

Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 6/2011

Overview

The proper collection of a specimen for culture is the most important step in the recovery of pathogenic organisms responsible for infectious disease. A poorly collected specimen may lead to failure in isolating the causative organism(s) and/or result in the recovery of contaminating organisms.

Basic Concepts for Specimen Collection

1. Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions.
2. Collect the specimen at optimal times (for example, early morning sputum for AFB culture).
3. Collect a sufficient quantity of material. Use appropriate collection devices: sterile, leak-proof specimen containers. Use appropriate transport media (anaerobe transport vials, Culturette for bacterial culture, Cary-Blair for stool culture, M4RT for viral and Chlamydia cultures, and urine boric acid transport. Check expiration date before inoculating collection device.
4. Whenever possible, collect specimens prior to administration of antimicrobial agents.
5. Properly label the specimen and complete the test request form. The specific source of specimen is required. Example: wound, left leg.
6. Minimize transport time. Maintain an appropriate environment between collection of specimens and delivery to the laboratory.
7. If appropriate, decontaminate the skin surface. Use 70-95% alcohol (ALC) and 2% chlorhexidine or 1-2% tincture of iodine (TIO) to prepare the site. Allow a contact time of two minutes to maximize the antiseptic effect.
8. For the orders with more than one test, ensure that the proper transport is utilized. (For example, anaerobic culture requests need to be submitted in anaerobic transport media; bacteriology requests should **not** be in viral media; AFB requests should not be in anaerobic transport media.

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Abscess

1. Decontaminate the surface with 70-95% alcohol and 1-2% tincture of iodine.
2. Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe. Open miliary abscesses with a sterile scalpel and collect the expressed material with a sterile needle and syringe.
3. Transfer 5-10 ml of the aspirated material to an anaerobic transport vial. Transport immediately. *Anaerobic transport media is not recommended for AFB culture. If requesting AFB culture, transfer at least 1 ml of the aspirated material into a sterile container.*
4. Swabs are a poor choice because they dry easily and because of the limited amount of material obtained. Swabs are not optimal for fungal, anaerobe cultures, or decubitus ulcers. Swabs are **not** accepted for mycobacterial cultures, perirectal abscesses, oral abscesses. Gram stains cannot be provided from a single swab. If a Gram stain is needed, collect two swabs.
5. Note requests to rule out *Actinomyces sp.* or *Nocardia sp.* on the requisition or in POE.

Amniocentesis

1. Usually collected by ultrasound method by physician. Send to lab in capped syringe or anaerobe transport medium.

Arthropod

1. Arthropod specimens should be collected using tweezers to remove or extract from the skin if attached. Place into a vial or container with or without a small volume of alcohol and secure the lid well to prevent leaking.

Blood

1. Determine the type of culture bottles to utilize, as indicated per physician's order (aerobic and anaerobic or resin bottles and anaerobic bottles), or other types as specified below. If necessary, discuss timing of cultures, sites, need for any special instructions on the lab slip, etc., with the physician. Please also refer to the JHH Interdisciplinary Clinical Practice Manual Infection Control Blood Culture Policy and Procedure.
2. Gather necessary equipment.
 1. If patient is not on antibiotics, a routine culture set consists of an aerobic and anaerobic culture bottle.
 2. If patient is on IV or PO antibiotics or has been off antibiotics for less than 24 hours or for suspected *Neisseria sp.* or other fastidious organisms, a resin aerobic bottle may be substituted for the aerobic bottle. An anaerobic culture bottle should also be sent.
3. Explain procedure to patient and/or significant other.
 1. Verify the patient's identification by checking the identification wristband for patient name and history number. If the patient does not have an identification wrist band, as in some

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outpatients, ask the patient to state his or her name and birth date and verify that these are the same as the name and birth date on the order.

4. Wash or sanitize hands before and after removing gloves. Follow Standard Precautions for all patients. Masks with face shields are recommended when drawing blood cultures.
5. Assemble necessary equipment before preparation of the patient's skin.
 1. Remove dust caps from culture bottles.
 2. Clean surface with alcohol wipe.
 3. Leave the alcohol wipe on the bottle top during skin preparation.
 4. Remove alcohol wipe just prior to inoculating the bottles - do not use iodine.
 5. Create a sterile field with the inside of the outer sterile glove package.
 6. Open supplies and place on the field.
6. Apply tourniquet to the extremity and identify the phlebotomy site.
7. Preparation of the phlebotomy site:
 1. Preferred: Use Chloraprep® One-Step Frepp
 1. Cleanse and scrub the site with 2-3 alcohol swabs. Allow it to dry for at least 30 seconds.
 2. Don sterile gloves.
 3. Squeeze the handle of the Chloraprep® Frepp to break the ampule and scrub the skin with the foam surface.
 4. Use a firm scrubbing motion for 30 seconds to disinfect the site.
 5. A 10 cm area of the skin should be disinfected.
 6. Allow the site to dry at least 30 seconds before venipuncture.
 2. If unable to utilize Chloraprep® One-Step Frepp, use 1% tincture of iodine frepp.
 1. Povidone-iodine swabs permit more contamination than either chlorhexidine or 1% iodine frepps.
 2. Using the 1% iodine frepp, cleanse a 10 cm area using circular motion starting at the site and working outward.
 3. Allow to dry for a at least 30 seconds to allow antiseptic effect
 4. If using iodine product, clean patient's skin with alcohol to remove excess iodine (to prevent iodine burns).
 3. If unable to use Chloraprep® One-Step Frepp or iodine (e.g., allergic):
 1. Use alcohol pad to cleanse the patient's skin, using a circular motion starting at the site and moving outward.
 2. Repeat times two.
 3. Allow to dry at least 30 seconds.
8. Do not touch the venipuncture site after skin preparation. If palpation is absolutely necessary, sterile gloves must be applied immediately prior to palpation.
9. Insert needle into vein and withdraw appropriate amount of blood (See 3.10). Draw blood cultures prior to drawing other blood samples.
 1. Insert the needle into the vein
 2. Try to keep the dominant hand sterile.
 3. Remove the alcohol pad from the top of the culture bottles
 4. Attach the vacutainer to the blood culture bottle and inoculate each culture bottle with exactly 8-10 ml of blood, using previously marked indicator line. If less than 10 ml of blood was obtained, inoculate aerobic bottle with total amount obtained.

