Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 6/2019

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, eSwabs for bacterial culture, Cary-Blair for bacterial stool NAT testing, VTM for viral and Chlamydia cultures, and urine boric acid transport for bacterial urine cultures). Check expiration date before inoculating collection device. For more information, see Specimen Collection Containers link which has collection device pictures and SAP numbers for ordering.
4. Whenever possible, collect specimens prior to administration of antimicrobial agents.
5. Properly label the specimen (a minimum two patient identifiers are required) and order appropriately in EPIC.
    - The specific specimen type is required in EPIC. Example: Specimen type-Wound.
    - To document completely, add a specimen source as well. Example: Specimen source-Left Foot.
6. Send the specimen to the laboratory as soon as possible after collection.
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 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 and swabs will not be accepted.

NOTE: eSwabs are a poor choice because of the limited amount of material obtained. Swabs are not optimal for fungal culture, anaerobe cultures, or decubitus ulcers. Procurement of tissue aseptically from the site of infection is recommended. Swabs are **not** accepted for mycobacterial cultures, perirectal abscesses, and oral abscesses. Gram stains cannot be provided from a single swab unless an eSwab is used.

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Contents: By Specimen Type

Abscess

1. Decontaminate the surface with 70-95% alcohol and 1-2% tincture of iodine.

2. Collect purulent material aseptically:

   - From an undrained abscess: use a sterile needle and syringe.

   - For large abscesses: open with a sterile scalpel and collect the expressed material with a sterile syringe.

3. Transfer 5-10 ml of the aspirated material to the appropriate transport media based on test being requested. See Test Directory for specific guidelines for each test. **Note:** *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. Transport immediately.

5. Note requests to rule out *Actinomyces* sp., *Cutibacterium* (formerly *Propionibacterium*) *acnes* sp. or *Nocardia* sp. on the requisition/EPIC.

Amniocentesis

1. Usually collected by ultrasound method by a physician. Send to lab in appropriate transport based on the tests requested.

Arthropod

1. Arthropod specimens (ticks, lice, nits, bed bugs, etc.) should be collected using tweezers to remove or extract from the skin if attached. Immerse parasite in 5-10 mL of 70-80 percent ethanol (or other alcohol) in a clean container and secure the lid well to prevent leaking. Make sure to keep the arthropod as intact as possible, identification is performed by visual analysis.
2. If scabies is suspected, scrape the skin from the leading edge of the lesion. Place in 2-3 mL of 70-80 percent ethanol (or other alcohol) in a clean container.
3. Identification is performed at a reference laboratory.

Blood

A. Blood Culture

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. Please refer to the JHH Interdisciplinary Clinical Practice Manual Blood Culture Procurement Policy and Procedure Appendix A. https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101712.pdf

1. **Adult Blood cultures:**

- Routine Blood Culture Set: BACTEC FX standard aerobic and BACTEC FX Lytic anaerobic bottle
  - Used for patients that are not on antibiotics
- Alternate Blood Culture Set: BACTEC FX Aerobic Plus (resin bottle) and BACTEC FX Lytic anaerobic bottle
  - Patient is on IV or PO antibiotics
  - Patient has been off antibiotics for less than 24 hours
  - Suspected *Neisseria* sp. or other fastidious organisms
- 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 A of the JHH Interdisciplinary Clinical Practice Manual Infection Control Blood Culture Policy and Procedure.

- 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.

- For complete procedure see: https://hpo.johnshopkins.edu/hopkins/policies/39/4282/policy_4282.pdf?_=0.083803167459

2. **Pediatric Blood cultures:**
   - Pediatrics shall follow the same policies and procedures as described in this policy. See Appendix B of the ICPM policy (PAT063) Blood cultures: ordering, procurement and transport.

     https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101714.pdf?_=0.921568357146

3. **Mycobacterial blood cultures (AFB)**
   - Use a Mycobacterial blood culture bottle (BD BACTEC Myco/F Lytic culture bottle). Because the media is unstable, bottles must be obtained from the Microbiology Lab (5-6510 option #1). Pneumatic tube can be used to obtain culture media. When returning the sample to the lab, make sure it is returned in the brown paper bag it is sent in to avoid exposure to light.

