syringe with safety needle, perform venipuncture and obtain a minimum of 10cc per blood culture bottle to be filled.
21. Release tourniquet.
22. After obtaining the necessary blood volume, remove needle from patient and activate safety device.
23. Apply pressure and cover venipuncture site with gauze and/or adhesive dressing.
24. Remove needle from syringe (be sure safety device is activated first) and discard needle in appropriate sharps container.

25. Remove alcohol swab from top of blood culture bottle then open blood transfer devices and place on top of each blood culture bottle.
26. Put hub of syringe into blood transfer device and activate the transfer device by pressing device firmly onto top of blood culture bottle. Vacuum from the blood culture bottle will pull blood from the syringe into the culture bottle.
27. Allow at least 10 cc of blood to transfer from syringe into blood culture bottle. Verify that the appropriate volume transfer has occurred by looking at the volume measurement indicators noted on the side of the culture bottle.
28. Remove syringe from transfer device and repeat the process (Steps 25-27) for the second blood culture bottle.
29. When second bottle has received the appropriate amount of blood (i.e., at least 10 cc for adult patients), remove syringe and the transfer device from both blood culture bottles and place them in the designated sharps container.
30. Check on patient's venipuncture site to ensure bleeding has stopped.
31. Remove gloves and perform hand hygiene.
32. Prior to leaving the patient's bedside, label bottles and ensure the order contains information including time of draw and site of draw as well as other information outlined in hospital blood collection policy.
33. Send blood cultures to the lab in accordance with hospital procedure for transport.

***Central line blood culture- adult***

1. Verify physician's order for blood specimen collection, and assess appropriateness of using central venous access device (CVAD) for this purpose.
2. Gather supplies
  - a. Exam gloves
  - b. Blood culture bottles
  - c. Alcohol swabs to disinfect top of blood culture bottle(s)
  - d. Syringes- sized to allow 10 cc of blood per culture bottle to be filled
  - e. Blunt cannula for each syringe (syringes for blood draw, syringe for priming new end cap, and for each flush)
  - f. Prefilled normal saline syringes (preservative free)
  - g. Sterile end cap
  - h. Blood transfer devices
  - i. Labels and transport bags for bottles
3. Perform hand hygiene.

4. Verify patient's identity, and explain procedure to patient.
5. Remove hard plastic top from blood culture bottles and swab rubber top with alcohol swab. Leave alcohol swab on top of bottle to prevent inadvertent recontamination of the top.
6. Don exam gloves.
7. Discontinue administration of all infusates via the CVAD **prior** to obtaining blood samples. If tubing disconnected, put a new sterile end cap or connecting device on the end of the tubing.
8. Attach a blunt cannula to the syringe containing the preservative-free normal saline.
9. Using an alcohol swab, scrub the end cap of the lumen that will be used to obtain the blood specimen for approximately 10 seconds then insert blunt cannula into the end cap. The distal lumen of the multi-lumen CVAD is the lumen of choice.
10. Flush the lumen of the CVAD with at least 3 – 5 ml of the preservative-free normal saline.
11. Discard that flush syringe in the sharps container.
12. Attach a blunt cannula to an appropriately sized syringe (capable of withdrawing at least 10cc of blood for each adult culture bottle).
13. Disinfect the end cap with alcohol, again using a vigorous scrubbing motion for approximately 10 seconds, then insert that newly prepared syringe with blunt cannula into the disinfected end cap. Save the blunt cannula cap so it can be used to recap the blunt cannula for removal from syringe as noted in step # 16.
15. Aspirate enough blood to allow 10 cc for each blood culture bottle (adults).
16. After enough blood has been drawn, remove syringe and replace blunt cannula cap so the cannula can be removed without contaminating the syringe tip and specimen. Even though the blunt cannula is not considered to be a sharp, recap carefully.
17. Remove alcohol swab from top of blood culture bottle. Open and place a sterile blood transfer device onto each blood culture bottle.
18. Put the hub of the syringe containing the blood into the blood transfer device and press down to active the device by piercing the top of the blood culture bottle. Using the blood transfer device, transfer blood into the first blood culture bottle(s). Check markings on the side of the bottle to ensure that at least 10 cc of blood has been used to inoculate the bottle.
19. Remove the syringe from the blood transfer device and insert it into the blood transfer device on the next blood culture bottle and repeat the activities noted in steps #17-18.
20. Discard blood transfer devices, syringes, and blunt cannulas into appropriate sharps containers.

21. Clamp the CVAD lumen in preparation for changing end cap.
22. Prime a new, sterile end cap with preservative free saline taking care to maintain sterility.
23. Remove the existing end cap and place aside for discard into regular waste.
24. Maintaining sterility of the components, connect the new, primed sterile end cap onto catheter lumen and unclamp the lumen.
25. Flush catheter lumen with at least 10 cc of preservative-free normal saline using the prefilled 10cc syringes. Make sure to flush all visible blood from the end cap.
26. Re-establish IV fluids in accordance with physician orders making sure to vigorously swab the end cap with an alcohol swab for approximately 10 seconds before connecting the fluids into the end cap.
27. Remove gloves and perform hand hygiene.
28. Prior to leaving the patient's bedside, label bottles and ensure order contains information including time of draw and site of draw as well as other information outlined in hospital blood collection policy.
29. Document procedure on the patient's nursing flowsheet.