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5. Remove the tourniquet and butterfly needle from the site and cover with gauze dressing. Apply pressure to site as needed.
10. If absolutely necessary to draw from a central catheter site, utilize the site that has been most recently inserted (unless ruling out catheter sepsis). Follow the procedure as outlined in Appendix B of the JHH Interdisciplinary Clinical Practice Manual Infection Control Blood Culture Policy and Procedure
11. For POE units:
 1. Label the culture bottles with a POE generated label in the presence of the patient. Indicate the time that the specimen was obtained.
 2. Do not place label over bar-coded area of the bottle.
 3. If using a VAD to draw the culture, and it is not indicated on the label, you must indicate the type and site of VAD in the comments section (eg. left subclavian triple lumen)
 4. Barcode scan specimens.
12. For non-POE units:
 1. Label the culture bottles with the patient's name and history number in the presence of the patient. Indicate the time that the specimen was obtained.
 2. Fill out Pathology 5 (Microbiology-routine) lab slip.
 3. Indicate the site from which blood was collected using comment section. If using a VAD to draw culture, you must indicate type and site of VAD in the comments section (eg., left subclavian triple lumen).
 4. Select appropriate test (i.e., Blood Culture - 7510).
 5. Indicate suspected diagnosis, if necessary (required for rule out endocarditis).
 6. Include date and time of collection.
 7. Document that cultures were obtained on appropriate nursing form (e.g., Eclipsys).
13. Send specimens to the laboratory as soon as possible (utilize pneumatic tube). Never refrigerate blood culture specimens. Send specimens directly to Microbiology Lab.
14. Send second set of blood cultures using the same procedure as above. If a different peripheral site is possible, the second set may be drawn immediately. If using the same site, wait at least 10 minutes for the second set, and if possible (i.e. not waiting to give antibiotics) draw a third set 1-3 hours later.
15. In order to rule out specific diagnoses, more specific blood culture procedures may be necessary. See below for recommendations:
 1. **Suspected catheter sepsis**
 1. Draw two blood culture sets.
 2. One set is obtained from the suspected catheter.
 3. At the same time, a second set must be from a separate peripheral site.
 4. Time of collection should be noted for both specimens.
 5. If the catheter is removed, a section of about 1 inch in length from an intradermal portion is to be cut aseptically and sent to Microbiology in a dry sterile container. **Do not send catheter tip without sending concomitant blood cultures. In such cases the catheter tip will not be cultured.**
 2. **Acute endocarditis**
 1. Draw 2-3 culture sets from separate sites within 30 minutes of each other and before beginning antimicrobial therapy.
 2. Begin therapy after cultures are obtained.
 3. **Subacute endocarditis**
 1. Draw 2-3 blood culture sets on day 1, spaced 30-60 minutes apart. This may help to document a continuous bacteremia.

