

WCP LABORATORIES, INC.

MICROBIOLOGY SPECIMEN COLLECTION AND HANDLING PROTOCOL

INTRODUCTION

WCP Laboratories, Inc. is committed to providing the highest quality laboratory services in the industry. In doing so a well defined and easy to follow specimen collection protocol is vital. Procedures related to specimen procurement, transport, and accessioning are vital in obtaining accurate testing results.

Guidelines for submitting specimens to the microbiology laboratory were developed to encompass all activities, from collection of the specimen to its acceptance by the laboratory. WCP Laboratories, Inc. only accepts specimens referred from licensed physicians or licensed organizations.

PREPARATION

MICROBIOLOGY SPECIMENS SHOULD BE SENT TO THE LABORATORY AS SOON AS POSSIBLE AFTER THEY ARE OBTAINED. MICROBIOLOGY SPECIMENS ARE PARTICULARLY SUSCEPTIBLE TO COMPROMISE AND CONTAMINATION.

SPECIMEN TYPE	SPECIMEN COLLECTION	TRANSPORT MEDIUM
Body Fluids Cultures (Joint, Pleural, Peritoneal, Pericardial, Amniotic, Cul-de-sac)	See Following Section – Page 4	Sterile leakproof container – Collection Syringe with a sterile cap (remove needle). Store RT.
Catheter Tip Cultures	See Following Section – Page 5	Sterile Plastic Container – Urine Collection Container. Store 4 C.
Cerebrospinal Fluid Cultures	See Following Section – Page 6	Sterile leakproof container. Store RT.
Genital Cultures	See Following Section – Page 7	ESwab Collection Kit. Store RT/Refrigerated.
Group B Streptococcus Cultures	See Following Section – Page 8	ESwab Collection Kit. Store RT/Refrigerated.
Ocular Cultures	See Following Section – Page 9	ESwab Collection Kit /Flexible mini-tip. Store RT/Refrigerated.
Otitis Cultures	See Following Section – Page 10	ESwab Collection Kit /Flexible mini-tip. Store RT/Refrigerated.
Respiratory Tract Cultures	See Following Section – Page 11	Refer to collection procedure.
Nasal Sinus Cultures	See Following Section – Page 12	Refer to collection procedure.
Urine Cultures	See Following Section – Page 13	Sterile leakproof container and transport tube with preservative. Store 4 C.
Wound, Cysts, Boils and Soft Tissue Cultures	See Following Section – Page 14	Refer to collection procedure.
Anaerobic Cultures	See Following Section – Page 15	ESwab Collection Kit. Store RT/Refrigerated.
Fecal Collection for C. diff Toxin	See Following Section – Page 16	Cary Blair Transport Medium with indicator
Fecal Collection for Enteric Pathogens	See Following Section – Page 17	Cary Blair Transport Medium with indicator
Fungus Only	See Following Section – Page 19	Refer to collection procedure.

Observe sterile, aseptic technique when collecting and handling microbiology specimens. If there are any questions as to the specimen criteria, please call the Manager of Microbiology, Brenda Cook, 314-991-4313 x244.

PREPARING SAMPLE FOR TRANSPORT TO LABORATORY

Do not add fixative to microbiology specimens.

1. Transport all microbiology specimens as quickly as possible after collection.
2. All specimens must be labeled appropriately: patient name, date of birth, date/time of collection, test requested, source, initials of person who collected specimen.
3. All microbiology requisitions and labels on swabs or containers must include the source and designated right or left when applicable.
4. Place specimen in a clear plastic BioHazard (OSHA approved) ziplock bag.
5. Put all paperwork in a separate pouch, and then place within the specimen bag.
6. Keep Microbiology specimens separate.
7. Check instructions for unacceptable anaerobic specimens under specimen requirement.
8. A requisition form must accompany all specimens.
9. Patient identification data should be correct and legible and should match that on the specimen container.

The initial responsibility for proper specimen collection, handling and labeling lies with the submitting physician. In general, the submitting physician is responsible for ensuring specimens are collected, labeled appropriately and comply with WCP Laboratories Inc. requirements for submission. While the laboratory cannot be responsible for the material until it is accepted, WCP Laboratories, Inc. works very closely with our clientele to train and provide to them the proper guidelines for submitting microbiology specimens. Our laboratory's main responsibility is to ensure adequate material for proper diagnosis. Any specimen referrals for special procedures or research will be done through the direction of our laboratory director and/or pathologists. In general, the timing of such procedures will take into account the work schedule for our laboratory personnel and internal policies.

***NOTE:** All specimens should be presumed to be infectious. Universal safety precautions are followed in handling all specimens.

The laboratory technician/clerk who accessions the specimen into the laboratory computer should not accept specimens that are either improperly labeled, incompletely labeled, or without proper accompanying specimen requisition. Only specimens that are properly obtained, placed into appropriate containers, and of adequate volume will be analyzed. Once a specimen arrives in the microbiology laboratory, personnel will ascertain that all pertinent information has been provided, that the specimen has been collected in the proper transport device, and that all other conditions for an acceptable specimen have been met. **Only appropriate specimens are suitable for anaerobic cultures. To attempt to use unsuitable specimens for this purpose is a waste of material and labor and will likely provide meaningless results.**

Only sterile containers are acceptable. Specimens are unacceptable when the outside of the container is grossly contaminated with the specimen. Most specimens collected with a swab and transported dry are unacceptable. Urine specimens must be transported in urine collection tubes with a preservative.

WCP Laboratories, Inc. has developed methods for obtaining and assuring correct identification in sample submission. These methods include timely follow up with the client to assure a correctly labeled specimen is obtained or resubmitted to the laboratory. WCP Laboratories, Inc. uses a "Specimen Rejection Form" to document and monitor improper specimen received in the lab. (Please see Attachment A).