A. Lumbar puncture:

1. Physicians should wear gown, mask, gloves and eye protection to collect specimen. Because an open tube is held to collect the fluid, other personnel should stand away or wear masks and eye protection in order to avoid respiratory and/or mucosal contamination.
2. Clean the puncture site with a skin disinfectant such as povidone-iodine and allow to dry before needle insertion to prevent introduction of microorganisms.
3. Insert a needle with a stylet at the L3 – L4, L4 – L5, or L5 – S1 interspace. When the appropriate placement is reached, remove the stylet and spinal fluid will appear in the needle hub.
4. Slowly drain the cerebrospinal fluid (CSF) into the sterile leak proof tubes. Three tubes are generally required for microbiology, hematology, and chemistry testing.
5. The first tube is sent to chemistry and the third to hematology. The second tube drawn should be submitted to microbiology, however, it is ideal to send them the most turbid tube. In traumatic taps, the CSF will often clear as the later tubes are collected.
6. It is essential that testing be done on the appropriately numbered tubes as ordered by the clinician.
7. Alternative: place the specimen in an aerobic blood culture bottle. Samples submitted in blood culture bottles cannot be gram stained. Submit fluid for gram stain separately.

B. Ommaya Reservoir Fluid:

1. Clean the Ommaya reservoir site with a skin disinfectant such as povidone-iodine prior to removal of fluid in order to prevent introduction of microorganisms into the reservoir and the specimen. Do not use chlorhexidine-alcohol combination for skin disinfection or any other such neurotoxic agent.
2. Remove the fluid for specimen via the Ommaya reservoir unit., and place in a sterile tube/container.
3. Transport the specimen immediately to the laboratory. Delays of thirty (30) minutes or more seriously compromise the detection and recovery of microorganisms such as *Neisseria meningitidis*.
4. Avoid exposing the specimen to preservatives such as formalin.
5. Maintain specimen at room temperature while awaiting and during transport.

C. Brain Abscess:

1. All brain abscess specimen should be submitted for anaerobic culture as anaerobes are the predominant etiologic organism.
2. Aspirate material from the lesion.

3. Place material in anaerobic transport medium and immediately transmit to the Laboratory.

## D. CNS Biopsy:

1. Obtain sample from the lesion during the surgical procedure.
2. Place in anaerobic transport medium and transport to the Laboratory IMMEDIATELY. Do not add formalin or other preservative.

**COLLECTION CONSIDERATIONS FOR CNS SPECIMEN**

<b>CULTURE</b>	<b>VOLUME (mL)</b>	<b>COMMENTS</b>
Bacteria	1 mL	Send cloudiest CSF specimen to Microbiology Laboratory; tube #2 is recommended but the most turbid tube is ideal.
Fungi	2 mL	Rule out <i>Cryptococcus spp.</i> , <i>Coccidioides immitis</i> , <i>Histoplasma capsulatum</i> . <i>Cryptococcal</i> antigen should also be ordered (in lieu of india ink exam).
Mycobacteria	2 mL	For detection of <i>Mycobacterium tuberculosis</i> , <i>Mycobacterium avium-intracellulare</i> complex.
Anaerobes	N/A	Brain abscess or CNS biopsy specimen are recommended.
Parasites	N/A	Brain abscess or CNS biopsy specimen for <i>Entamoeba histolytica</i> , <i>Toxoplasma gondii</i> , <i>Naegleria</i> species, <i>Acanthamoeba</i> spp.
Virus	1 – 2 mL	Transport on ice. Order <i>Herpes/Enterovirus</i> PCR.

N/A = not applicable

A. Fecal Specimen:

Fecal specimen are the primary specimen in suspected case of gastroenteritis that can result from bacterial, viral, or parasitic infection. Primary community-acquired bacterial pathogens include *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157-H7. In addition, *Clostridium difficile* produces Toxins A and B, the primary virulence factors produced by this organism. *C. difficile* toxin-mediated colitis is the primary cause of diarrhea in hospitalized patients whereas the aforementioned bacterial agents, along with the *Vibrio* species, account for the majority of community-acquired disease.

General considerations:

1. If possible, have the patient obtain the stool specimen.
2. Instruct the patient to pass the stool into a clean, dry pan or specimen container that is mounted on the toilet for this purpose.
3. Avoid contact with the toilet bowl, toilet or tap water, or urine as these are a good source for waterborne bacteria and parasites that could lead to false-positive laboratory results.
4. Transfer a minimum of 5ml of diarrheal stool , or one (1) gram of material (about a walnut sized portion) to a clean, leak proof container with a tight fitting lid.
5. If the specimen is to be examined for parasites, include the suggested preservative as a means of inhibiting bacterial and viral growth in that specimen. Contact the laboratory for assistance in obtaining a container with the appropriate preservative.
6. If there is to be a delay in transporting the specimen to the laboratory, contact them for special instructions that will assist in ensuring that the specimen will yield the desired information.
7. Note: bacterial culture cannot be performed once the specimen has been placed in a preservative.
8. Keep stool specimen at room temperature. Do not refrigerate or incubate them.
9. Do not use toilet paper or diapers to collect stool. Toilet paper or diaper may be impregnated with barium salts which are inhibitory for some fecal pathogens.

