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	Procedure: Microbiology Specimen Collection from Different Anatomical Sites	

Specimen Collection Manual Collection Instructions for Different Anatomic Sites

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I. BLOOD

A. Numbering and timing

Most cases of bacteremia are detected by using three separately collected blood cultures. More than three cultures yield little additional information. Conversely, a single blood culture may miss intermittently occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms.

1. Acute sepsis

Collect two or three cultures from separately prepared sites prior to starting therapy.

2. Endocarditis

a. Acute

Obtain three blood cultures with three separate venipunctures over 1 to 2 hr. and begin therapy.

b. Subacute

Obtain three blood cultures on day 1 (15 minutes or more apart). If all are negative 24 hours later, obtain 3 more.

3. Fever of unknown origin

Obtain two separate blood cultures at least 1 hour apart. If these are negative, then 24 to 36 hrs. later obtain two more blood cultures 1 hr. apart. The recovery of microorganisms beyond four blood cultures is usually minimal.

B. 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 blood is required for culture.

1. Neonates 0.5-2 mL
2. Children: 1 to 5 mL
3. Young adults: 5-8 mL

A. Collection of Blood Cultures

Disinfect the venipuncture site **and** stoppers of culture bottles or collection tubes prior to blood collection using the technique below for both:

1. Clean the site with 70% isopropyl alcohol.
2. Swab concentrically, starting at the center with Chloroprep or 1-2% tincture of iodine. If the patient is hypersensitive to iodine, prepare the skin by using a double application of 70% alcohol.)
3. Allow the disinfectant to dry or at least one minute. (Do not palpate the vein after disinfecting skin prior to inserting needle.)
4. Draw blood through a syringe and needle and transfer to collection tube or bottle.

5. Wipe any residual iodine from the skin with alcohol to prevent irritation of skin. Dispose of collection system in accordance with standard precautions.

II. CNS-CENTRAL NERVOUS SYSTEM SPECIMENS

A. CSF

Suggested volumes are 1-3 cc for routine, fungal and acid fast, viral and PCR tests.

1. Lumbar puncture

Routine in sterile tube

2. Brain abscess

Ninety percent of brain abscesses will grow anaerobic bacteria. Fluid collected in a syringe is acceptable if the needle is removed and brought to the lab immediately. Screw capped sterile containers are also acceptable. Culturette and anaerobic culturettes are acceptable if aspirates cannot be obtained.

III. GASTROINTESTINAL TRACT

A. Fecal specimen

Fecal specimens are submitted primarily for the detection of bacteria such as: Salmonella, Shigella, Yersinia, Campylobacter, Aeromonas, Plesiomonas, and Vibrio. Also viral isolation.

1. General considerations

Keep stools at room temperature. If more than one hour transport time, collect pathogenic material such as blood and mucous on a swab and submit culturette. Separate culturettes for each type of culture provide better recovery rates for bacteria.

Do not submit diapers.

Plastic wrap between diaper and infant may be used to collect stool and then transferred to a container.

Rectal swabs are collected by passing the tip of a sterile swab approximately 1 inch beyond the anal sphincters.

2. **Rectal Chlamydia culture.** Use a rectal swab and submit in Chlamydia transport media. If delays are expected refrigerate or transport on ice.

3. Clostridium difficile PCR

C. difficile should be considered in children over 6 months with clinically significant diarrhea and a history of antibiotic exposure. Collect 1-3 cc. or grams in a clean container. Refrigerate if transport is delayed.

4. Giardia and Cryptosporidium EIA

Collect in culturette or clean container. Any visible amount of stool is adequate.

5. Stool for WBCs

Collect in culturette or clean container. Any visible amount of stool is adequate

6. Stool Culture Routine

Includes Salmonella, Shigella, E. coli shiga-toxin assay, Aeromonas, Plesiomonas

Recommend no more than 2 specimens/patient. Not recommended for patients in the hospital when diarrhea begins after the third day of hospitalization.

Collect in culturette. Any visible amount of stool is adequate.

7. Rectal swabs for Group A Streptococcus, Neisseria gonorrhoeae, Shigella

Pass the tip of a sterile swab approximately 1 inch beyond the anal sphincter. Carefully rotate the swab to sample the anal crypts, and withdraw the swab. Submit in a culturette.

8. Viral culture

Use a rectal swab and submit in Viral Transport. If delays are anticipated, submit on ice or refrigerate.

9 Gastrointestinal Disease Panel by PCR. Only loose stools should be tested. Submit 1 mL in a clean container or use Carey Blair transport with stool filled to the specimen fill line on the container.

B. Gastric aspirates

The patient should fast prior to each of the following procedures.

1. Gastric lavage

Submitted primarily for the detection of Mycobacterium tuberculosis in patients 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.