4. **Fungal Cultures**
   - ***Candida* spp. -** If a physician orders fungal cultures, follow routine procedure for bacterial cultures as described above.
   - ***Cryptococcus, Histoplasma*, or other filamentous organisms--**obtain an Isolator tube from the Microbiology Lab (call 5-6510 option #1).
     - **Adults:**
       - Inoculate isolator tube with 10ml of blood.
     - **Pediatric From children < 8 kg body weight**
       - Inoculate Pediatric isolator tube with 1.5 ml of blood.

5. **Viral blood cultures**: See Molecular Section

6. **When rare organisms such as *Brucella*, *Campylobacter* or *Bartonella* are suspected:**
    - An ID physician shall be consulted.
    - The Microbiology laboratory shall be consulted to advise which type of specimen is most likely to support the suspected organism.

B. Blood Parasites: Malaria, Babesiosis, Trypanosomiasis, and Filariasis

1. Draw 3 ml of blood in a lavender top (EDTA) vacutainer tube using the standard venipuncture procedure.
2. Deliver the tube to the Microbiology lab immediately or within 2 hours of collection.
3. Indicate patient's travel history (if available) and suspected pathogen (i.e., rule-out *Loa loa* filariasis).

NOTE: The submission of a single blood specimen does NOT rule out malaria (especially in immunologically naïve patients); submit additional bloods every 24 hours for up to 3 days if malaria remains a consideration.

C. Aspergillus galactomannan antigen

1. Collect 3-5 mL blood in a serum separator tube (SST) without anticoagulants.
2. Transport the specimen to the lab as soon as possible.

D. 1-3, Beta-D-glucan test

1. Collect 3-5 mL blood in serum separator tube (SST) without anticoagulants or in a gold top tube.
2. Transport the specimen to the lab as soon as possible.

E. HIV Serology

1. Collect 6 mL blood in a pink top tube with EDTA
2. Transport the sample to the lab as soon as possible.

Body Fluids, Sterile (except urine and cerebrospinal fluid)

1. Prepare the skin as for Blood culture.
2. Collect the fluid using a sterile needle and syringe and place in transport container based on test being requested:
    - For aerobic organisms submit 10 ml in a sterile container. (30 mL for pleural fluid)
    - For aerobic and anaerobic organisms, submit 10 ml in anaerobe transport. (30 mL for pleural fluid)

- For viral isolation, send 3 ml or less fluid in a sterile vial (1 ml minimum).
- If mycobacterial or fungal infections are suspected, collect a minimum of **5 ml** of fluid into a sterile container.
- If testing for multiple labs, add up the volume needed, based on the above volumes, collect in a sterile container and deliver to the lab within 1 hour.

3. Transport immediately.

******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 Culture.
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:

    - Bacterial culture requests, inoculate into a blood culture bottle - do not send in a heparin tube.
    - AFB culture and fungal culture into a mycobacteria/fungal blood culture bottle (Myco/F Lytic bottle – available in microbiology lab, call 5-6510 option #1).
    - Viral culture-collect into a heparinized (green top) tube.
    - 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 B-171, 955-6510 option #1.
2. Provided in the collection system are two swab/swab transport packages.

    - The package with collection materials for *Bordetella pertussis* culture contains a swab with flexible wire shaft (orange handle) and a charcoal

tube for swab transport containing black medium into which the swab should be placed once the specimen has been collected.
    - THE ORANGE HANDLED SWAB IS OPTIMIZED FOR BACTERIAL CULTURE AND CONTAINS MATERIAL THAT INHIBITS PCR. DO NOT USE FOR SPECIMENS TO BE TESTED BY PCR.
- The package with collection materials for *Bordetella pertussis* PCR contains a swab with a flexible wire shaft (blue handle) that will be placed into the accompanying tube containing a sponge for dry transport.
- Both collection systems can be stored at room temperature.