B. Rectal Swabs:

These specimen are submitted primarily for the detection of *Neisseria gonorrhoeae*, Herpes simplex virus, Vancomycin resistant enterococcus (VRE) and anal carriage of Group B streptococci.

1. Insert the tip of a sterile swab approximately 1 inch beyond the anal sphincter.
2. Carefully rotate the swab to sample the anal crypts, then withdraw the swab.
3. Place the swab into a culturette. Samples for Herpes simplex should be placed into viral or M4 transport immediately.

Gastric aspirate specimen are submitted primarily for detection of *Mycobacterium tuberculosis* in patients unable to produce quality sputum. Obtaining this specimen should be performed after the patient wakes in the morning so that sputum swallowed during sleep is still in the stomach.

1. Pass a well lubricated nasogastric tube orally or nasally to the stomach of the patient, and aspirate at least 10 cc of gastric contents using a clean syringe. If lavage is needed to obtain a specimen, use a preservative-free saline solution.
2. Before removing the nasogastric tube, release the suction and clamp to prevent mucosal trauma and/or aspiration. Ask the patient to take a deep breath and hold it during tube removal to prevent aspiration.

D. Pinworm Preparation:

Diagnosis of pinworm infection is usually based on the recovery of typical eggs. The goal of the specimen is to capture eggs onto a slide.

1. Place a strip of clear cellulose tape (adhesive side down) on a microscope slide as follows:
  - a. Starting 1.5 cm from one end, run the tape toward the same end, and wrap the tape around the slide to the opposite end.
  - b. Tear the tape even with the end of the slide. Attach a blank label to the tape at the end torn flush with the side.
2. To obtain a sample from the perianal area, peel back the tape by gripping the labeled end, and, with the taped looped (adhesive side outward) over a wooden tongue depressor that is held firmly against the side and extended about 2.5 cm beyond it, press the tape firmly several times against the right and left perianal folds.
3. Smooth the tape back on the slide, adhesive side down.
4. Label the slide with the patient name, date and time of collection and patient number.
5. Send to the laboratory in a plastic bag or specimen cup.

**COLLECTION CONSIDERATIONS FOR GASTROINTESTINAL SPECIMEN**

<b>CULTURE</b>	<b>COMMENTS</b>
Bacteria	Stool: Three specimen recommended Gastric Biopsy: Rule out <i>Helicobacter pylori</i> Rectal Swab: Rule out enteric pathogens
Fungi	Gastric aspirate, gastric biopsy, esophageal brush, esophageal biopsy.
Pinworm Prep	Collect from perianal area early in the morning before the patient bathes or defecates.
Mycobacteria	Gastric aspirate or gastric biopsy. Feces in immunocompromised patients.
Parasites	If transport to laboratory is delayed for more than 30 minutes, place the stool in preservative. Duodenal aspirates are useful in detecting <i>Giardia spp.</i> and larvae of <i>S. stecoralis</i> and <i>A. lumbricoides</i> . Use rectal biopsy specimen for <i>E. histolytica</i> and <i>B. coli</i> . Use small bowel biopsy specimen for <i>Giardia</i> , <i>Cryptosporidium</i> , and <i>Microsporidium spp.</i>
Virus	Use esophageal specimen for CMV and HSV and rectal biopsies for HSV. Send to Laboratory on ice. Do not freeze.

**Female:** Genital tract specimen are submitted primarily for detection of sexually transmitted pathogens. If infection is not caused by one of these pathogens, anaerobic bacteria may be involved. If anaerobic infection is suspected, transport the specimen in an anaerobic collection system.

**Male:** For urethral cultures, collect specimen at least 2 hours after the patient has urinated. Obtain specimen by inserting a thin urogenital swab 2 – 4 cm into the endourethra, gently rotate it, leave in place for 1 – 2 seconds, then withdraw it.

**COLLECTION CONSIDERATIONS FOR GENITAL CULTURES**

ORGANISM	RECOMMENDED SPECIMEN	TRANSPORT SYSTEM
<i>Neisseria gonorrhoeae</i>	Cervical, urethral, vaginal, anal (culture only)	Culturette, Martin-Lewis plate (culture) M4 transport (PCR) – send on ice, and transport to the Microbiology Laboratory immediately.
Bacterial	Prostatic fluid, cervical, vaginal	Culturette, or sterile container
<i>Trichomonas vaginalis</i>	Vaginal, prostatic fluid	Sterile container
Fungi	Anal, vaginal or cervical	Culturette or sterile container
Anaerobes	Epididymis aspirate, amniotic fluid, abscess fluid	Anaerobic transport
Herpes Simplex Virus (HSV)	Genital or perianal lesion	Viral or M4 transport
<i>Chlamydia trachomatis</i>	Urine (male/female) Urethral (male), cervical, vulva (culture only)	Viral or M4 transport for culture M4 transport (PCR) – send on ice and transport to the Microbiology Laboratory immediately.
<i>Treponema pallidum</i>	Genital lesion (Note: secondary lesions of syphilis are most commonly found on mucous membranes and skin including palms of hands and soles of feet, but any body organ may be involved.)	Prepared slide in sterile container.
<i>Ureoplasma urealyticum</i>	Urethral, epididymis or prostatic fluid.	Contact Laboratory for transport medium.