Pass a well-lubricated tube orally or nasally into the stomach of the patient, and perform the lavage. Before removing the tube, release the suction and clamp to prevent mucosal trauma and/or aspiration.

2. Duodenal aspiration

Submitted primarily for the detection of Giardia species and the larvae of Strongyloides stercoralis and Ascaris lumbricoides.

Pass a tube orally into the duodenum of the patient.

To aspirate a sample for giardiasis, the tube should be at least in the third portion of the duodenum.

IV. GENITAL TRACT SPECIMENS

A. Female

Genital tract specimens are submitted primarily for the detection of sexually transmitted pathogens (such as N. gonorrhoeae, Chlamydia trachomatis, lymphogranuloma venereum, trichomonads, Haemophilus ducreyi, Group B streptococci, and Candida infections). If infection is not caused by any of these pathogens, anaerobic bacteria may be involved. If an anaerobic infection is suspected, transport the specimen in an anaerobic swab. For Chlamydia use Chlamydia Transport. Trichomonas EIA can be submitted in a culturette.

For any of the bacteria, use a culturette.

1. Bartholin gland

Decontaminate the skin with providone-iodine, and aspirate material from the ducts(s).

2. Cervix

- a. Do not use lubricant during procedure.
Wipe the cervix clean of vaginal secretion and mucus.
- b. Rotate a sterile swab, and obtain exudate from the endocervical glands.
- c. If no exudate is seen, insert a sterile swab into the endocervical canal, and rotate the swab.

3. Endometrium

Collect endometrium specimens by transcervical aspiration through a telescoping catheter.

4. Fallopian tubes

Obtain aspirates, (preferably) or swab specimens during surgery. Bronchoscopy cytology brushes may be used if exudate is not expressed.

5. Rectal swabs for sexually transmitted diseases:

Used primarily to detect *N. gonorrhoeae*, *Shigella* species, HSV and anal carriage of *S. pyogenes*. Pass the tip of a sterile swab approximately 1 in. beyond the anal sphincter. Carefully rotate the swab to sample the anal crypts, and withdraw it. Send the swab in appropriate transport (see Specimen Transport Guide).

6. Urethra

- a. Collect specimens 1 hr. or more after patient has urinated.
- b. Stimulated discharge by gently massaging the urethra against the pubic symphysis through the vagina.
- c. Collect the discharge with a sterile swab.
- d. If discharge cannot be obtained, wash external urethra with betadine soap and rinse with water. Insert an urethrogonital swab 2-4 cm into the endourethra, gently rotate the swab, and leave it in place for 1-2 sec. Withdraw the swab, and submit it in the appropriate transport system for culture (See Specimen Transport Guide).

7. Vagina

Specimens are also useful in the detection of group A streptococci in children.

Use a speculum without lubricant. Collect secretions from the mucosa high in the vaginal canal with sterile swab.

8. Vulva

- a. Clean the surface of the lesion with 0.85% non-bacteriostatic saline. If there is a crust on the lesion, remove it.
- b. Wipe away fluid and debris with sterile gauze. (Try to avoid bleeding.)
- c. Press the base of lesion until clear fluid is expressed.
- d. Aspirate vesicular fluid with a 26- to 27- g needle **OR** unroof the vesicle, and collect fluid with a sterile swab, **OR** scrape the base of an open vesicle with a sterile scalpel blade, and then rub the base vigorously with a sterile swab.

B. Male

1. Anal swab

Submitted primarily for the detection of *N. gonorrhoeae*, *Shigella* species, HSV, and anal carriage of Group A *Streptococcus*. Pass the tip of a sterile swab approximately 1 in. beyond the anal sphincter. Carefully rotate the swab to sample the anal crypts, and withdraw it. Send the swab in the appropriate transport (see Specimen Transport Guide).

2. Epididymis

Used primarily to diagnose nonspecific bacterial epididymitis and sexually transmitted epididymitis. Bacterial epididymitis is most commonly due to members of the family Enterobacteriaceae or pseudomonads. *M. tuberculosis* infections generally occur after involvement of the prostate or seminal vesicles. Sexually transmitted epididymitis is most commonly due to *C. trachomatis* and *N. gonorrhoeae*.

Use a needle and syringe to aspirate material from the epididymis.

3. Penile lesion

Used primarily to detect sexually transmitted pathogens such as *N. gonorrhoeae*, *C. trachomatis*, lymphogranuloma venereum, HSV, *T. pallidum*, and *H. ducreyi*.

- a. Clean the surface of the lesion with 0.85% non-bacteriostatic 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 lesion until clear fluid is expressed.
- e. Aspirate vesicular fluid with a 26 to 27- gauge needle. **OR**
- f. Unroof the vesicle, and collect fluid with a sterile swab (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.
- h. Refer to Specimen Transport Guide for transport system.