PROMPTNESS OF DELIVERY TO THE LABORATORY

In general, specimens should be delivered to the laboratory as soon as possible after they are obtained. WCP Laboratories, Inc. follows a courier pickup system where specimens are picked up from our clients on a periodic basis. If a specimen is referred to as a “stat”, a special pickup is performed at an additional charge.

TURN-AROUND-TIME

In order to assure prompt handling of all specimens the Microbiology personnel are on site from 8:00 am - 12:00 am Monday –Friday. Laboratory hours on Saturday and Sunday vary according to the work load.

The turn-around-time for positive cultures will vary according to the number of bacterial pathogens isolated. Pure cultures or cultures containing well isolated organisms, in most cases, will be reported within 48 hours. Cultures with slow growing organisms or requiring isolation of different colony types will require at least 3-4 days for accurate results. Preliminary results and notification by phone of all highly contagious organisms are standard unless requested otherwise by the client.

Negative urine cultures are reported in 24 hours. All other negative cultures are held for 4-7 days according to the growth rate of organism known for causing infections in some specimens (e.g. cerebral spinal fluids).

Title: BLOOD CULTURES

PRINCIPLE: Laboratory diagnosis of bacteremia and fungemia depends on blood cultures, which are probably the most important cultures performed by the microbiology laboratory. Because the culture methods are so sensitive, the procedure must be carefully controlled beginning at the collection, to avoid the misinterpretation of a procurement-associated skin commensal microorganism as an agent of infection.

In this instance, a blood culture consists of blood from a single venipuncture inoculated into *one* of the Oxoid Signal blood culture bottles. The Signal blood culture system is unique in that it requires only *one bottle per blood culture order*. One bottle provides growth conditions for both aerobes and anaerobes.

PROCEDURE

Although drawing blood cultures before or during the fever spike is optimal for recovery, volume is more important than timing in the detection of agents of septicemia. Draw two blood cultures of maximum volume (up to 10ml) consecutively from different anatomic sites before starting antimicrobial therapy. When it is necessary to draw blood cultures from patients on antimicrobial therapy, they should be drawn when antimicrobial agents are at their lowest concentration.

Skin antisepsis and collection of blood from venipuncture:

1. Select a different venipuncture site for each blood culture.
 - a. If poor access requires that blood for culture be drawn through a port in an indwelling catheter, the second culture must be from a peripheral site, because cultures drawn through catheters can indicate catheter colonization but may not be indicative of sepsis.
 - b. Do not draw blood from a vein into which an intravenous solution is running.
2. Prepare the site.
 - a. Vigorously cleanse with 70% isopropyl or ethyl alcohol to remove surface dirt and oils. Allow to dry.
 - b. Swab or wipe concentric circles of tincture of iodine, moving outward from the center of the site.
 - c. Allow the iodine to dry (about a minute), and avoid touching the site.
 - d. For pediatric patients, omit the iodine step and clean two additional times with separate preparation pads saturated with 70% isopropyl alcohol or ethyl alcohol.
3. Examine the bottle of broth before taking the blood sample and discard it if any evidence of contamination can be seen.
4. Prepare the bottle for inoculation before taking the blood sample. Remove the green plastic ‘flip-off’ cap and disinfect the exposed part of the rubber stopper.
5. Label the bottles with the patient name and the date, time of draw and site of draw.
6. Vigorously wipe septa with 70% alcohol and allow to dry completely, usually for 30 to 60 s.
7. While wearing gloves, insert the needle into the vein and withdraw the blood. Use a new needle if the first attempt is not successful. *Do not repalpate the skin after it is disinfected.*
8. Aseptically inject a maximum volume of 10 ml of blood through the central ring of the rubber stopper.
9. Thoroughly mix the blood with the broth in the bottle.
10. After phlebotomy, dispose of needles in sharps container and remove residual tincture of iodine from the patient’s skin by cleansing with alcohol to avoid development of irritation.

11. Immediately transfer the inoculated blood culture bottle to the laboratory. In the event of transportation being delayed, the bottle should be incubated at $36\pm 1^{\circ}\text{C}$. Transport of blood cultures to the laboratory must be within 24 hours.

Collection of blood from intravascular catheters:

NOTE: The comparison of cultures that are drawn through an indwelling intravenous catheter and through a peripheral site may be useful for diagnosis of catheter-related sepsis.

1. Examine the bottle of broth before taking the blood sample and discard it if any evidence of contamination can be seen.
2. Prepare the bottle for inoculation before taking the blood sample. Remove the green plastic ‘flip-off’ cap and disinfect the exposed part of the rubber stopper.
3. Label bottle with patient name, site of draw, and date and time of draw.
4. Disinfect the septum of the blood culture bottle and the rubber stopper on the bottle with 70% alcohol as for peripheral draw. Allow to dry completely, usually for 30 to 60 s.
5. Using two separate alcohol preps, scrub catheter hub connection for 15 s with 70% alcohol. Air dry.
6. While wearing gloves, disconnect tubing or cap of catheter and attach syringe to collect discard blood (suggested amounts are 3 ml for adults and 0.2 ml for pediatric patients), which is not used for culture.

NOTE: Avoid drawing from lines within an hour of completion of antimicrobial agent administration.

7. Using a new syringe, collect blood for culture through the hub. Quickly reconnect tubing.
8. Connect filled syringe to safety system adapter.
9. Holding the syringe plunger for control, inoculate the Signal blood culture bottle with no more than the amount recommended by the manufacturer (up to 10 ml).
10. Thoroughly mix bottles to avoid clotting.