**OCULAR SPECIMEN:**

General Considerations:

1. Obtain viral and chlamydial samples before topical anesthetics are instilled.
2. Obtain culture media for bacterial culture from the Laboratory for direct inoculation.

A. Conjunctival Scrapings:

1. One or two drops of topical anesthetic are generally instilled.
2. Scrape the lower tarsal conjunctiva with a sterilized kimura spatula.
3. Inoculate the appropriate media directly.
4. 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.
5. Alternatively, use a calcium alginate swab or a cotton tipped applicator to swab the inferior tarsal conjunctiva (inside surface of the eyelid) and the fornix of the eye. However, organisms are more readily detected in scrapings than from a swab.

B. Corneal Scrapings:

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

C. Intra-ocular Fluid

1. Use a needle aspiration technique to collect intraocular fluid.
2. Inoculate appropriate media directly, and/or transmit the samples to the laboratory in an anaerobic transport system.
3. Prepare smears by applying scrapings in a gentle circular motion over a glass slide or by compressing material between two glass slides and pulling the slides apart.

**COLLECTION CONSIDERATIONS FOR OCCULAR SPECIMEN**

CULTURE	COMMENTS
Bacteria	Inoculate media directly with ocular scrapings. (If <i>Neisseria gonorrhoeae</i> is suspected, inoculate a Martin-Lewis plate also.
Fungi	Inoculate media directly with ocular scrapings
Anaerobes	Use anaerobic transport medium, or inoculate medium directly. If inflammation occurs after extracapsular cataract extraction, rule out <i>Propionibacterium</i> spp.
Parasites	Used to detect <i>Acanthamoeba</i> spp.
Chlamydia	Do not use cotton swabs for specimen collection.
Virus	Do not use calcium alginate swabs for specimen collection. Submit in viral or M4 transport medium
Mycobacterium	Inoculate medium directly with ocular scrapings.

**RESPIRATORY SPECIMEN:**

## General Considerations

1. Twenty-four hour sputum collections (e.g., all sputum collected over an extended period of time) are not acceptable for culture and will be rejected.
2. If *Corynebacterium diphtheriae*, *Arcanobacterium hemolyticum*, *Bordetella pertussis*, *Neisseria gonorrhoeae*, *legionellae*, *chlamydiae*, or *mycoplasmas* are suspected, the physician should contact the Microbiology Laboratory prior to specimen collection because special techniques and/or media are required for isolation of these agents.

## A. Expecterated Sputum:

1. The optimal time for collection of an expecterated sputum specimen is in the early morning.
2. If possible, have the patient rinse mouth and gargle with water or sterile saline prior to sputum collection.
3. Instruct the patient not to expectorate saliva or postnasal discharge into the container.
4. Collect specimen resulting from deep cough in sterile screw-cap cup or other sterile, leak proof container.
5. The optimal volume for an expecterated sputum is 4-6 ml. If the specimen is being cultured for mycobacteria, a minimum of 4ml is needed.

## B. Induced Sputum:

1. Using a wet toothbrush, brush the buccal mucosa, tongue, and gums prior to the procedure.
2. Rinse the patient's mouth thoroughly with sterile water or sterile saline.
3. Using an ultrasonic nebulizer, have the patient inhale approximately 20 – 30 ml of 0.85% saline then ask the patient to cough. Respiratory therapy can be of important assistance in this process and are skilled at collection of respiratory specimen.
4. Collect the induced sputum in a sterile screw-cap cup or other leak proof container.
5. The optimal volume of this specimen is 4-6 ml.

C. Tracheostomy and endotracheal aspirations:

Tracheostomy is followed by colonization within 24 hours of insertion of the tube. Results must be correlated with clinical findings such as fever or infiltrate on chest x-ray.

1. Using a sterile suction catheter and glove, aspirate 4-6 ml of sputum into a sterile specimen cup and replace the lid tightly.
2. If lavaging is done, make sure to use sterile, preservative-free saline.

D. Bronchial Washing/Bronchial Brushings/Bronchoalveolar Lavage/Lung Aspirate/Biopsy:

*Bronchial Washing or Bronchoalveolar Lavage:* Bronchial Washing and bronchoalveolar lavage specimen are usually obtained before brushing or biopsies to avoid excess blood in the recovered fluid, as blood may alter the concentration of cellular and non-cellular components.

1. Inject sterile non-bacteriostatic 0.85% NaCl (generally 5 – 20 ml aliquots) from a syringe through a biopsy channel of the bronchoscope.
2. Gently suction the 0.85% saline into a sterile container before administering the next aliquot. (In general, 50 – 75% of the saline instilled is recovered in the lavage effluent.) Keep the aliquots separate during collection. Combine aliquots from the same site for microbiology cultures and smears, but aliquots from separate sites (ex. right upper and right lower lobe) should be combined only after consultation with the physician of record.
3. Transport all lavages directly to the Microbiology Laboratory.