3. To collect the nasopharyngeal swab specimen for culture, remove the orange handled swab from the package and:
    - Seat the patient comfortably. Tilt the head back.
    - If available, insert a nasal speculum. Press the swab through the nares until resistance is met due to contact with the nasopharynx.
    - Rotate the swab gently and allow the swab to maintain contact with the nasopharynx for 20-30 seconds or until coughing is induced.
    - 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.
4. To collect the nasopharyngeal swab for PCR, remove the blue handled swab from the package and repeat collection steps above after inserting the swab into the alternate nares. Place the swab into the sponge-containing tube. Label this tube with the patient's name and identification number. Leave the swab embedded in the tube during transport.

Bronchial Brush/Wash/Lavage

1. This technique should be performed by an experienced individual. See HPO procedure: Bronchoscopy and Associated Procedures ENDO628.

2. Transport in a sterile container immediately at ambient temperature.
    - **Immunocompromised host protocol**: 40 mL of BAL fluid
    - ***Aspergillus* galactomannan antigen** (Bronchial lavage): Please send 1-2mL for galactomannan testing (minimum 1mL).
    - ***Pneumocystis jirovecii*,** Direct Immunofluorescence stain
        - Submit bronchial washings and bronchoalveolar lavage for PCP testing in a sterile container. Testing is performed once daily on weekdays and

weekends. A second run is performed on BAL's and bronchial washings on Fridays if received by 7:00 PM. On holidays and weekends testing will be performed on specimens received by 2:00 PM.

Bullae, Cellulitis, Vesicles

1. Cleanse the skin as for Blood culture.
2. Aspirate the fluid/purulent material using a sterile needle and syringe.
    - If an aspirate is obtained, place in appropriate viral or bacterial transport tube or vial.
    - If no material is obtained, unroof vesicle or bullous lesion and use an eSwab to collect cells from the base of the lesion. Place in appropriate viral or bacterial transport media.

A. 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. Place the biopsy in a sterile container with a small volume of non-bacteriostatic saline and transport to the lab as soon as possible.

B. Vesicle Fluids and Scrapings for Viral Testing

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 viruses since neutralizing antibodies may be present in the serum.

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% tincture of iodine, followed by 70-90% alcohol 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:

    - bacterial culture 1-5 ml
    - fungal culture 5-10 ml
    - molecular 1.5-2 ml
    - mycobacterial culture 5-10 ml
    - viral culture 1.5-2 ml

5. Cap the tubes tightly. Submit the third tube for culture to reduce the possibility of contamination due to skin-microbiota. Transport immediately at ambient temperature.

A. CSF for *Acanthamoeba* and *Naegleria* sp: Microscopic exam and culture

1. Collect 1 ml of spinal fluid in a sterile container.
2. Seal tightly and submit to the lab immediately for microscopic examination and culture.
3. Transport CSF immediately at ambient temperature.

Cutaneous (Fungal only)

A. 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:
    - Hair stubs
    - Contents of plugged follicles
    - Skin scales
    - Hair plucked from the scalp with forceps

***Cut hair is NOT an acceptable specimen.**

B. Nails

1. Cleanse the nail with 70-95% alcohol.
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:
    - Clippings from any discolored or brittle parts of the nail
    - Deeper scrapings and debris under the edges of the nail

C. Skin

1. Cleanse the skin with 70-95% alcohol.
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.

Ear

1. External ear cultures are processed as superficial wounds.

2. Middle ear fluid will be processed as a sterile 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 an eSwab (green cap-mini tip).
   - Place the swab into the eSwab transport media and transport at ambient temperature.
   - This is **NOT** an acceptable specimen for anaerobe culture.
   - Nucleic acid testing – *Chlamydia* only: collect specimen with bacteriology culturette swab and place in 3 ml of viral transport media. **Do not use Cobas Collection Kit.**

3. Corneal infections:
   - Obtain Cornea Pack from the Microbiology Laboratory, Meyer B-171, 5-6510 option #1.
   - 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.
   - Transport at ambient temperature.
   - Gram stain is not routinely performed.