4. Prostatic massage

Used primarily to diagnose acute or chronic prostatitis. For both diseases, gram-negative enteric organisms are the most frequently isolated pathogens. *N. gonorrhoeae* is found infrequently but is sometimes implicated in acute prostatitis.

- a. Perform a digital message through the rectum.
- b. Collect the specimen in a sterile tube or on a sterile swab.

5. Urethra

Used primarily to detect *N. gonorrhoeae* and *C. trachomatis*

- a. Collect specimens at least 2 h after the patient has urinated.
- b. Insert a thin urethrogenital swab 2-4 cm into the endourethra, gently rotate it, leave it in place for 1-2 sec, and withdraw it.
- c. Refer to Specimen Transport Guide for transport system.

V. OCULAR SPECIMENS

A. General considerations

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1. Obtain viral and chlamydial samples before topical anesthetics are instilled.
2. Obtain samples for chlamydial culture and viral culture using Dacron or Cotton swabs with non-wood shafts.

B. Conjunctival scrapings

1. One or 2 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 cotton-tipped applicator to swab the inferior tarsal conjunctiva (inside the eyelid). However, organisms are more readily detected in scrapings than from a swab.

C. Corneal scrapings

1. Obtain conjunctiva samples prior to corneal scrapings. Sometime a conjunctiva culture is helpful in assessing the possibility of contamination of corneal cultures.
2. One or 2 drops of topical anesthetic are generally instilled.
3. Using short, firm strokes in one direction, scrape multiple areas of ulceration and suppuration with a sterile kimura spatula. (Keep the eyelid open, and be careful not to touch the eyelashes.)
4. Prepare smears by applying the scrapings in a gentle circular motion over a clean glass slide or by compressing material between two clean glass slides and pulling the slides apart.

D. Intraocular fluid

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

VI. RESPIRATORY SPECIMENS

A. General considerations

1. Twenty four hour sputum collections are not recommended for culture.
2. If *Corynebacterium diphtheriae*, *Arcanobacterium haemolyticum*, *N. gonorrhoeae*, *legionellae*, *mycoplasma* or *ureaplasma* are suspected, please contact the microbiology lab for special handling.

B. Lower respiratory tract

1. Expecterated sputum

- a. If possible, have the patient rinse mouth and gargle with water prior to sputum collection.
- b. Instruct the patient not to expectorate saliva or postnasal discharge into the container.
- c. Collect specimen resulting from deep cough in a sterile screw-cap cup or other suitable sterile collection assembly.

- d. Sputum for Acid Fast should be collected X3 early morning specimens. The lab recommends gastric aspirates for young children. Refrigerate if transport is delayed longer than 30 minutes.

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 20-30 ml of 3-10% of 0.85% non-bacteriostatic saline. Collect the induced sputum in a sterile screw-cap cup.

3. 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. Aspirate the specimen into a sterile sputum trap.

4. Bronchoscopy specimens

Bronchoscopy specimens include bronchoalveolar lavage, bronchial washing, bronchial brushing, or transbronchial biopsy specimens

- a. Pass the bronchoscope transnasally or transorally in nonintubated patients or via the endotracheal tube for intubated patients.
- b. Wedge the tip of the bronchoscope in a segmental (for bronchial wash) or subsegmental (for bronchoalveolar lavage) bronchus.
- c. To obtain specimens

(1) Bronchial wash and bronchoalveolar lavage-

generally obtained before brushing or biopsy specimens to avoid excess blood in the recovered fluid, because blood may alter the concentration of cellular and noncellular components

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

(2) Bronchial brush specimens

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.

(3) Transbronchial biopsies

Obtain the biopsy sample through the biopsy channel of the bronchoscope, and transport it in a sterile container with a small amount of non-bacteriostatic sterile 0.85 saline.

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(4) Lung aspirations

Obtain lung aspirates by inserting a needle through the chest wall into a pulmonary infiltrate. Aspirate material from the lesion. If the lesion is large or if there are multiple lesions, collect multiple specimens from representative sites.

C. Upper respiratory tract specimens

1. Throat (pharyngeal specimens)

Submitted primarily for the detection of group A streptococci. Can also be used to detect *N. gonorrhoeae*, *H. influenzae*, (for epiglottitis).

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 the swab back and forth across the posterior pharynx, tonsillar area, and any inflamed or ulcerated areas to obtain sample.

2. Nasal/Nasopharyngeal swabs

Submitted primarily for the detection of group A streptococcus, and *B. pertussis*. Notify laboratory if submitted for detection of carriers of *Staphylococcus*.

- a. Insert a sterile swab into the nose until resistance is met at the level of the turbinates (approximately ½-1 inch)
- b. Rotate the swab against the nasal mucosa.
- c. Repeat the process on the other side.