*Bronchial Brush Specimen:*

Insert a telescoping double catheter plugged with polyethylene glycol at the distal end (to prevent contamination of the bronchial brush through the biopsy channel of the bronchoscope.

1. Transbronchial biopsies:
  - a. Obtain the biopsy sample through the biopsy channel of the bronchoscope.

- b. Transport it in a sterile container with a small amount of preservative-free saline..
  2. Lung Aspirations:
    - a. Use a computed tomography scan to obtain lung aspirates by inserting a needle through the chest wall into a pulmonary infiltrate.
    - b. Aspirate material from the lesion.
    - c. If the lesion is large or if there are multiple lesions, collect multiple specimen from representative sites.
  3. Lung Biopsy:
    - a. Obtain a 1 – 3 cm square piece of tissue if possible. If the lesion is large, or there are multiple lesions, collect multiple specimen from representative sites.
    - b. Submit in sterile containers without formalin.
    - c. Transport all material to the Laboratory **IMMEDIATELY**.
- E. Throat:
  1. Do not obtain throat samples if epiglottitis is inflamed, as sampling may cause serious respiratory obstruction.
  2. Depress tongue gently with tongue depressor.
  3. Extend sterile swab between the tonsillar pillars and behind the uvula. (Avoid touching the cheeks, tongue, uvula, or lips.)
  4. Sweep back and forth across the posterior pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain sample.
- F. Nasal Swabs:
  1. Moisten a sterile swab with preservative-free saline.
  2. Insert the swab into one nare until resistance is met at the level of the turbinates (approximately 1 inch into the nose).
  3. Rotate the swab against the nasal mucosa.
  4. Repeat the process in the other nare.
- G. Nasopharyngeal Suction
  1. Suction material from the nasopharynx.
  2. Place in a sterile leakproof container.

## H. Nasopharyngeal Swabs:

1. Carefully insert a flexible-wire calcium alginate-tipped swab through the nose into the posterior nasopharynx, and rotate the swab.
2. Keep the swab near the septum and floor of the nose.

## I. Nasal Washings (submitted primarily for viral culture):

1. Instruct the patient not to swallow during the procedure.
2. With the patient's head hyperextended, instill approximately 5 ml of sterile preservative-free saline into each nostril.
3. To collect the material, tilt head forward and allow fluid to run out of the nares into a sterile container, or aspirate the fluid by inserting a rubber syringe into each nostril.
4. Place in viral transport media, or transport in a sterile, leakproof container.

## J. Tympanocentesis Fluid:

1. Clean the external canal with mild detergent.
2. Using a syringe aspiration technique, the physician will obtain fluid from the ear drum.
3. Place contents of the syringe into a sterile container.
4. If eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum.

**CULTURE CONSIDERATIONS FOR RESPIRATORY SPECIMEN**

CULTURE	VOLUME (mL)	COMMENTS
Bacteria	N/A	Contact Microbiology if Legionella is suspected. Submit sputum only. Saliva is unacceptable.
Fungi	3-5 mL	Collect three early morning fresh specimen resulting from deep cough or sputum induction. Lung biopsy specimen or lung aspirates are also appropriate.
Anaerobes	1 mL	Sinus aspirate, tympanocentesis fluid, transtracheal aspirate, lung aspirates, and biopsy samples are appropriate.
Mycobacteria	5-10 mL	Collect three fresh specimen (one every day) resulting from deep cough or sputum induction. Lung biopsy specimen or lung aspirates are also appropriate.
Pneumocystis	2 mL	Use induced sputum, bronchoalveolar lavage fluid or lung biopsy specimen.
Parasites	3-5 mL	Can be examined for amoebae, helminth eggs, hooklets of <i>Echinococcus</i> spp., larvae of hookworm and <i>Ascaris</i> and <i>Strongyloides</i> spp.

POLICY / PROCEDURE		
Subject: <b>Nasal Washing, How to Perform</b>		24 of 2
Author: <b>INFECTION CONTROL</b>		

**PURPOSE:** To provide instructions for obtaining a nasal washing specimen for laboratory analysis. A correct specimen will be obtained by washing the posterior pharyngeal area, not the outer nasal area. An incorrectly collected specimen may yield inaccurate results.