Specimen transport:

1. Do not refrigerate blood cultures.
2. Incubate bottles at $36\pm 1^{\circ}\text{C}$ until they can be transported to the laboratory or within 24 hours.
3. Provide method of transport that will ensure that bottles are not broken in transit.

Rejection criteria:

1. Blood cultures that are received unlabeled will be rejected.
2. Cracked or broken bottles will not be processed.
3. Labeled blood cultures are not rejected even if medium is expired.
4. If bottle does not contain at least 1 ml of specimen.

REPORTING

1. Gram stain results for all positive blood cultures will be reported immediately with as much interpretive information as possible.
2. No Growth cultures will be reported in 7 days.

Title: COLLECTION OF BODY FLUID CULTURES

PRINCIPLE: Infection of normally sterile body fluids often results in severe morbidity and mortality; therefore, rapid and accurate microbiological assessment of these samples is important to successful patient management.

SPECIMEN TYPES:

1. Joint (Synovial), viscid fluid of the joint cavity.
2. Pleural (Empyema, Thoracentesis), within the membrane surrounding the lungs.
3. Peritoneal (Abdominal, Ascites, Paracentesis), within the membrane lining the abdominal cavity.
4. Pericardial, within the membrane lining the cavity of the heart.
5. Cul-de-sac (Culdocentesis), a blind pouch between the anterior wall of the rectum and the posterior wall of the uterus.
6. Amniotic (Amniocentesis), within the membrane of the fetus.

PROCEDURE:

NOTE: Use care to avoid contamination with commensal microbiota.

1. Clean the needle puncture site with alcohol, and disinfect it with an iodine solution to prevent introduction of specimen contamination or infection of patient.
2. Aseptically perform percutaneous aspiration with syringe and needle to obtain pleural, pericardial, peritoneal, or synovial fluid. Use safety devices to protect from needle exposure.
3. Immediately place fluid into a sterile container for transport, retaining some in syringe for Gram stain. The collection syringe, from which the air has been expelled and the needle removed are acceptable. Cap the syringe with a sterile cap prior to transporting it to the laboratory. **NOTE:** Syringes that are capped with a Luer-Lok (with needle removed) are acceptable. Ensure that there is no leakage during transport, which could result in contamination of the culture.
4. All or the transport methods listed above are acceptable for aerobic, anaerobic, fungal, and acid-fast bacillus (AFB) cultures, as well as stains. **NOTE:** Swabs afford the least desirable sample for culture of body fluids since the quantity of sample may not be sufficient to ensure recovery of a small number of organisms.
5. Routine bacterial culture is sufficient for culture for *Candida* species.
6. Invasively collected specimens in leaky containers must be processed but the physician will be alerted to the possibility of contamination.

REPORTING:

1. Physicians will be notified of all positive body fluid cultures.
2. All No Growth cultures will be held for 4 days.

Title: COLLECTION OF CATHETER TIP CULTURES

PRINCIPLE: Intravascular (intra-arterial or intravenous) catheter insertions cause a break in the skin barrier amenable to infection. The continued presence of this foreign body predisposes further to infection. Since infected catheters are usually exposed directly to sterile spaces, there is a risk that the infection will result in bacteremia. Intravascular catheter-related infections are a major cause of morbidity and mortality in the United States.

PROCEDURE:

1. Clean the skin with 70% alcohol prior to catheter removal.
2. Observing aseptic technique, hold the exposed end of the catheter and carefully remove the catheter from the patient with a sterile instrument, taking care to avoid contact with exposed skin.
3. Holding the distal end over a sterile container cut the tip with sterile scissors, dropping the last 2 to 3 in. into the container.
4. Recap container immediately and submit to the laboratory as soon as possible.

NOTE: Smears will not be performed on catheter tips.

Rejection criteria:

1. Foley catheter tips are not acceptable specimens.
2. Catheter tips that arrive in saline or transport medium.

REPORTING:

1. Catheter tips will be cultured using a semi-quantitative technique and will be reported to distinguish infection from contamination, with counts of > or = to 15 CFU (colony forming unit).
2. Positive cultures will be reported as soon as work up is complete.
3. No Growth cultures will be reported after 4 days.

Title: COLLECTION OF CEREBROSPINAL FLUID CULTURES

PRINCIPLE: Bacterial meningitis is the result of infection of the meninges. Identification of the infecting agents is one of the most important functions of the diagnostic microbiology laboratory because acute meningitis is life-threatening.

PROCEDURE:

NOTE: A lumbar puncture is a medical procedure that is performed by a physician guided by appropriate precautions.

1. After specimen collection slowly drain the CSF into the sterile leakproof tubes.
2. Submit the most turbid tube to microbiology. Otherwise n. 2 is the preferred tube.
3. Submit an appropriate amount commensurate with the tests required to make the diagnosis, using a guideline of 2 ml of fluid for each culture request: routine, fungal, and acid-fast bacillus (AFB).
4. Submit to laboratory as soon as possible and alert laboratory the specimen is in transit.
5. Do not refrigerate.

NOTE: Fungal and AFB cultures of the CSF are infrequently indicated in acute community-acquired meningitis.

Rejection criteria:

1. Specimens in leaky containers must be processed, but the physician will be alerted of the possibility of contamination.
2. Direct antigen testing is not recommended.

REPORTING:

1. Gram stains will be reported as soon as possible, usually within 1 h of receipt.
2. Positive cultures will be reported and called to the physician as soon as preliminary tests are complete.
3. No Growth cultures will be reported in 7 days.