4. Intraocular fluid:
   - Collect fluid by surgical needle aspiration.
   - Transport cultures at ambient temperature.

A. Amoeba Culture: Contact lens, contact lens solution, corneal scrapings/tissue

Please contact the Microbiology Laboratory at 410-955-6510 (option #2) to coordinate amoeba culture. Special media is required. Culture media will be provided to the requesting healthcare provider to inoculate the specimens at bedside (optimal for recovery). If bedside inoculation is not possible, please follow directions below for specimen collection:

Contact lens and corneal scraping or corneal tissue

1. Submit the specimen in a sterile container with 1 mL of sterile saline.
2. Keep at room temperature.
3. Deliver to the lab immediately.

Contact lens solution

1. Submit 2 ml of contact lens solution in a sterile container.
2. Keep at room temperature.
3. Deliver to the lab immediately.

Gastric Biopsy

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. This is sent to a reference laboratory.

Genital Sources

Routinely processed only for gonococcal infections. Predominance of *S. aureus*, Beta hemolytic streptococci and yeast reported 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.

A. Bartholin's Glands

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

B. 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 an eSwab into the cervical os, rotate gently, and allow to remain for 10 to 30 seconds.
6. Remove eSwab and place in bacterial transport medium.
7. Transport at ambient temperature.

C. Cervix (Endocervix) for HPV DNA Testing

1. Samples are referred to Molecular Microbiology from the Johns Hopkins Cytopathology laboratory. Samples are sent in SurePath collection devices.

D. *Chlamydia trachomatis/Neisseria gonorrhoeae/Trichomonas vaginalis* Nucleic Acid Amplification Tests - Acceptable Sources

	Cervix	Vagina	Rectum (male/female)	Pharynx (male/female)	Urine (male/female)
Chlamydia trachomatis	√	√	√	√	√
Neisseria gonorrhoeae	√	√	√	√	√
Trichomonas vaginalis	√	√			√

- ***Chlamydia trachomatis/Neisseria gonorrhoeae/Trichomonas vaginalis*** **Nucleic Acid Amplification Tests - Specimen Collection Procedures**

  - **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 Cobas 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.

  - **Female cervical**

1. Obtain collection pack from Consolidated Service Center (CSC), SAP number 233352.

2. Wipe exocervix with the white-stemmed sterile swab, removing the excess mucus. **Discard** this swab.
3. Insert the flocked swab into the endocervical canal. Rotate 10-30 seconds and withdraw.
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit 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 Consolidated Service Center (CSC), SAP number 233352.
2. Insert either flocked swab **or** woven 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. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit 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.

Note: A single cervical or vaginal swab may be submitted for Chlamydia, Gonorrhea, and Trichomonas testing.

- **Pharyngeal (Male/Female)**

1. Obtain collection pack from Consolidated Service Center (CSC), SAP number 233352.
2. **Use the flocked swab for collection.**
3. Swab area between the tonsillar pillars and the region posterior to the pillars.
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit 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 (Male/Female)**

1. Obtain collection pack from Consolidated Service Center (CSC), SAP number 233352.
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 flocked swab or woven swab for collection.**
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit 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

- Obtain charcoal swab from microbiology, Meyer B-171, 5-6510 option #1.

  - ***Endometrium***

    - Place the patient in the lithotomy position.
    - Insert speculum and visualize the cervical os.
    - Place a narrow-lumen catheter within the cervical os.
    - 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.
    - Place the culture swab into bacterial transport media and transport at ambient temperature.

  - **Urethra**
    - Instruct patient not to urinate at least 2 hours prior to sampling.
    - Insert the swab 2-to 4 cm into the urethra. Rotate 3 to 5 sec and withdraw.
    - Place the culture swab into bacterial transport media and transport at ambient temperature.

  - **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/Trichomonas. If yeast infection is suspected, a fungal culture should be ordered rather than a routine culture.