3. Nasal washings

Primarily submitted for Viral PCR and EIA. Obtain nasal wash kit from Microbiology lab.

4. Sinus aspirates

Use a syringe aspiration technique, a specially trained physician or an otolaryngologist will obtain material from maxillary, frontal, or other sinuses.

Specimens may be submitted in closed syringe.

5. Typanocentesis fluid

Submitted primarily to diagnose middle ear infections if previous therapy has failed.

- a. Clean the external canal with mild detergent.
- b. Using a syringe aspiration technique, the physician will obtain the fluid from the ear drum. Send the specimen in a trap, sterile container, or send it in a closed syringe.
- c. If the eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum.

6. Oral cultures

Used primarily for the detection of yeast or fusospirochetal disease.

- a. Rinse mouth with sterile saline.
- b. Wipe the lesion with dry sterile gauze.

- c. Swab or scrape areas of exudation of ulceration.
- d. Submit in culturette.

VII. STERILE BODY FLUIDS (EXCLUDING CSF, URINE, BLOOD)

- A. Clean the needle puncture site with alcohol, and disinfect it with an iodine solution 1-2% tincture of iodine, or a 10% solution of povidone-iodine to prevent introduction of infection.
- B. The physician will aseptically perform percutaneous aspiration to obtain plural, pericardial, peritoneal, or synovial fluids.
- C. Expel any air bubbles from the syringe, and immediately inject the specimen into an anaerobic transport system or send the specimen in the needleless syringe with cover.

VIII. SUBCUTANEOUS TISSUE AND SKIN SPECIMENS

A. Burn specimens

The surfaces of burn wounds will become colonized by the patient's macrobiotic or by environmental organisms. When the organism load is large, infection of underlying tissue may occur, and bacteremia may ensue. 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. Disinfect the surface of the burn with 70% alcohol and then with an iodine solution (tincture or povidone). Allow iodine to dry before collecting. Collect a biopsy specimen.

B. Wounds, Abscesses, Aspirates, Tissue specimens

1. Syringe aspiration is preferable to swab collection.
2. Disinfect the surface of the wound with 70% alcohol and then with an iodine solution (tincture or povidone). Allow the disinfectant to dry prior to collecting the specimen.
3. Use a 3-5 ml syringe with a 22-23 gauge needle. The 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, nonbacteriostatic 0.85% saline subcutaneously.
5. If no material is obtained, rinse needle and syringe with non-bacteriostatic saline and submit.

C. Superficial lesions for fungus

1. Clean the area with 70% alcohol and then with an iodine solution (tincture or povidone).
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.
- 5.

IX. 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 procedures to ensure that the specimen obtained is not contaminated with colonizing microorganisms in this area.
3. Soap, rather than disinfectants is recommended for cleaning the urethral area.
4. Transport specimen to laboratory within 1 h of collection , refrigerate or use boric acid transport tube
5. Use sterile cups or tubes.

B. Clean catch urine

FEMALES: Separate labial folds of urinary opening with fingers and clean inside with towelettes or soap and water. Using downward strokes only; hold folds separated during urination.

MALES: Clean head of penis using towelettes or soap and water.

1. Remove container. DO NOT TOUCH INSIDE OF CONTAINER.
2. Begin urination into toilet. As urination continues, bring container into stream. FILL CONTAINER ONLY HALF FULL.
3. Remove cap from package with thumb and forefinger. DO NOT TOUCH INSIDE OF CAP. Screw cap on container and affix label.

C. Straight catheter urine (in/out)

In/out catheter urine specimens are useful when clean-catch urines cannot be obtained or when results from clean-catch urine specimens are equivocal and a diagnosis is critical.

1. Prior to catheterization, the patient should force fluids until the bladder is full
2. Clean the patient's urethral opening (and in females, the vaginal vestibule) with soap, and carefully rinse the area with water.
3. Using sterile techniques pass a catheter into the bladder.
4. Collect a sample from the mid-or later flow of urine in a sterile container.

D. Indwelling catheter urine

Indwelling catheters are placed in patients who are unable to pass urine.

1. Clean the catheter collection port with a 70% alcohol wipe.
2. Using sterile technique, puncture the collection port with a needle attached to a syringe.
3. Aspirate the urine, and place it in a sterile container.

NOTE: DO NOT COLLECT URINE FROM COLLECTION BAG.

E. Suprapubic aspiration (SPA)

SPA 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. SPA is useful in pediatric patients when clean-catch urine specimens are difficult to obtain.

1. Before SPA, the patient should force fluids until the bladder is full. (Forcing fluids may reduce the organism number.)
2. Shave and disinfect the suprapubic skin overlying the urinary bladder.
3. The physician will make a small lance wound through the epidermis just above the symphysis pubis.

4. Aspirate urine from the bladder by using a needle aspiration technique.