Title: COLLECTION OF GENITAL CULTURES

PRINCIPLE: Specimens from genital sites are sent to the clinical microbiology laboratory for detection of microorganisms from females presenting with clinical syndromes such as cervicitis, vulvovaginitis, urethritis, bacterial vaginosis, salpingitis (pelvic inflammatory disease), endometritis, or genital ulcers. From males exhibiting urethritis, epididymitis, prostatitis, or genital ulcers. Specimens are also submitted from pregnant females to diagnose the presence of organisms that may cause disease in neonate.

PROCEDURE:

NOTE: The following collection procedures are for vaginal, cervical, rectal, throat (for *N. gonorrhoeae*) and urethral cultures only. For collection of other cultures in and around the genital area please contact the Microbiology department at WCP Laboratories, Inc. at 991-4313, ext. 244.

1. Vaginal
 - a. Collect discharge or vaginal secretion using an ESwab collection kit that contains modified Liquid Amies medium provided by WCP Laboratories, Inc.
 - b. Successful self-collection of vaginal swabs can be done.
2. Cervical
 - a. Clear away vaginal mucus and exudate with large swab.
 - b. Moisten speculum with warm water, not lubricants, which can be antibacterial.
 - c. Insert swab from culturette (Amies) through a speculum and obtain exudate from the endocervical canal. Avoid the vaginal walls during collection.
3. Rectal
 - a. Insert swab past anal sphincter, move swab from side to side, allow 10 to 30 s for absorption, and withdraw.
 - b. If contaminated with feces, recollect.
 - c. Order *N. gonorrhoeae* culture.
4. Throat
 - a. Depress tongue gently with tongue depressor.
 - b. Extend swab between the tonsillar pillars and behind the uvula, avoiding the tongue, inner cheeks, and uvula.
 - c. Sweep the swab back and forth across the posterior pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain sample.
 - d. Order *N. gonorrhoeae* culture.
5. Urethral
 - a. Express exudate onto swab from distal urethra. ESwab collection kits with flexible mini-tip swabs are available.
 - b. If there is no exudate, collect 1 h after urination. Wipe area clean, insert the urethrogenital swab 2 to 4 cm into the endourethra, gently rotate the swab, leave in place for 1 to 2 s, and withdraw it.
 - c. Order *N. gonorrhoeae* culture.

TRANSPORT:

1. Submit swab in the ESwab transport tube.
2. Store at room temperature or refrigerated.

NOTE: Recent literature indicates that *N. gonorrhoeae* survives best at refrigerated temperature.

Rejection criteria

1. Specimens not received in proper transport medium will be rejected, since the agents of genital infections lose viability easily.

REPORTING:

1. Genital cultures will be reported after 48-72 h.

Title: GROUP B STREPTOCOCCUS CULTURES

PRINCIPLE: Group B streptococcus (*Streptococcus agalactiae*, GBS) has been recognized as the leading infectious cause of perinatal morbidity and mortality in the United States. In pregnant women, it is associated with asymptomatic bacteriuria, urinary tract infection, and amnionitis. In women who have recently delivered, it causes endometritis and wound infection. Early-onset neonatal disease (during the first week of life) results from transmission of GBS during labor or delivery from mother to infant; late-onset disease (from 1 to 3 months after birth) is thought to be acquired in the nursery. Both are characterized by septicemia, pneumonia, or meningitis and can result in death or permanent neurological sequelae.

GBS disease is increasing in nonpregnant adults, especially the elderly and those with significant underlying disease. Diabetes mellitus, neurological impairment, and cirrhosis appear to be risk factors. Skin, soft tissue infections, pneumonia, and urosepsis are common presentations, although meningitis and endocarditis are reported. Disease is frequently nosocomial, possibly related to catheter placement.

PROCEDURE:

1. Collect specimen at 35 to 37 weeks' gestation.
2. Using the ESwabs collection kit, swab the distal vagina (vaginal introitus), followed by the rectum (insert swab through the anal sphincter).

NOTE: For diagnosis of GBS disease in nonpregnant adults and in neonates, routine culture of the symptomatic body site will detect this pathogen along with the other potential pathogens which can be isolated from those cultures.

TRANSPORT:

1. Submit swabs to the laboratory in transport medium .
2. Store at room temperature or refrigerate at 4 C.
3. Order culture for Group B Streptococcus.

REPORTING:

Cultures for Group B Streptococcus will be reported in 24-48h.

Title: Ocular Cultures

PRINCIPLE: Inflammatory eye conditions may be due to a variety of diseases, and microorganisms play a major role in both acute and chronic diseases. The detection of infectious agents depends on knowledge of the site of infection and the severity of the process, because a variety of organisms cause infections of the eye.

NOTE: Several types of specimens may be collected for the microbiological analysis of eye infections, including conjunctival scrapings obtained with a swab or sterile spatula for the diagnosis of conjunctivitis, corneal scrapings collected with a sterile spatula for the diagnosis of keratitis, vitreous fluid collected by aspiration for the diagnosis of endophthalmitis, and fluid material collected by aspiration or tissue biopsy for the diagnosis of periorbital cellulitis. Most eye specimens are collected by an ophthalmologist. Direct inoculation of culture media and immediate incubation are recommended; for more information regarding this technique please contact the Microbiology laboratory at WCP Laboratories, 314-991-9313.

PROCEDURE:**Eye Conjunctiva:**

1. Sample each eye with separate swabs (premoistened with sterile saline) by rolling over each conjunctiva.
2. Transport specimen (ESwab collection kit/flexible mini-tip swab) as soon as possible.

Corneal Scrapings:

1. Specimen collected by ophthalmologist.
2. Using sterile spatula, scrape ulcers or lesions, direct inoculation onto culture medium is recommended. However, the ESwab collection kit is an excellent alternative.
3. Prepare 2 smear by rubbing material from spatula onto 1-2 dm area of slide if inoculation is performed on-site. Otherwise, smears will be performed in the Microbiology lab.