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2. If all are negative additional sets can be drawn on days 2 and 3 (no more than 4 sets in a 24 hour period).
3. Immediate antibiotics are less important than establishing a specific microbial diagnosis.
4. **Mycobacterial blood cultures (AFB)**
 1. Use a Mycobacterial blood culture bottle (BD Bactec Myco/F Lytic culture bottle). Because the media is unstable, bottles must be obtained from the Mycobacteriology (AFB) Lab (Meyer B1-124, 5-6470) on dayshift and the Microbiology Lab (5-6510) during evening hours. Pneumatic tube can be used to obtain culture media.
 2. Prepare skin as for routine blood cultures.
 3. Inoculate 5 ml blood into culture bottle (**do not exceed 5 ml of blood**). Do not place patient label over bottle bar code label.
 4. Deliver the blood bottle in the *brown bag* promptly to the Microbiology Lab. Pneumatic tube can be used as long as sample is sent directly to the Microbiology Lab.
 5. An authorized prescriber must have specifically placed an order for Mycobacterial blood culture.
5. **Fungal Cultures**
 1. **Candida spp.** - If physician orders fungal cultures, follow routine procedure for bacterial cultures as described above.
 2. **Histoplasma, Cryptococcus, or other filamentous organisms**--obtain an isolator tube from Microbiology Lab (call 5-6510).
 1. Isolator media is light sensitive and is stored in brown paper bags.
 2. Inoculate isolator tube with 5cc of blood (minimum 3cc).
 3. Send culture in brown paper bag directly to Microbiology Lab.
6. **Viral blood cultures** Only herpes simplex viruses (HSV) are tested by conventional culture (**heparin tube not acceptable for PCR please see [Molecular testing guidelines](#)**)
 1. Obtain one 3 ml green top tube (heparinized) after preparing the skin as above.
 2. Deliver the tube promptly to the Microbiology Lab. Pneumatic tube can be used as long as sample is sent directly to the Microbiology Lab.
 3. Indicate suspected HSV virus on the requisition to optimize culture technique and reduce cost.
 4. Viral blood cultures must be specifically ordered by the physician.
7. **Malaria, Babesia or other blood parasites**
 1. Draw 3 ml of blood in a lavender top (EDTA) vacutainer tube.
 2. Deliver the tube to the Microbiology lab within 2 hours of collection.
 3. Indicate patient's travel history (if available).
8. **When rare organisms such as Brucella, Campylobacter or Bartonella are suspected:**
 1. An ID physician shall be consulted.
 2. The Microbiology laboratory should be notified for special instructions.
9. **Antibiotic Activity (Serum)**
 1. **Requires authorization by Infectious Disease, Microbiology Faculty or Clinical Pathology Resident.**
 2. Collect 2-5 ml of blood in a red top tube **pre** and one hour **post** infusion of the antimicrobial agents.
 3. Include: patient isolate, culture number, specimen collection time, dosage time, and antibiotic therapy.
 4. Process requirements: aliquot 2 ml of serum into a plastic vial and freeze.
10. **Aspergillus galactomannan antigen**
 1. Collect 3-5 mL blood in serum separator tube (SST) without anticoagulants.
 2. Transport the specimen to the lab as soon as possible

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Body Fluids, Sterile (except urine and cerebrospinal fluid)

1. Prepare the skin as for [blood cultures](#).
2. Collect the fluid using a sterile needle and syringe.
3. For aerobic and anaerobic organisms, submit 10 ml in a sterile container.
4. For viral isolation, send 3 ml or less fluid in a sterile vial (1 ml minimum).
5. If tuberculosis or fungal infections are suspected, collect a minimum of **5 ml** of fluid into a sterile container.
6. Transport immediately.
7. **Do not send Sterile Body Fluids on swabs.**

Bone Marrow

1. Physicians should wear gowns, masks, and gloves during specimen collection.
2. Prepare skin as for [blood cultures](#).
3. Drape the surrounding skin with sterile linen.
4. Aspirate the marrow percutaneously using a sterile needle and syringe.
5. Transfer 3-5 ml for each:
 1. Bacterial test into a blood culture bottle - do not send in a Heparin tube.
 2. AFB culture and fungal culture into a mycobacteria/fungal blood culture bottle (Myco/F Lytic bottle – available in microbiology lab, call 5-6510)
 3. Viral test into a Heparin (green top) tube
 4. Parvo B19 molecular test into an EDTA (purple top) tube
6. Transport specimens immediately at ambient temperature.

Bordetella pertussis

Culture and PCR

1. Obtain collection system from Microbiology lab, Meyer B1-111, 955-6510.
2. Provided in the collection system are: 1 charcoal culture swab for culture; and 1 culture swab for PCR. Store collection system at room temperature.
3. Use two swabs on a flexible wire handle to collect the specimen. One swab is used to inoculate the charcoal transport medium. The other swab is for PCR testing (Calcium alginate swabs cannot be used for PCR testing.)

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4. Seat the patient comfortably. Tilt the head back.
5. If available, insert a nasal speculum. Press the swab through the nares until resistance is met due to contact with the nasopharynx.
6. Rotate the swab gently and allow the swab to maintain contact with the nasopharynx for 20-30 seconds or until coughing is induced.
7. Place the swab into the transport medium. Label the tube with the patient's name and identification number. Leave the swab embedded in the tube during transport.

PCR

1. Preferred specimen is a dry swab. Do not use calcium alginate.
2. Transport immediately.

Bronchial Brush/Wash/Lavage

1. This technique should be performed by an experienced individual. Descriptions of the methodology are readily available in the literature.
2. Transport in a sterile container immediately at ambient temperature.

Bullae, Cellulitis, Vesicles

1. Cleanse the skin as for [blood cultures](#).
2. Aspirate the fluid/purulent material using a sterile needle and syringe.
3. If an aspirate is obtained, place in appropriate viral or bacterial transport tube or vial.
4. If no material is obtained, unroof vesicle or bullous lesion and use a Dacron swab to collect cells from the base of the lesion. Place in appropriate viral or bacterial transport media.

Cellulitis

Swabs and leading-edge aspirates with or without injection of saline fail to yield etiologic agents in the majority of cases. If an unusual organism is suspected, a leading-edge (advancing margin) punch biopsy is the recommended specimen of choice. Place biopsy in sterile container with small volume of non-bacteriostatic saline.

Vesicle Fluids and Scrapings

Select a fresh vesicle, wipe gently with alcohol, dry thoroughly with sterile gauze. Using a tuberculin syringe with a small needle (26 g x 1/2 inch) aspirate vesicular fluid; transfer the fluid to the viral transport medium by filling the remainder of the syringe with the medium, then flush the solution into the transport tube or by swabbing the vesicle and breaking the swab off into a tube of viral transport media. USE STERILE TECHNIQUE AT ALL TIMES. Care should be taken to avoid any bleeding as this can impair recovery of virus in diseases such as Herpes simplex or Varicella zoster in which neutralizing antibodies are present in the serum.