**POLICY STATEMENTS:**

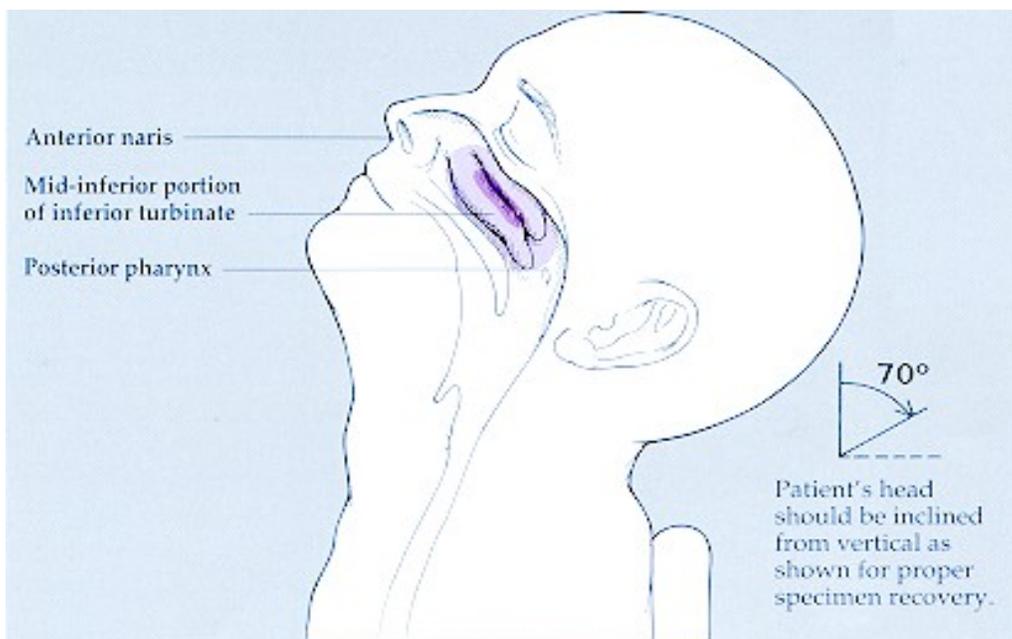
1. Nasal washings provide an optimal specimen for the performance of some laboratory testing.
2. When performed, it is important to use the correct equipment to promote both the safety of the patient as well as ensure an optimal specimen.
3. Washing should be done with a preservative-free, non-bacteriostatic saline solution. This can be obtained through the use of the prefilled saline syringes available in the patient care areas or the saline squirts available through the cardiopulmonary services department.

**NASAL WASH USING SUCTION**

Materials needed:

Sterile, non-bacteriostatic saline (0.85% NaCl). 1-Bulb syringe (1-2 oz. Tapered tip as in the bulb syringes used in the nurseries)

2 Sterile specimen containers. One container is to be used to transport the collected specimen and the other to hold the saline for nasal instillation



POLICY / PROCEDURE		
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1. Wash hands and explain procedure to patient.
2. Instruct the patient that we need him/her to avoid swallowing the saline and the procedure will be performed as quickly as possible
3. Gently squirt 5 cc sterile, preservative-free saline into sterile specimen cup
4. Using sterile bulb syringe, aspirate the saline into the bulb syringe and invert so the saline remains inside.
5. Tilt the patient's head back and instruct them not to swallow.
6. Gently insert the entire tip of the bulb syringe into one of the patient's nostrils (until the nostril is occluded).
7. Gently instill the saline into the nostril and immediately release pressure on the bulb so recovery of the saline occurs immediately. If recovery of the saline does not occur immediately, the instilled saline will rapidly drain down the throat.
8. Empty recovered saline into the sterile specimen container.
9. Repeat the procedure using the other nostril.
10. Combine both specimen into a single container and prepare for transport to the lab. Ensure that the specimen is labeled as a nasal wash along with the other information required for laboratory analysis.

## STERILE BODY FLUIDS (EXCLUDING CSF, URINE AND BLOOD)

- A. Clean the needle puncture site with alcohol, and follow with a skin disinfectant such as chlorhexidine-alcohol or povidone-iodine to prevent introduction of microorganisms. (If tincture of iodine is used, remove with 70% ethanol after the procedure to avoid burn.)
- B. The physician will aseptically perform percutaneous aspiration to obtain pleural, pericardial, peritoneal, or synovial fluids.
- C. Expel any air bubbles from the syringe and immediately inject the specimen into an anaerobic transport system. Make sure to use protective equipment in expectation of splash or spray.
- D. Transport any additional fluid or pus in a sterile, leakproof, screw cap container.
- E. When submitting CSF to the laboratory, tubes should be labeled carefully so analysis is performed on each tube as indicated by the clinician order (e.g., cell count performed on tube #1, glucose on tube #2)

### COLLECTION CONSIDERATIONS FOR STERILE BODY FLUIDS

CULTURE	VOLUME (mL)	COMMENTS
Bacteria	1-5 mL	If gonococcal arthritis is suspected, notify Microbiology to include a Martin Lewis plate
Fungi	>10 mL	<i>Histoplasma capsulatum</i> (AIDS), <i>Cryptococcus</i> spp, <i>Candida albicans</i> , and <i>Candida tropicalis</i> can also be isolated from blood.
Anaerobes	1-5 mL	Use anaerobic transport system
Mycobacteria	>10 mL	<i>Mycobacterium haemophilum</i> may be isolated from synovial fluid.