Vitreous Fluid Aspirates:

1. Specimen collected by ophthalmologist.
2. Prepare eye for needle aspiration of fluid.
3. Transfer specimen to a sterile screw –cap tube or direct inoculation of small amount of fluid onto media.

Rejection criteria:

1. If inoculated plates are used and delayed in transport the physician will be notified that the culture may be compromised or contaminated.

REPORTING:

1. All positive reports from invasively collected specimens will be telephoned to the physician as soon as possible.
2. No Growth cultures will be reported in 72 h except for vitreous fluids which will be held for 7 days.

Title: OTITIS CULTURES

PRINCIPLE: Two types of ear specimens are received most commonly by the laboratory, swab specimens for the diagnosis of otitis externa and middle ear fluid specimens for the diagnosis of otitis media. Potential pathogens at these two sites differ. Since anaerobic bacteria may be involved in middle ear infections, anaerobic culture should be performed on properly collected and transported specimens when requested.

PROCEDURE:

External Ear:

1. Insert sterile swab into ear canal until resistance is met.
2. Rotate swab and allow fluid to collect on swab.

Middle Ear:

1. Clean the external canal with mild detergent.
2. Using a syringe aspiration technique, the physician will obtain the fluid from the eardrum.
3. Send the specimen in a sterile container or in the syringe capped with needle removed.

Outer Ear:

1. Use moistened swab to remove any debris or crust from the ear canal.
2. Obtain a sample by firmly rotating the swab in the outer canal. NOTE: For otitis externa, vigorous swabbing is required since surface swabbing may miss streptococcal cellulitis.

REPORTING:

1. Positive cultures will be reported after work up of organisms is complete. Generally 2-4 days.
2. No Growth cultures will be reported in 48 h. for aerobic bacteria.

Title: RESPIRATORY TRACT CULTURES

PRINCIPLE: Specimens from the lower respiratory tract are submitted to determine the etiology of airway disease (tracheitis and bronchitis), pneumonia, lung abscess, and empyema. Usual specimens consist of lower respiratory tract secretions and inflammation in the form of expectorated sputum; induced sputum; endotracheal tube aspirations; bronchial brushings, washes, or alveolar lavages and pleural fluids.

Upper respiratory tract specimens include the external nares, nasopharynx, throat, oral ulcerations, and inflammatory material from the nasal sinuses. Although few serious diseases involve these areas, many pathogens colonize or persist in these sites while causing symptomatic infection in deeper, less accessible sites.

PROCEDURE:**Lower Respiratory:**

1. Collect washing or aspirate in a sputum trap.
2. Place brush in sterile container with 1 ml of saline.
3. If transport is greater than 24 h. store at 4 C.
4. Minimum amount >1 ml.

Sputum expectorated:

1. Have patient rinse or gargle with water to remove excess oral flora.
2. Instruct patient to cough deeply to produce a lower respiratory specimen (not postnasal fluid).
3. Collect in a sterile container.
4. If transport is greater than 24 h store at 4 C.
5. Minimum amount >1 ml.
6. Unacceptable specimens include: 24-hour sputum collections and swabs.

NOTE: *A systematic evaluation will be performed to ensure that only specimens representative of lower respiratory tract secretions are processed.*

Sputum induced:

1. Have patient rinse mouth with water after brushing gums and tongue.
2. With the aid of a nebulizer have patient inhale approximately 25 ml of 3- 10% sterile saline.
3. Collect in a sterile container.
4. Store at RT.
5. Minimum amount >1 ml.

Nasal:

1. Insert a swab, premoistened with sterile saline, approximately 1-2 cm into the nares.
2. Rotate the swab against the nasal mucosa.
3. Store at RT.

Nasopharynx:

1. Gently insert a small swab (e.g., calcium alginate) into the posterior nasopharynx via the nose.
2. Rotate swab slowly for 5 s to absorb secretions.
3. Store at RT.

Throat or pharynx:

1. Depress tongue with a tongue depressor.
2. Sample the posterior pharynx, tonsils, and inflamed areas with a sterile swab.
3. Store at RT.
4. Unless otherwise indicated, will only be evaluated for the presence or absence of Group A Strep.

REPORTING:

1. Respiratory cultures will be reported 2-4 days after receipt.

Title: NASAL SINUS CULTURES

PRINCIPLE: Acute rhinosinusitis, an infection of one or both of the paranasal sinuses, is among the most common health problems. It is manifest by an inflammatory response of the mucous membranes of the nasal cavity, seen as edema and hypersecretion of mucus following a common upper respiratory viral infection. Specimens are collected by an otolaryngologist; this procedure deals only with invasively collected specimens for diagnosis of acute sinusitis.

PROCEDURE

Rigid endoscopy:

1. Provide patient with an intranasal decongestant and then a topical anesthetic.
2. Identify the middle meatus adjacent to the maxillary sinus ostium ipsilateral to the side to be aspirated.
3. Collect drainage from the middle meatus with a small swab on a wire.
4. Store at RT.

Maxillary sinus puncture and aspiration.

1. Clean the anterior nares with antiseptic solution.
2. Apply topical anesthetic.
3. Puncture the maxillary antrum and aspirate secretions with a needle and syringe.
4. If no material is aspirated, irrigate with 2 ml of nonbacteriostatic saline.
5. Submit aspirates in the original syringe with a Luer-Lok to prevent leakage.
6. Store at RT.

NOTE: These specimens are acceptable for *B. pertussis* and for viral culture.

REPORTING

3. Positive cultures will be reported after work up is complete, approximately 2-4 days.
4. No Growth cultures will be reported in 4 days.