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Cerebrospinal Fluid

1. Physicians should wear gowns, masks, and gloves to collect the specimen. Because an open tube is held to collect the fluid, other personnel should stand away or wear masks in order to avoid respiratory contamination.
2. Decontaminate the skin with 1-2% TOI, followed by 70-90% ALC using an increasingly outward circular movement.
3. Drape sterile linen over the skin surrounding the puncture site.
4. Insert the needle. Collect the fluid into three sterile leak-proof tubes. Collect an adequate volume of fluid as recommended below.
 1. bacterial culture > 1 ml
 2. fungal culture 2-5 ml
 3. molecular > 1 ml
 4. mycobacterial culture 5-10 ml
 5. viral culture > 1 ml
5. Cap the tubes tightly. Submit the third tube for culture to reduce the possibility of contamination due to skin flora. Transport immediately at ambient temperature.

Cutaneous (Fungal only)

Hair

1. Scrape the scalp with a blunt scalpel.
2. Place specimen in a dry sterile container.
3. Transport at ambient temperature.
4. The following specimens are also acceptable:
 1. Hair stubs
 2. Contents of plugged follicles
 3. Skin scales
 4. Hair plucked from the scalp with forceps**Cut hair is NOT an acceptable specimen.**

Nails

1. Cleanse the nail with 70-95% ALC.
2. Remove the outermost layer by scraping with a scalpel.
3. Place specimen in a dry, sterile container.
4. Transport at ambient temperature.
5. The following specimens are also acceptable:
 1. Clippings from any discolored or brittle parts of nail
 2. Deeper scrapings and debris under the edges of the nail

Skin

1. Cleanse the skin with 70-95% ALC.
2. Collect epidermal scales with a scalpel, at the active border of the lesion.
3. Place specimen in a dry sterile container. Do not tape specimen to slide.
4. Transport at ambient temperature.

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Ear

1. External ear cultures are processed as superficial [wounds](#).
2. Middle ear fluid will be processed as a miscellaneous body fluid. If the diagnosis is otitis media, the specimen of choice is middle ear fluid collected by tympanocentesis.
3. Please indicate specific ear source.

Eye

1. Cleanse the skin around the eye with a mild antiseptic.
2. Purulent conjunctivitis: Collect purulent material with a regular cotton swab.
 1. Place the swab into appropriate transport media and transport at ambient temperature.
 2. This is **Not** an acceptable specimen for anaerobe culture.
 3. PCR – Chlamydia only; collect specimen with bacteriology culturette and place in 3 ml of viral transport media. **Do not use Aptima Kit.**
3. Corneal infections:
 1. Obtain Cornea Pack from microbiology laboratory, Meyer B1-111, 5-6510.
 2. Swab the conjunctiva as described above.
 3. Collect multiple corneal scrapings and inoculate directly onto bacterial agar media (chocolate agar, brain heart infusion with gentamicin agar, sheep blood agar, and Schaedler's broth) and/or viral transport media.
 4. Transport at ambient temperature.
 5. Gram stain not routinely performed.
4. Intraocular fluid:
 1. Collect fluid by surgical needle aspiration.
 2. Transport cultures at ambient temperature.

Gastric Biopsy

1. Appropriate for *Helicobacter pylori* culture only. Contact the microbiology laboratory at (410)-955-6510 for appropriate transport media. Must be transported to the microbiology laboratory within one hour of collection.

Genital Sources

Routinely processed only for gonococci. Predominance of *S. aureus*, Beta hemolytic strep and yeast upon request. Specimens from normally sterile sites (e.g., transabdominal amniocentesis fluid) can be submitted for anaerobic culture if the specimen is transported to the lab in anaerobe transport medium.

For sexually transmitted diseases testing, refer to [Chlamydia/Gonorrhea](#).

Bartholin's Glands

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1. Do not use alcohol for mucous membranes. Skin prep as for regular skin sites.
2. Aspirate material from Bartholin gland abscess.
3. Send to lab in anaerobic transport medium.

Cervix (Endocervix) for Culture

1. Place the patient in the lithotomy position.
2. Prepare the speculum, avoiding the use of a lubricant other than warm water.
3. Insert the speculum and visualize the cervical os.
4. Remove excess mucus with a cotton ball.
5. Insert a Dacron swab into the cervical os, rotate gently, and allow to remain for 10 to 30 seconds.
6. Remove swab and place in bacterial transport medium.
7. Transport at ambient temperature.
8. Vaginal cultures, in general, do not often produce meaningful results and are not recommended, except for group B streptococcal screen. Vaginal cultures are not acceptable for anaerobic culture.