## SUBCUTANEOUS AND SKIN SPECIMEN:

- A. Burn Specimen:
  - 1. Disinfect the surface of the burn with 70% alcohol followed by povidone-iodine or povidone-iodine alone. Allow the skin disinfectant to dry prior to collection of the specimen. Remove the iodine with alcohol after the procedure to prevent additional irritation to the tissue.
  - 2. Blood cultures should be used to monitor patient status concerning sepsis.
- B. Superficial Wound (bacterial)
  - 1. Syringe **aspiration** is preferable to swab collection.
  - 2. Disinfect the surface of the wound with 70% alcohol followed by povidone-iodine or povidone-iodine alone. Allow the skin disinfectant to dry prior to collection the specimen. Remove iodine with alcohol after procedure to prevent irritation.
  - 3. Using a 3 – 5 ml syringe with a small gauge needle, 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.
  - 4. If the initial aspiration fails to obtain material, inject sterile, preservative-free saline subcutaneously then repeat aspiration if necessary.
  - 5. If no material is obtained, rinse needle and syringe with broth by drawing the culture medium through the needle into the syringe.
- C. Superficial Lesions (fungal)
  - 1. Clean the surface with sterile water.
  - 2. Using a scalpel blade, scrape the periphery of the lesion border. Samples of scalp lesions should include hair that is selectively collected for examination. If there is nail involvement, obtain scrapings of debris or material beneath the nail plate.
  - 3. Transport in a sterile container with a screw cap.
- D. Ulcers and nodules:
  - 1. Clean the area with 70% alcohol, followed by povidone-iodine or povidone-iodine alone. Remove iodine with alcohol after the procedure to prevent irritation.
  - 2. Remove overlying debris.
  - 3. Curette the base of the ulcer or nodule.
  - 4. If exudate is present from ulcer or nodule, collect it with a syringe or sterile swab.

## COLLECTION CONSIDERATIONS FOR SUBCUTANEOUS TISSUE AND SKIN SPECIMEN

CULTURE	COMMENTS
Bacteria	Syringe aspirates or biopsy specimen are preferable to swab specimen.
Anaerobes	Uncommon in burn, ulcer nodules, or superficial skin infections; useful in bites and trauma.
Fungi	Useful in diagnosing dermatophytes, yeast, filamentous fungi and dimorphic fungi.
Mycobacteria	Useful in diagnosing <i>Mycobacterium marinum</i> , <i>Mycobacterium fortuitum</i> , and <i>Mycobacterium chelonae</i> .
Virus	Useful in diagnosing HSV and <i>Varicella zoster</i> virus.

**URINE:**

A. General Considerations:

1. Never collect urine from a bedpan or urinal.
  2. Thoroughly clean the urethral opening (and vaginal vestibule in females) prior to collection procedure to ensure the specimen obtained is not contaminated with colonizing microorganisms.
  3. Plain soap rather than a disinfectant is recommended for cleaning the urethral area. If disinfectants are introduced into the urine during collection, they may be inhibitory to the growth of the microorganisms.
  4. Transport the specimen to the Laboratory within 90 minutes of collection. If it cannot be transported within 90 minutes of collection, the urine should be refrigerated. (Bacterial counts remain stable for at least 24 hours at 4°C.) Do not freeze.
  5. Use sterile, leakproof cups or tubes to transport urine.
  6. Transport suprapubic bladder aspirate specimen for anaerobic culture in an anaerobic transport system.
  7. Always transport urine for viral cultures on wet ice in a sterile, leakproof container.
  8. Any urine collection procedure involving catheterization must be done with scrupulous aseptic technique to avoid introducing microorganisms.
  9. Send the first morning voided urine. Three consecutive first morning specimen are recommended for mycobacterial culture.
  10. Do not submit 24 hour urine collections for culture.
- B. Collection Techniques:
1. Clean catch urine specimen (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 prior to specimen collection.
    - 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 wipes while holding labia apart to avoid recontamination.
    - d. Continue to hold labia apart during voiding.
    - e. Allow a few milliliters of urine to pass. Do not collect this initial amount of urine and do not stop the flow of urine.
    - f. Collect the midstream portion of urine in a sterile, leakproof container.
  2. Clean catch urine (male):

- a. The person obtaining the urine should wash hands with soap and water, rinse and dry. If the patient is collecting the specimen, he should be given detailed instructions prior to specimen collection.
  - b. Cleanse the penis, retract the foreskin (if not circumcised), and wash with soapy water.
  - c. Rinse the area well with water.
  - d. Keeping the foreskin retracted (to minimize contamination with skin flora), allow a few milliliters of urine to pass. Do not collect this initial amount of urine and do not stop the flow of urine.
  - e. Collect the midstream portion of urine in a sterile container.
3. Ileal conduit urine:
- a. Specimen obtained via this method are not recommended as results are difficult to interpret due to inherent contamination with fecal organisms.
  - b. If this method is selected, remove the external urinary appliance and discard urine within the appliance.
  - c. Gently swab and clean the stomal opening with a 70% alcohol pad and then with a povidone-iodine solution. Remove excess iodine with 70% alcohol to avoid irritation.
  - d. Using sterile technique, insert a urinary catheter into the stoma.
  - e. Catheterize the ileal conduit to a depth beyond the fascial level.
  - f. Collect the urine drained into a sterile, leakproof container.
4. Straight catheter urine (in/out catheter urine specimen):

A specimen from this method is useful when a clean catch specimen cannot be obtained or when results from clean catch specimen are equivocal and a diagnosis is critical.