- **Group B *Streptococcus* screening**: collect combined vaginal and rectal swab using a culturette. Testing is performed using Lim broth enrichment followed by a nucleic acid test for detection.
- **Vaginosis/Vaginitis**: Collect vaginal swab specimens from **symptomatic** women prior to the speculum examination. **Note:** No lubricant should be used for the sample technique. If lubricant must be used for speculum insertion, the lubricant should be used sparingly and applied only to the exterior sides of the speculum blades, avoiding contact with the tip of the speculum.

  Use the BD MAX UVE specimen collection kit (available from the CSC: SAP # 242373) and follow the instructions. Hold the collection swab by the cap and insert it into the vaginal opening about 2 inches (5 cm) gently rotating the swab for 10-15 sec. Make

sure the swab touches the walls of the vagina so that moisture is absorbed by the swab. Withdraw the swab and place it in the UVE sample buffer tube

Molecular Testing

Virus	Sources	Specimen
Qualitative NAT		
Adenovirus	▪ Conjunctiva ▪ Urine ▪ Respiratory(BAL, Endo/naso trach, NP swab, NP wash ▪ Stool ▪ Rectal swab	▪ Swab in 2.0 mL viral transport media (VTM or UVTM) ▪ Sterile container ▪ Sterile container or mini flocked swab in viral transport for NP swab. ▪ Sterile container ▪ Swab in 2.0 mL viral transport media (VTM or UVTM)
BK Virus	▪ Urine	▪ Sterile container
CMV	▪ CSF ▪ Amniotic fluid, urine ▪ Sterile Eye	▪ CSF collection container, tube #3 ▪ Sterile container ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM)
EBV	▪ CSF	▪ CSF collection container, tube #3
Enterovirus	▪ CSF ▪ Plasma ▪ NP aspirate ▪ Throat, NP swab	▪ CSF collection container, tube #3 ▪ Lavender top (EDTA) tube or pearl top (PPT/EDTA) tube ▪ Viral transport media (VTM or UVTM) ▪ Dacron or flocked swab in viral transport media (VTM or UVTM)
HSV 1+2	▪ CSF ▪ Sterile eye ▪ Lesion	▪ CSF collection container, tube #3 ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM) ▪ Dacron or flocked swab in viral transport media (VTM or UVTM)

	▪ Vesicle fluid	▪ Viral transport media (VTM or UVTM)
JCV	▪ CSF	▪ CSF collection container, tube #3
Virus	**Sources**	**Specimen**
VZV	▪ CSF ▪ Sterile eye ▪ Lesion ▪ Vesicle fluid	▪ CSF collection container, tube #3 ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM) ▪ Dacron or flocked swab in viral transport media (VTM or UVTM) ▪ Viral transport media (VTM or UVTM)
Respiratory Pathogen Panel: Flu A/B, RSV, Para flu 1/2/3/4, Rhino/Enterovirus, Metapneumo, Adenovirus, Mycoplasma	▪ BAL, Endo/Naso trach, NP wash ▪ NP swab	▪ Sterile container ▪ Mini flocked swab in viral transport
HPV High Risk + genotyping	▪ Cervix	▪ Original SurePath vial
Send out Tests (Qualitative NAT Viruses)		
Parvovirus B19	▪ Bone marrow, Amniotic fluid, Plasma	▪ Send to Customer Service for testing by Quest Diagnostics
CMV,HSV, VZV	▪ BAL	▪ Send to Customer Service for testing by Quest Diagnostics

JCV	▪ Plasma	▪ Send to Customer Service for testing by Quest Diagnostics
HIV	▪ Whole blood	▪ Send to Customer Service for testing by Quest Diagnostics
Virus	**Sources**	**Specimen**
Quantitative NAT		
Adenovirus	▪ Plasma	▪ Lavender top EDTA tube or Pearl top PPT tube.
BK virus	▪ Plasma	▪ Pearl top tube (PPT)
CMV	▪ Plasma	▪ Pearl top tube (PPT)
EBV	▪ Plasma	▪ Lavender top EDTA tube or Pearl top PPT tube.
HBV	▪ Plasma	▪ Pearl top tube (PPT)
HCV	▪ Plasma	▪ Pearl top tube (PPT)
HIV	▪ Plasma	▪ Pink top tube (EDTA)