Cervix (Endocervix) for HPV DNA Testing

1. Samples are referred to microbiology from the Johns Hopkins Cytopathology laboratory.

Chlamydia/Gonorrhea Swab PCR testing by Gen-Probe Aptima Combo 2 CT/NG

Female cervical

1. Obtain collection pack from Medical Distribution Center, item number ESI85958.
2. Wipe exocervix with the white-stemmed sterile swab, removing the excess mucus. **Discard** this swab.
3. Insert the **blue**-stemmed swab into the endocervical canal. Rotate 10-30 seconds and withdraw.
4. Transfer the specimen swab to the Aptima Swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

Female vaginal

1. Obtain collection pack from Medical Distribution Center, item number ESI87648
2. Insert swab about 2 inches past the introitus and gently rotate the swab for 10-30 seconds. Ensure the swab touches the walls of the vagina.
3. Carefully withdraw the swab without touching the skin.
4. Transfer the specimen swab to the Aptima Swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

Male Urethral

1. Obtain collection pack from Medical Distribution Center, item number ESI85958.
2. Instruct patient not to urinate at least 2 hours prior to sampling.
3. Insert the **blue**-stemmed swab 2-to 4 cm into the urethra. Rotate 3 to 5 sec and withdraw.

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4. Transfer the specimen swab to the Aptima Combo swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

Pharyngeal

1. Obtain collection pack from Medical Distribution Center, item number ESI85958.
2. **Use the blue shaft swab for collection.**
3. Swab area between the tonsillar pillars and the region posterior to the pillars.
4. Transfer the specimen swab to the Aptima Combo swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

Rectal

1. Obtain collection pack from Medical Distribution Center, item number ESI85958.
2. For **ASYMPTOMATIC** men: Moisten swab with sterile saline and insert into anus and rectum. Leave for 20 seconds. For **SYMPTOMATIC** men: Swab rectal mucosa through the anoscope.
3. **Use the blue shaft swab for collection.**
4. Transfer the specimen swab to the Aptima Combo swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

Gonorrhea Culture

1. Obtain charcoal swab from microbiology, Meyer B1-111, 5-6510.

Chlamydia/Gonorrhea Urine Male/Female

1. Instruct patient not to urinate at least 2 hours prior to sampling.
2. Provide a plastic, preservative-free, sterile urine collection cup with a secure lid.
3. Instruct the patient to catch the **FIRST 10-30mL** of the urine stream. (You may want to mark the outside of the cup to show the desired volume.) Caution the patient not to begin urinating until the collection cup is in position.
4. Close the lid securely.
5. Transfer urine from the urine cup into the Aptima Urine Collection tube using a transfer pipet (provided) until the liquid level rises to **between** the 2 black lines on the tube.
6. Cap and label the tube with patient ID and date.
7. Transport the specimen to the lab as soon as possible.

Endometrium

1. Place the patient in the lithotomy position.
2. Insert speculum and visualize the cervical os.
3. Place a narrow-lumen catheter within the cervical os.

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4. Insert the tip of a culture swab through the catheter and collect the endometrial specimen. This method prevents touching the cervical mucosa and reduces the chance for contamination.
5. Place the culture swab into bacterial transport media and transport at ambient temperature.

Urethra

Refer to [Chlamydia/Gonorrhea](#).

Vaginal

Vaginal cultures do not often produce meaningful results. Group B Streptococcus will be ruled out on all vaginal cultures. If gonorrhea is suspected, testing by nucleic acid detection is recommended. Refer to [Chlamydia/Gonorrhea](#). If yeast infection is suspected, a yeast culture should be ordered rather than a routine culture. Herpes Simplex Virus only will be ruled out on all vaginal viral cultures. Please place HSV cultures in viral transport media.

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Molecular Microbiology Lab / JHH Medical Microbiology Division

Viruses

Test	Test code	Specimen types *	Vol requested	Analytical sensitivity [†]	Cut off: must receive by
EBV Quantitative (viral load)	7927	whole blood (at least two 3-ml purple top tubes)	2 x 3 ml (more if low WBC)	50 copies/10 ⁵ lymphs, measure 1.7-7 log ₁₀ copies/10 ⁵ lymphs	8 AM Tues, Thurs
EBV Qualitative	7122	CSF	1.0 ml total if multiple tests ordered ‡ Exception for vesicle: swab fluid & send in 2 ml viral transport medium (VTM)	6-60 copies/rxn	8 AM Mon - Fri
CMV	7130	CSF vitreous humor amniotic fluid BAL		20-200 copies/rxn	
HSV 1+2	7135	CSF vitreous humor BAL		10 copies/rxn	
VZV	7131	CSF vitreous humor vesicle fluid (VTM ok [‡]) BAL retinal biopsy		10 copies/rxn	
JC Qualitative	7901	CSF		100 copies/rxn	
JC Quantitative (viral load)	7305	Plasma (purple or PPT)	1.0 ml	400 copies/ml, measure 2.6 to 7.5 log ₁₀ copies/ml	8 AM Thurs
Enterovirus	7113	CSF respiratory specimens in Viral Transport stool or rectal swabs plasma (purple or PPT)	1.0 ml	0.1 pfu/ml	8 AM Mon - Fri
CMV Quantitative (viral load)	7929	<u>Plasma - use PPT tubes!!</u> <i>Separate & freeze within 4 hr of collection!!</i> PPT = plasma preparation tube	1.0 ml	300 copies/ml, measure 2.5-8.0 log ₁₀ copies/ml	12 Noon Mon - Fri
CMV Genotype	7921	whole blood (purple) culture isolates BAL vitreous fluid	Fluids: 0.5 ml	1000 copies/mL	Call lab 5-2642 for more info
Parvovirus B19	7127	Plasma (purple or PPT) or serum (red) bone marrow (purple) amniotic fluid	1.0 ml	100 copies/rxn	8 AM Mon - Fri
BK Quantitative (viral load)	7951	Plasma (purple or PPT)	1.0 ml	200 copies/ml, measure 2.3 to 7.0 log ₁₀ copies/ml	9 AM Mon, Wed, Fri
BK Qualitative	7129	Urine	1.0 ml total for one or both viruses	10 copies/rxn	
Adenovirus	7109	Urine		1 copy/rxn	
Adenovirus Quantitative (viral load)	7955	Plasma (purple or PPT)	1.0 ml	100 copies/rxn	