- a. Prior to catheterization if possible, the patient should force fluids until the bladder is full. (Forcing fluids may reduce organism number.)
  - b. Clean the patient's urethral opening (and in females, the vaginal vestibule) with soap and carefully rinse the area with water.
  - c. Repeat the process using a skin disinfectant such as povidone-iodine taking care to hold the labia apart during the process.
  - d. Using sterile technique, pass a catheter into the bladder.
  - e. Discard the initial 15 – 30 ml of urine then collect the sample from the mid or later flow of urine into a sterile, leakproof container.
5. Indwelling catheter, urine:

- a. Clean the catheter collection port with a 70% alcohol wipe.
  - b. Using sterile technique, puncture the collection port using the type of safety device appropriate for that type of urinary drainage system. Do not collect urine from the collection bag.
  - c. Aspirate the urine and place in a sterile, leakproof container.
6. Suprapubic aspirate (SPA) of the urinary bladder:

A specimen collected in this manner is useful in determining urinary infection in adults in whom infection is suspected and for whom results from routine procedures have been equivocal and diagnosis is critical.

- a. Before SPA, if possible the patient should force fluids until the bladder is full. (Forcing fluids may reduce the organism number.)
- b. The physician will make a small lance wound through the epidermis, just above the symphysis pubis.
- c. Aspirate urine from the bladder by using a needle aspiration technique.

#### COLLECTION CONSIDERATIONS FOR URINE SPECIMEN

CULTURE	VOLUME (mL)	COMMENTS
Bacteria	0.5 – 1 mL	Do not use urine from a 24 hour specimen for culture. After proper cleansing of patient, use first morning midstream void.
Fungi	>20 mL	Do not use urine from a 24 hour specimen for culture.. First morning void is recommended.
Mycobacteria	>20 mL	Do not use urine from a 24 hour specimen for culture. Three consecutive first morning voids are recommended.
<i>Chlamydia trachomatis</i>	>20 mL	Do not use urine from a 24 hour specimen for culture. First morning void is recommended. Send on ice, and transport to the Microbiology Laboratory immediately.
<i>Neisseria gonorrhoeae</i>	>20 mL	Do not use urine from a 24 hour specimen for culture. First morning void is recommended. Send on ice, and transport to the Microbiology Laboratory immediately. <b>Male ONLY</b>
Anaerobes	1 mL	Use suprapubic aspirate. Use anaerobic transport system.
Virus	10 – 50 mL	Do not use urine from a 24 hour specimen for culture. First morning void is recommended. Useful for adenovirus, mumps and CMV detection. Send on ice, and transport to the Microbiology Laboratory immediately.

Parasites	24 hour collection	Used for detecting <i>Shistosoma haematobium</i> eggs, <i>Trichomonas vaginalis</i> trophozoites in males, and <i>Onchocerca volvulus</i> microfilariae.
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**WOUNDS, ASPIRATES, AND TISSUE SPECIMEN:**

A. Bite Wounds:

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

B. Bone:

1. Obtain bone sample at surgery.
2. Submit in sterile container without formalin. Specimen may be kept moist by placing sterile gauze pads soaked with with sterile preservative-free saline in the bottom of the specimen container.

C. Deep Wounds or Abscesses:

1. Disinfect the surface with 70% alcohol and a povidone-iodine solution. Remove iodine with alcohol after procedure to prevent irritation.
2. Aspirate the deepest part of the lesion, avoiding contamination by the wound surface. If collection is done at surgery, a portion of the abscess wall should also be sent for culture.

D. Punch Biopsy:

1. Disinfect the skin surface with 70% alcohol and then with a povidone-iodine solution. Remove iodine with alcohol to prevent irritation.
2. Collect 3 – 4 mm sample with a dermal punch.
3. Submit for culture in a sterile container without formalin.
4. Place specimen onto a sterile gauze pad moistened with preservative-free saline in order to keep the specimen from drying.

E. Soft Tissue Aspirate

1. Disinfect the skin surface with 70% alcohol and then with a povidone-iodine solution. Remove tincture of iodine with alcohol to prevent irritation.
2. Aspirate the deepest portion of the lesion or sinus tract. Be careful to avoid contamination by the wound surface.

**COLLECTION CONSIDERATIONS FOR DEEP WOUND, ASPIRATE AND TISSUE SPECIMEN**

CULTURE	COMMENTS
Bacteria	Biopsy specimen or aspirates are better than swab specimen

Anaerobes	Useful in diagnosing actinomycosis; place specimen in anaerobic transport system.
Fungi	Useful in diagnosing <i>Pseudoallescheria boydii</i> , <i>Bipolaris spp.</i> , <i>Exophiala spp.</i> and <i>Fusarium spp.</i> and other fungi.
Mycobacteria	Useful in diagnosing <i>Mycobacterium tuberculosis</i> , <i>Mycobacterium bovis</i> , and <i>Mycobacterium kansasii</i> , <i>Mycobacterium chelonae</i> , <i>Mycobacterium haemophilum</i> , and <i>Mycobacterium marinum</i> .