Title: URINE CULTURES

PRINCIPLE: Diseases of the urinary tract include prostatitis, urethral syndrome, cystitis, and pyelonephritis. Urine, prostatic secretion, or urethral cells/secretin specimens are needed to diagnose these diseases. Urine can be collected by midstream collection, catheterization (straight/in-out or indwelling), cystoscopic collection, or suprapubic aspiration. Foley catheter tips should not be submitted or accepted for culture since they are always contaminated with members of the urethral flora and quantitation is not possible.

PROCEDURE:

NOTE: A first-voided morning urine is optimal, since in most cases bacteria have been multiplying in the bladder for several hours. Clean-catch urine, implying cleansing of periurethral areas, has not been shown to improve the quality of urine culture and is not recommended.

Urine Female, midstream:

1. While holding the labia apart, begin voiding.
2. After several milliliters has passed, collect a midstream portion without stopping the flow of urine.
3. The midstream portion is used for bacterial culture. Minimum amount 1 ml.

Male, midstream:

1. While holding the foreskin retracted, begin voiding.
2. After several milliliters has passed, collect a midstream portion without stopping the flow of urine.
3. The midstream portion is used for culture. Minimum amount 1 ml.

Straight catheter:

1. Thoroughly cleanse the urethral opening with soap and water.
2. Rinse area with wet gauze pads.
3. Aseptically, insert catheter into the bladder.
4. After allowing approximately 15 ml to pass, collect urine to be submitted in a sterile container.

Indwelling catheter:

1. Disinfect the catheter collection port with 70% alcohol.
2. Use needle and syringe to aseptically collect 5-10 ml of urine.
3. Transfer to a sterile tube or container.

WCP Laboratories, Inc., provide sterile leak-proof containers and transport tubes with boric acid preservative (gray top). Please transfer all urine specimens into gray top vacutainer tube. If specimen cannot be transported to the laboratory within 24 h refrigerate at 4 C. Urine specimens that have not been transferred into gray top tubes must be refrigerated within 2 h of collection.

Rejection criteria:

1. Foley catheter tips and urine from bag of catheterized patients.
2. Specimens in leaky containers.
3. Specimens received in collection cup (no preservative) with no evidence of refrigeration.

REPORTING:

1. Urine cultures will be reported in 2-3 days.

Title: WOUND AND SOFT TISSUE CULTURES

PRINCIPLE: A wide variety of microorganisms that reside on the skin and mucous membranes of the body, as well as those found in the environment, can cause skin and soft tissue infections. These organisms enter the body through breaks in the skin or mucous membranes, through wounds made by trauma or bites (exogenous) or as a complication of surgery or foreign-body implants (endogenous), or they can be spread through the vascular system (hematogenous).

PROCEDURE:

NOTE: *Tissue or fluid is always superior to a swab specimen. If it is necessary to use a swab WCP Laboratories, Inc. provides our clients with the ESwab collection kit/Amies liquid preservative. One tube can be used for the isolation of aerobic, anaerobic, fungus and fastidious bacteria.*

1. Remove surface exudate by wiping with sterile saline or 70% alcohol.
Open – Aspirate if possible or pass a swab deep into the lesion to firmly sample the lesion’s “fresh border.” Samples of the base of the lesion and abscess wall are most productive.

Closed – Aspirate abscess material with needle and syringe; aseptically transfer all material into a sterile screw-top container. **WCP Laboratories, Inc. provides an anaerobic transport vial (Port-A-cul) for the collection of small tissue and fluids. However, the ESwab with Amies preservative can be used if aspiration is not possible.**
2. Sterile cup with nonbacteriostatic saline for tissue or bone is acceptable.

NOTE: Organisms may not be distributed evenly in a burn wound, so sampling different areas of the burn is recommended.

Rejection criteria:

1. Specimens for microbiological analysis will not be accepted in formalin.
2. A recollect will be requested if specimen is not received in proper transport system.

REPORTING:

1. Special request for Gram stain will be reported within 1 h of receiving specimen in laboratory.
2. Preliminary reports will be available after 24 h by request.
3. Positive cultures will be reported in 2-4 days.
4. No Growth cultures will be reported in 4 days.

Title: ANAEROBIC CULTURES

PRINCIPLE: Anaerobes characteristically produce purulent infections in areas adjacent to mucous membranes containing anaerobes from the normal flora. Anaerobic bacteria cause a variety of infections in humans, including appendicitis, cholecystitis, otitis media, dental and oral infections, endocarditis, endometritis, brain abscesses, myonecrosis, osteomyelitis, peritonitis, empyema, salpingitis, septic arthritis, liver abscesses, sinusitis, wound infections following bowel surgery or trauma, perirectal and tuboovarian abscesses, and bacteremia. Many reports associate 50 to 60% of important infections with anaerobic bacteria.

PROCEDURE:

NOTE: Proper collection of specimens and prompt transport to the laboratory for processing are extremely important. Isolating anaerobes from clinical specimens, determining the numbers of anaerobes in the specimen, and establishing the clinical significance all depend on proper collection and transport of the specimen. **Anaerobic specimens must be protected from oxygen.**

1. The best specimen for culture is obtained by using a needle and syringe.
2. Tissue samples and biopsy samples are also very good specimens for anaerobic culture.
3. The least desirable specimen is collected by swab. Generally the specimen volume when collected by a swab is small, reducing the probability of isolating organisms.
4. If collecting a specimen with a swab is unavoidable, then, collect as much specimen as possible.
5. Use an ESwab collection system (the same specimen can be used for the aerobic, fungus and acid-fast cultures) and use special care to sample the active site of infection to prevent contamination.
6. Avoid extremes of heat or cold.
7. Do not transport material for culture in the needle and syringe. There is always the risk of a needle stick injury and syringe transport poses a risk because the specimen may be expelled.
8. Transfer aspirated material to an anaerobic transport vial. Large volumes of purulent material may be transported in a sterile screw-cap tube.
9. Store at RT.