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Adenovirus Genotype	7952	Plasma (purple or PPT)	1.0 ml	unknown	Call lab 5-2642 for more info
HPV high risk (Hybrid Capture)	7919	Post-Autocyte Pap-smear preps cervical samples (Digene devices only)	Requests must go through Cytopathology !!!		7:30 AM Tues & Thurs

Hepatitis

Test	Code	Specimen: plasma*	Vol	Analytical sensitivity [†]	Cut off: must receive by
HBV DNA Quantitative	6046	<u>Use PPT tubes!!</u> <u>Centrifuge, Separate & freeze within 4 hr of collection!!</u>	1.0 ml	29 IU/mL, measure 1.46 to 8.04 log ₁₀ IU/mL	7:30 AM Thurs
HCV RNA Quantitative	2915		1.0 ml	43 IU/mL, measure 1.6 to 7.8 log ₁₀ IU/mL	7:30 AM Tues, Wed, Thurs
HCV Genotype	7195		0.5 cc	≈ 4,000 IU/ml	7:30 AM Mon

Bacteria

Test	Code	Specimen types / requested volumes	Vol.	Analytical sensitivity [†]	Cut off: must receive by
Ehrlichia (HGE & HME)	8526	whole blood (purple)	1.0 ml	Send Out	8 AM Mon - Fri
Mycoplasma pneumoniae	7106	throat swab (dry) throat swab (in Viral Transport) BAL	BAL= 2.0 mls		
Legionella pneumophila	7107	BAL	BAL = 1.0 ml		
Chlamydia pneumoniae	7110	throat swab (dry) throat swab (in Viral Transport) BAL	BAL= 1.0 ml		
Bordetella pertussis	7111	NP swab using rayon swab in pertussis pack		0.2 cfu/rxn	7 PM Mon - Fri
CT/NG (Chlamydia trachomatis / Neisseria gonorrhoeae)	7196-CT 7197-NG	Endocervical, urethral, rectal or throat swabs in Aptima Unisex Swab Kit Conjunctival swabs: in 3 ml of M4 media Urine: 10 to 15 cc with 2ml transferred into Aptima Urine Kit		CT: 5 IFU/ml (urine), 7.25 IFU (swab) NG: 250 cells/ml (urine) 362 cells (swab)	Anytime (assay Mon - Fri)
CDIFF PCR	7079	Liquid or soft stool (takes shape of sterile container)		10 copies/rxn	Anytime (assay Sun - Sat)

* No green top tubes! Heparin interferes with PCR.

† Analytical sensitivity for purified DNA - this may or may not correlate with clinical sensitivity; for culturable viruses, sensitivity is generally similar to or higher than that of cell culture. "Measure" indicates measurable range for quantitative assays.

Methodologies employed: HCV Genotyping and CMV Genotyping are sequenced-based, CT/NG are TMA – Transcription-Mediated Amplification, and the remaining assays are Real Time PCR-based.

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Nares Surveillance

Instructions for Proper Nares Cultures Technique:

When obtaining a swab sample for surveillance culture (MRSA), the technique is as follows:

1. Grasp the swab cap with fingers. (Be careful to avoid contacting the swab or stick with your fingers).
2. Withdraw the swab; sweep around the interior surface of the anterior nares. (Do both sides with one swab.)
3. Carefully plunge the swab into the media tube.
4. Label the tube with the patient's name, specimen (nares culture) and date.
5. Send to microbiology lab with a requisition slip.
6. Nares swabs are only acceptable for MRSA surveillance, not routine culture.

Nasopharyngeal

Aspirates/Washings (virus only)

1. For aspirate, attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter. Turn on suction and adjust to suggested pressure.
2. Without applying suction, insert catheter into the nose, directed posterior and toward the opening of the external ear.

Note: Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior nares and external opening of the ear.

3. Apply suction. Using a rotating movement, slowly withdraw the catheter.
4. Rinse tubing with M4RT for viral culture.
5. For washings, suction 3-5 ml of sterile saline into a new sterile bulb.
6. Insert bulb into one nostril until nostril is occluded.

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7. Instill saline into one nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
8. Empty bulb into suitable dry, sterile specimen container or add 3 ml or less to viral transport media (M4RT).
9. Transport immediately at ambient temperature.

Swab (viral culture and Pertussis PCR - not acceptable for rapid viral testing)

1. Seat the patient comfortably and tilt the head back.
2. Insert a nasal speculum.
3. Insert a nasopharyngeal swab (on a malleable wire) through the speculum into the nasopharyngeal area.
4. Rotate the swab gently and allow to remain for 20-30 seconds.
5. Remove the swab and place in a nongrowth- promoting transport medium (such as the culette container from which the original swab has been removed). Place swab in viral transport media for viral cultures.
6. Transport at ambient temperature.