Nares Surveillance

Instructions for Proper Nares Cultures Technique:

When obtaining an eSwab (SAP 173665 – Adult), (SAP 173666- Peds) sample for surveillance culture (MRSA), the technique is as follows:

- Grasp the swab cap with fingers. Be careful to avoid contacting the swab or stick with your fingers.
- Withdraw the swab; sweep around the interior surface of the anterior nares. (Perform both sides with one swab.)
- Carefully place swab in collection container and snap off shaft of swab. Make sure the cap is securely fastened.

- Label the tube with the patient's name, specimen or specimen bar-code (nares culture) and date.
- Send to microbiology lab with a requisition slip.
- Nares swabs are only acceptable for MSSA/MRSA surveillance, not routine culture.

A. Pre-op MRSA/MSSA Nasal Screen:

1. Collect anterior nares culture with a Dacron swab.
2. Transport at ambient temperature.

Note: This is an inappropriate specimen for anything other than the assessment of staphylococcal colonization.

Nasopharyngeal

A. Nasopharyngeal swabs for *Bordetella pertussis* Culture and PCR – see *Bordetella Pertussis* section**.**

B. Viral Respiratory Testing – Flocked Swab

- 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.

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 and additional education, click here:
http://www.copanusa.com/downloads/education/

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. If this is done, please indicate "pre" or "post" massage when ordering the urine culture.
    - Specimen type: Urine, other
    - Specimen source:
        - Pre-Prostatic Massage
        - Post-Prostatic Massage

Sputum

1. Assure patient cooperation to get an adequate specimen.
2. Instruct the patient as follows:
    - Rinse mouth with tap water to remove food particles and debris.
    - Have patient breathe deeply and cough several times to achieve a deep specimen.
    - Patient should expectorate into dry, sterile container.
    - Patients suspected of having tuberculosis should expectorate sputum in the early morning, into a sterile container with lid sealed tightly. Leaking specimens may be cancelled.
3. Transport immediately at ambient temperature. Refrigerate if a delay of more than one hour is anticipated.
4. Expectorated 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 expectorated specimens.

5. In patients with clinical and chest x-ray findings compatible with tuberculosis, collect 3 first morning sputum specimens (on 3 separate days) for AFB culture.

Induced Sputum

Induced sputum is collected by respiratory therapists and trained nursing staff. Induced sputum is acceptable for *Legionella* culture, *Pneumocystis jirovecii* (PCP), fungal, and AFB testing. It is not acceptable for viral cultures or routine bacterial cultures.

***Pneumocystis jirovecii*, Direct Immunofluorescence stain**

Submit induced sputum for PCP testing in a sterile container on ice. Induced sputum specimens will only be accepted on weekdays (M-F) from 3:00 AM until 3 PM. **No** induced sputum specimens will be accepted/tested on weekends or holidays. Testing is performed once daily on weekdays.

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.

    - **Unpreserved stool in a sterile container:**
        - Norovirus testing
        - *C. difficile*
        - Fecal lactoferrin testing
        - *H. pylori* stool antigen testing

      Transport to lab immediately. Stools sent in transport media, swabs or preservatives are not acceptable specimens.

    - **Cary-Blair transport medium:**
        - Bacterial pathogens (not including C. *difficile*): *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., Enterotoxigenic *E. coli*, *Yersinia enterocolitica*, *Vibrio* sp., Shiga-toxin producing *E. coli*, *Plesiomonas shigelloides*

      Transfer specimen into a Cary-Blair transport medium container. Transport at ambient temperature within two hours of collection.