Rejection Criteria

Anaerobic bacteria are normally prevalent in the mucous membranes of the mouth, genital and gastrointestinal tract. Specimens from these sites will not be cultured for anaerobes unless the area is abscessed or there is a lesion. The following is a list of sites which will **NOT** be examined for anaerobic bacteria because of the relatively abundant normal anaerobic flora present:

- Throat, nasopharynx, sputum, gingival or other internal mouth surfaces, bronchoscopic specimens (unless obtained via a protected double-lumen catheter)
- Feces, rectal swab, fistula, any specimen obviously contaminated with feces, gastric contents, vagina, cervix (unless visualized via a speculum)
- Voided or catheterized urine.

REPORTING:

1. Positive cultures will be reported after testing is complete, approximately 4-7 days.
2. No Growth cultures will be reported in 4 days.

Title: Fecal Collection for Clostridium difficile Toxin A/B

PRINCIPLE: Clostridium difficile-associated disease (CDAD) primarily occurs in hospitalized patients. While diarrhea caused by C. difficile is usually developed during hospitalization it does occur as a community-acquired disease following hospital discharge or the use of outpatient antimicrobial therapy. Individuals with CDAD shed spores in the stool, which can survive for as long as five months in the environment. Infection with toxigenic C. difficile is a potentially life-threatening disease process; however, when properly treated, patient mortality rates are low. Thus, rapid diagnosis, allowing clinicians to initiate appropriate therapy and implement adequate measures to control nosocomial spread, is important.

TYPE: Fresh stool specimens should be collected in clean, airtight, leak-proof containers. Stool specimens collected in modified Cary Blair Transport Medium with indicator (or equivalent) are recommended in the event of transport delays. Contact WCP Laboratories for collection medium.

Stool specimens collected in modified Cary Blair Transport Medium with indicator may be stored refrigerated (2-8°C) or stored at room temperature (20-25°C) and should be tested within 5 days of collection.

Fresh, untreated stool specimens should be stored at 2-8°C and tested within 72 hours of collection. If fresh specimens cannot be tested within 72 hours, they should be frozen at -20°C or below in a non-defrosting freezer and tested within 2 months of collection. Avoid multiple freeze-thaw cycles.

NOTE: This collection can also be used for stool cultures.

PROCEDURE:

Collection using the Cary Blair Transport Medium:

NOTE: Patient should not use antacids, barium, bismuth, antibiotics, anti malarial agents, ant diarrheal medication or oily laxatives prior to specimen collection. After administration of any of these compounds, specimen collection should be examined to insure recovery of organisms.

1. Several specimens, collected intermittently over several days, should be examined to insure recovery of organisms.
2. Specimens must be collected properly to avoid contamination with urine or water. Specimens are best collected in a bedpan or a clean, dry, leak-proof container.
3. Fill the transport medium container with sufficient stool to bring the liquid level up to the "Fill" line.
4. Stir each specimen with the spoon provided, tighten the cap and shake firmly until the specimen is adequately mixed. When mixing is complete the specimen should appear uniform.
5. Complete the label on the vial and replace the vial in the plastic bag. Transport the specimen to the laboratory. Specimen may be refrigerated or kept at room temperature.

REPORT:

Specimens will be processed daily. All positive results will be reported to the physician or physician's designee as soon as results are available. Negative results will be reported within 24 h. of receipt of specimen.

Title: Fecal Collection for Enteric Pathogens (Stool Cultures)

PRINCIPLE: Feces specimens are submitted to the microbiology laboratory to determine the etiologic agent of infectious diarrhea or food poisoning. Feces should be collected in a clean container with a tight lid and should not be contaminated with urine, barium, or toilet paper. Because intestinal pathogens can be killed by the metabolism of members of the fecal flora rapidly acidifying, the specimen should be transferred to Cary Blair transport medium soon after collection.

Diagnosis of enteric bacterial disease is confirmed by isolation and identification of pathogenic organisms in stool specimens. Procedures such as freezing, incubation and refrigeration do not insure recovery and identification of enteric bacterial pathogens. Cary Blair transport medium has been formulated to facilitate collection and transportation while maintaining the bacterial population for optimum recovery up to 96 hours after passage. Proper use of the system assures the microbiologist that enteric pathogens such as Yersinia, Salmonella, Shigella, Campylobacter, Cholera Vibrio, if present, will be preserved.

If Yersinia, Vibrio or E. coli 0157 is the suspected agent of infection please stipulate on the requisition so that special attention can be given to these specimen.

NOTE: This collection can also be used for the recovery of Clostridium difficile toxin.

TYPE: Fresh stool specimens should be collected in clean, airtight, leak-proof containers. Stool specimens collected in modified Cary Blair Transport Medium with indicator (or equivalent) are recommended in the event of transport delays. Contact WCP Laboratories for collection medium.

Stool specimens collected in modified Cary Blair Transport Medium with indicator may be stored refrigerated (2-8°C) or stored at room temperature (20-25°C) and should be tested within 5 days of collection.

Fresh, untreated stool specimens should be stored at 2-8°C and tested within 72 hours of collection. If fresh specimens cannot be tested within 72 hours, they should be frozen at -20°C or below in a non-defrosting freezer and tested within 2 months of collection. Avoid multiple freeze-thaw cycles.

PROCEDURE:

Collection using the Cary Blair Transport Medium:

NOTE: Patient should not use antacids, barium, bismuth, antibiotics, anti-malarial agents, anti-diarrheal medication or oily laxatives prior to specimen collection. After administration of any of these compounds, specimen collection should be examined to insure recovery of organisms.