Notes:

- Transport media must be used because the swab tip is small and vulnerable to drying. The organisms likely to be present are fastidious.
- For infants, special bulb suction procedures are available.
- If unusual organisms such as *Bordetella pertussis* are suspected, special culture media is necessary for collection and transport. (Refer to [Bordetella pertussis Culture and PCR.](#))

Swab (Flocked swab for rapid respiratory virus testing)

- 1 Copan[®] brand flexible **flocked sterile swab applicator** (SAP #114949)
- 1 **Viral Transport Medium** tube (SAP # 44674)

1. Peel open the pouch containing the collection swab and remove the swab. Holding the swab near the patient's head, **visualize the distance from the patient's nostril to the front of the ear.**
2. Use the thumb and forefinger of a gloved hand to grip the swab shaft at a point **equivalent to half the distance measured in step 1.** This distance approximates the mid-inferior turbinate sampling site.
3. Tilt the head of the patient backwards slightly. Have the patient close their eyes as this helps minimize discomfort. Gently insert the swab through one of the nostrils and horizontally into the nasal passage up to the measured distance on the swab shaft or until resistance is met. Rotate the swab 2 or 3 times and then hold the swab in place for 5-10 seconds to absorb the sample material.

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4. Remove the swab and insert into the **Viral Transport Medium Tube**. **Break the plastic shaft swab at the break point line**. Replace cap and screw on tightly. Apply label. Place in biohazard transport bag and send to lab via the pneumatic tube.

For video demo of the procedure, click here (no speakers/headphones required):

<http://copanusa.com/products/flockedswabs/animation.php>

Dr. Kevin Fonseca's videos have the best depictions of this procedure.

Additional education:

<http://www.copanusa.com/downloads/education/>

Nose

1. Collect anterior nares culture with a regular cotton swab. In small children, use a nasopharyngeal swab to facilitate collection.
2. Transport at ambient temperature.
3. **Note: This is an inappropriate specimen for anything other than the assessment of staphylococcal colonization.**

Prostate

1. Cleanse the glans with soap and water.
2. Obtain prostate fluid by digital massage through the rectum.
3. Collect fluid using a sterile swab.
4. Transport at room temperature.
5. Alternatively, a urine specimen obtained immediately before and after massage may be submitted for culture.

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Sputum

1. Assure patient cooperation to get an adequate specimen.
2. Instruct the patient as follows:
 1. Rinse mouth with tap water to remove food particles and debris.
 2. Have patient breathe deeply and cough several times to achieve a deep specimen.
 3. Patient should expectorate into dry, sterile container.
 4. *Tuberculosis patients should expectorate sputum in the early morning, into a sterile container with lid sealed tightly. Leaking specimens may be cancelled. Collection containers are to be obtained from Central Storage. Microbiology lab does not supply collection containers.*
3. Transport immediately at ambient temperature. Refrigerate if a delay of more than one hour is anticipated.
4. Expecterated sputum is acceptable for bacterial, mycobacterial, and fungal cultures. Not acceptable for viral cultures. Microbiology will determine the number of squamous epithelial cells present for specimen adequacy and reject samples for bacterial culture that are not indicative of deeply expecterated specimens.
5. Patients with clinical and chest x-ray findings compatible with TB should collect 3 first morning sputums (preferably on 3 separate days) for AFB culture.

Induced Sputum

Induced sputum is collected by Pulmonology and nursing staff on OSL-8. Induced sputum is acceptable for Legionella, PCP (on ice), fungal, and AFB testing. Not acceptable for viral cultures or routine bacterial cultures.

Stool, Feces

1. Collect specimen in a clean bed pan or use plastic wrap placed between the toilet seat and the bowl. Do not submit feces contaminated with urine or toilet water.
2. Transfer specimen into a clean, dry container or the appropriate preservative.
3. Transport at ambient temperature within two hours of collection.

Notes:

- Stool samples collected on patients hospitalized longer than 3 days prior to collection are not acceptable for routine enteric culture.
- Only loose or diarrheal stools are recommended for routine bacterial and *C. difficile* cultures and PCR. A limit of one sample per week for *C. difficile* testing
- Place the specimen in an appropriate stool preservative or transport media, immediately after collection. For ova and parasite, use 10% formalin and modified PVA; for routine stool culture, use Cary-Blair transport media.
- If a stool specimen is not available, the following are suitable alternatives for culture:
 1. A swab of rectal mucus, or
 2. A rectal swab inserted one inch into the anal canal (not acceptable for Rotavirus/ Adenovirus EIA or *C difficile* testing).

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- For CMV colitis, culture of biopsy tissue is preferred. Stool is frequently toxic to cultured cells and virus is infrequently recovered from this source.

Throat

1. Use a cotton, Dacron (for viral culture), or calcium alginate swab.
2. Use a tongue blade and a good light source to ensure good visualization.
3. Reach behind the uvula and swab:
 1. both tonsillar fauces, and
 2. the posterior pharynx, and
 3. any ulceration, exudate, lesion, or area of inflammation.
4. Place the swab into the appropriate transport media and transport at ambient temperature.
5. Bacterial culture requests are only tested for Beta-hemolytic streptococci and *Arcanobacter hemolyticum*.