- **Total Fix transport media:**
  - Enteric Protozaon Panel: *Giardia, Cryptosporidium, E. histolytica*
  - *Microsporidium*

  Transfer specimen into a Cary-Blair transport medium container. 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 testing.
- Repeat testing within the same diarrheal episode or within 7 days of a previous result are not acceptable for bacterial enteric testing.
- Only loose or diarrheal stools are recommended for routine bacterial and *C. difficile* cultures and PCR. There is a limit of one sample per week for *C. difficile* testing. Minimum testing volume is 1 mL.
- Place the specimen in an appropriate stool preservative or transport media, immediately after collection.
- If a stool specimen is not available, the following are suitable alternatives for testing:
  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).
- For CMV colitis, culture of biopsy tissue is preferred. Stool is frequently toxic to cultured cells and virus is infrequently recovered from this source.

A. Cyclospora/Isospora (syn. *Cystoisospora*) Stains – Stool, intestinal fluid or sputum

Staining for the intestinal gut coccidia is not performed with the standard stool O&P procedure. If gut coccidia are suspected, special staining procedures must be ordered and performed. Optimally, 3 fecal specimens collected over a 7 to 10 day period must be submitted in a Total-Fix container as intestinal parasites are shed intermittently. It is not acceptable to send more than one specimen collected on any given day.

1. Collect stool or intestinal fluid in the Total-Fix vial.
2. Keep at room temperature.
3. Testing is performed at Quest.

B. Microsporidia Stain

Staining for microsporidia is not performed with the standard stool O&P procedure. If intestinal microsporidia are suspected, special staining procedures must be ordered and performed.

1. Collect stool or intestinal aspirates in the Total-Fix vial. Keep at room temperature.
2. Collect respiratory specimens (BAL, sputum, bronchial wash, pleural fluid) in a sterile container. Keep refrigerated.

3. Collect fresh tissue (lung, eye, rectal, intestinal, colon, skin, muscle, kidney) in a sterile container and add a small amount of sterile saline (3-mm biopsy in 0.1 mL sterile saline).

C. Pinworm Exam, Perianal

Detection of the eggs of *Enterobius vermicularis* on the skin of the perianal folds.

1. Time of collection is best immediately upon arising in the morning. The patient should not shower or bathe, have a bowel movement, wipe or clean the rectal area, or apply ointment to the skin in the rectal area until after collection of the specimen.
2. Note: Up to 6 pinworm exams are required prior to considering the patient negative for pinworm.

Pinworm paddle kit (a paddle coated with adhesive material)

1. These can be obtained from the Microbiology laboratory. Please call 410-955-6510.
2. Please wear gloves while collecting the specimen.
3. Hold the paddles by the cap and remove it from the tube.
4. Using gentle pressure, press the sticky side of the paddle against the skin around the rectum 3 or 4 times.
5. Insert the paddle into its protective tube and tighten the lid.
6. Keep at room temperature.

D. Stool Enteric Protozoan Panel:

The Stool Enteric Protozoan Panel is a rapid and sensitive molecular test for the detection of *Giardia duodenalis*, *Cryptosporidium* species and *Entamoeba histolytica* from fecal specimens.

<u>This test is recommended as the first line diagnostic in all patient populations where intestinal parasites are being considered especially for immunocompetent patients with no travel history outside the US and Canada.</u>

Ordering prompts in EPIC will help guide practitioners to order the appropriate tests for intestinal parasites that are stratified by patient risk factors.

1. Stool in Total-Fix preservation system is preferred. Unpreserved stool specimens and Para Pak (10% Formalin (Pink Top) are acceptable.
2. Fill the Total-Fix or Para Pak 10% Formalin vial to the fill line. Please <u>DO NOT</u> overfill.
3. Keep at room temperature.
4. Indicate patient's travel history and if the patient is immunocompromised or not (if applicable).
5. Testing is performed on weekdays.

E. Comprehensive Stool Enteric Parasite Panel (EPIC test codes for JHMI inpatients: O307810 and JHMI outpatients: O307811):

This panel will include the Enteric Protozoan Panel (as described above), Ova & Parasite exam and Microsporidium staining. This test will be available to immunocompromised patients or to patients with a travel history outside of the US or Canada. Ordering prompts in EPIC will help guide practitioners to order the appropriate tests for intestinal parasites that are stratified by patient risk factors.