1. Several specimens, collected intermittently over several days, should be examined to insure recovery of organisms.
2. Specimens must be collected properly to avoid contamination with urine or water. Specimens are best collected in a bedpan or a clean, dry, leak-proof container.
3. Fill the transport medium container with sufficient stool to bring the liquid level up to the "Fill" line.

4. Stir each specimen with the spoon provided, tighten the cap and shake firmly until the specimen is adequately mixed. When mixing is complete the specimen should appear uniform.

5. Complete the label on the vial and replace the vial in the plastic bag. Transport the specimen to the laboratory. Specimen may be refrigerated or kept at room temperature.

REPORT: Routine reports for cultures negative for Salmonella, Shigella or Campylobacter spp. and special orders for E. coli 0157 and Yersinia will be reported between 48-72 hours. All positive cultures for isolates of Salmonella, Shigella, E coli 0157, Yersinia and Vibrio cholera (if present) will be reported immediately to the physician or physicians designee and to the local health department.

Title: FUNGUS CULTURES – Specimen Selection, Collection, and Transport

PRINCIPLE:

The collection, transport, and processing of clinical specimens encompass some of the most important considerations in determining the etiology of fungal disease. Only with the appropriate handling of specimens can the recovery of fungal organisms be clearly associated with a disease process. Since inappropriate specimen collection may introduce potentially confusing members of the indigenous flora and jeopardize culture results interpretation, it is critical to use only those practices most likely to facilitate the direction of etiologic agents.

To establish or confirm the diagnosis of a suspected fungal infection, it is essential for the clinician to provide the laboratory with adequate specimens for evaluation. The microbiology laboratory should be notified if an unusual pathogen or organism that can be a significant laboratory hazard (e.g. *Coccidioides* spp. and *Histoplasma capsulatum*) is suspected, as some require special handling or special stains.

TRANSPORT OF FUNGAL CULTURES:**A. General considerations**

1. Collect specimens aseptically and place in sterile, leakproof containers.
2. Deliver the specimen to the laboratory as soon as possible.
3. For normally sterile specimens (e.g., blood, bone marrow, CSF, or deep lesion material) call for a stat pick up.
4. Refrigerate at 4°C specimens that are potentially contaminated with bacterial microbiota (e.g., dermatological specimens, transtracheal aspirates, ear [internal] aspirates, and conjunctiva cultures).

PROCEDURE:**Abscess, Drainage, Wound**

1. Whenever possible, aspirate sample in a syringe and submit in a sterile screw-cap container.
2. If it is necessary to use a swab, use an aerobic transport system, preferably, the ESwab collection kit provided by WCP Laboratory.

Eye – Corneal Scrapings

1. For optimum recovery, direct inoculation onto the appropriate fungal medium is recommended. If this is not possible submit in a sterile screw-top container.

Hair

1. Select an infected area of the scalp.
2. Remove at least ten hairs and scrape scalp, scales if present. Invaders of the scalp and hair are best isolated by culturing the basal portion of the infected hair.
3. Transport in a clean envelope or screw top container.

Nails

1. Clean nail with an alcohol wipe.
2. Scrape infected nail area deeply enough to obtain recently invaded nail tissue.
3. Discard the initial scrapings which are usually contaminated.
4. Transport in a clean envelope, screw top container or between two clean glass slides taped together.
5. Transport slides in slide carrier.

Skin

1. Clean the surface of the skin with 70% alcohol.
2. Scrape the active, peripheral edge of a lesion with a scalpel or the end of a microscope slide.
3. Place the scrapping in a clean screw top container, or place in a sterile Petri plate.

Sterile Fluids

1. Collect a minimum of 2 ml. in a sterile container (the more fluid obtained the better the recovery rate of fungal elements).

Tissue and Bone

1. Collect specimen and transport in a sterile screw cap container with a small amount of sterile saline to prevent drying.

Intraocular Fluid

1. Collect in a sterile screw cap container.

REPORTING:

1. No growth fungal cultures are reported in 4 weeks.
2. Positive fungal cultures will be reported when work up is complete. Some fungal cultures may take 6-8 weeks depending on the type of fungus isolate.

REFERENCES

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Isenberg, H.D., Clinical Microbiology Procedures Handbook. 2nd Edition. Vols. I<II &III. 2004 ISBN 1-55581-246-0. ASM. Washington, D.C.

BioMed Diagnostics, Inc. Technical bulletin no. 101 (document 100-001). BioMed Diagnostics, Inc., San Jose, Calif.

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Larone, D.H., Medically Important Fungi A Guide to Identification. 4th Edition. 2002. ASM Press. Washington, D.C.

Oxoid. Technical bulletin. Signal-for Detection of Bacteria in Body Fluids. Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, England.

ATTACHMENT A

Submitting Location or Physician _____ Accession# _____

Date Received _____ Received From _____

REASON FOR REJECTED/UNSATISFACTORY SPECIMEN
--

- _____ No Client or Physician Identified on Requisition or Container
- _____ Container Not Labeled with Complete Patient Name
- _____ Container Not Labeled at All
- _____ Patient's Age/DOB Not on Requisition
- _____ No Date of Service (Specimen Taken) Given
- _____ Incorrectly Labeled (Container vs. Requisition)
- _____ All Specimen Container(s) Not Indicated/Labeled as per Requisition
- _____ No Fixative or Improper Fixative for Test
- _____ No Specimen Identified in Container
- _____ Specimen Received Broken Beyond Repair
- _____ No Sites Listed on Requisition on Container(s)
- _____ Multiple Accession Numbers for Same Patient
- _____ Other (Please Describe under Comment Section below)

COMMENTS:

Reviewed by _____ Date _____