Tissue

1. Tissue collection is an invasive procedure and requires surgery by a trained physician.
2. Collect tissue aseptically. Include material from both the center and the edge of the lesion.
3. Place the specimen in a sterile container on sterile gauze moistened with sterile **nonbacteriostatic** saline.
4. Transport in less than an hour at ambient temperature, in a manner to ensure recovery of anaerobic organisms. For virology cultures, do not allow the tissue to dry and transport in viral transport media (M4RT).
5. Do not submit tissue in formalin.
6. **Do not jam the tissue into a Culturette using the swab; this is not an acceptable transport device.**

Urine for Bacterial, Fungal, AFB, Parasite and Viral Cultures

1. Instructions for female patients to collect midstream urine for bacterial culture:
 1. Remove undergarments.
 2. Wash hands thoroughly with soap and water, rinse them, and dry them on a disposable paper towel or shake off excess water.
 3. Spread labia, with one hand, and keep them continuously apart.
 4. Open the WASH PACK and wash the urinary opening and the surrounding area. Discard the cloth in the waste basket.

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5. Take the open sterile cup in the other hand without touching the rim or inner surface of the cup or lid.
 6. Void 20 to 25 ml into the toilet and catch a portion of the rest of the urine in the container without stopping the stream. Do not touch the legs, vulva, or clothing with the cup.
 7. Place the lid securely on the cup.
 8. Immediately transfer to the urine bacterial culture transport media.
2. Instructions for male patients to collect midstream urine for bacterial culture:
1. Wash hands.
 2. Retract the foreskin completely.
 3. Wipe head of penis in a single motion with first towelette. Repeat with second towelette. If not circumcised, hold foreskin back before cleansing.
 4. Void 20 to 25 ml into the toilet and catch a portion of the remaining urine in the cup without stopping the stream. Do not touch the cup with the penis.
 5. Place the lid on the cup securely
 6. Immediately transfer to the urine bacterial culture transport media.
3. First void urine for nucleic acid amplification tests (Chlamydia/ Gonorrhea).
1. Patient must not have urinated during the previous two hours.
 2. Collect the **first 10 to 30 ml** of the urine stream in a clean, empty plastic cup.
 3. Transfer 2 mls of urine in test-specific transport media.
4. Suprapubic aspiration:
1. This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in the literature.
 2. Faculty approval required for anaerobic culture, call 5-5077. Specimen should be submitted in an anaerobic environment if an anaerobic culture is approved.
5. Indwelling catheter urine:
1. Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
 2. Clean the catheter with an alcohol pad.
 3. Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
 4. Transfer the urine to a sterile specimen container or appropriate transport media.
 5. Urine catheter tip cultures are not acceptable.
6. Specimen handling:
1. Label the container immediately.

Notes:

- a. Urine for CMV culture must be received within 1 hour of collection.
- b. Minimum urine volume for AFB culture is 40 ml.

Viral Transport Media (M4RT)

Some samples can be submitted, without utilizing a transport media, with a reasonable expectation of virus viability. Specimens in this category include, sterile fluids such as cerebrospinal fluid, pleural fluid, blood, urine, as well as some nonsterile specimens such as bronchoalveolar lavage, and feces. Whenever there is a question of stability, the specimen should be placed in a suitable virus transport

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media such as M4RT. Refer to specific test in the alphabetical test list of this User's Guide for more information.

1. Tissue and biopsy material can be placed directly into the viral transport media (M4RT). Each sample need not be more than 1-2 cm in diameter.
2. Abscess material, bullae, pustules, vesicles, lesions, and skin scrapings can be collected on a Dacron swab and placed directly into viral transport media. If the material has been aspirated, place no more than 3 ml (equal to the amount of transport media) in the vial of M4RT.
3. CSF should be submitted in a sterile container.
4. Urine should be submitted in a sterile container.
5. Rectal swab (Dacron only) should be submitted in M4RT.
6. Blood should be submitted in a Heparin tube.
7. Swabs that are made of calcium alginate and wood are known to interfere with the recovery of some viruses. These can also act as PCR inhibitors and are not appropriate for this type of testing.
8. For CMV colitis, culture of biopsy tissue is preferred. Stool is frequently toxic to cultured cells and virus is infrequently recovered from this source.
9. Mumps: Acceptable specimens for culture include buccal swab after parotid gland massage and urine.

Wounds

1. For closed wounds, refer to [Abscess](#) and [Bullae, Cellulitis, Vesicles](#).
2. For open wounds:
 1. Clean the sinus tract opening of the wound surface mechanically, without using a germicidal agent, to remove as much of the superficial flora as possible.
 2. Attempt to culture the base or edges of the wound to avoid collecting "normal flora" organisms.
 3. The following are preferred specimens for sinus tracts:
 1. Aspiration of material obtained by needle or catheterization.
 2. Curettings from the lining of the sinus tract.
 4. Swabs of the sinus tracts are acceptable only if the above cannot be obtained. Swabs of sinus tracts may not accurately reflect the underlying disease process.
 5. Do not submit cultures of superficial lesions for anaerobic culture. Biopsy of advancing margin of wound is the preferred specimen for anaerobes, mycobacteria and fungi.