1. Stool in Total-Fix preservation system is required. Stools in Para Pak 10% Formalin (Pink Top) and PVA (Blue Top) are also are unacceptable (both tubes must be submitted). Unpreserved stool is unacceptable.
2. Fill the vials to the fill line. Please DO NOT overfill.
3. Keep at room temperature.
4. Indicate patient's travel history and if the patient is immunocompromised or not (if applicable).
5. Testing is performed on weekdays.

F. Stool Ova and Parasite Examination:

Standalone Ova and Parasite Exam (O&P) orders are discouraged as a first line diagnostic for intestinal parasites. *Giardia duodenalis* (syn. *G. lamblia* or *G. intestinalis*) and *Cryptosporidium* spp. are the most common pathogenic intestinal parasites identified in the US and other industrialized countries. O&P exam casts a broad diagnostic net for intestinal parasites; nonetheless it **is not** the diagnostic method of choice for the two most common parasites. Only patients with a previous Enteric Protozoan Panel result (either standalone or part of the Comprehensive Stool Enteric Parasite Panel as described above) within the last 30 days will be able to order a standalone O&P result. Ordering prompts in EPIC will help guide practitioners to order the appropriate tests for intestinal parasites that are stratified by patient risk factors.

If O&P exam is appropriate, 3 fecal specimens collected over a 7 to 10 day period must be submitted as intestinal parasites are shed intermittently. It is not acceptable to send more than one specimen collected on any given day.

1. Stool in Total-Fix preservation system is required. Stools in Para Pak 10% Formalin (Pink Top) and PVA (Blue Top) are also are unacceptable (both tubes must be submitted). Unpreserved stool is unacceptable.
2. Fill the vials to the fill line. Please DO NOT overfill.
3. Keep at room temperature.
4. Indicate patient's travel history and if the patient is immunocompromised or not (if applicable).
5. Testing is performed at Quest Diagnostics.

Throat

1. Use an eSwab (SAP 173665- Adult or SAP 173666- Peds).

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 eSwab collection tube provided and transport at ambient temperature.

Beta-hemolytic streptococci requests will be completed using the Rapid Strep Group A Nucleic Acid test for the detection of *Streptococcus* Group A only or Rapid Strep Group A Nucleic Acid test/reflex to culture for the detection of beta-hemolytic streptococci and *Arcanobacterium haemolyticum.*

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 **non-bacteriostatic** 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 (VTM).

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, Parasitology and Viral Cultures

1. **Instructions for female patients to collect midstream urine for bacterial culture:**
    a. Remove undergarments.
    b. Wash hands thoroughly with soap and water, rinse them, and dry them on a disposable paper towel or shake off excess water.
    c. Spread labia, with one hand, and keep them continuously apart.

d. Open the WASH PACK and wash the urinary opening and the surrounding area. Discard the cloth in the waste basket.
e. Take the open sterile cup in the other hand without touching the rim or inner surface of the cup or lid.
f. 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.
g. Place the lid securely on the cup.
h. Immediately transfer to the urine bacterial culture transport media (gray top tubes).
i. Low volume urine (less than 3 mL) send in sterile cup.

2. **Instructions for male patients to collect midstream urine for bacterial culture:**
   a. Wash hands.
   b. Retract the foreskin completely.
   c. Wipe head of penis in a single motion with first towelette. Repeat with second towelette. If not circumcised, hold foreskin back before cleansing.
   d. 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.
   e. Place the lid on the cup securely.
   f. Immediately transfer to the urine bacterial culture transport media.
   g. Low volume urine (less than 3 mL) send in sterile cup.

3. **First void urine for nucleic acid amplification tests – males and females (Chlamydia/ Gonorrhea).**
   a. Patient must not have urinated during the previous two hours.
   b. Collect the **first 10 to 30 ml** of the urine stream in a clean, empty plastic cup.
   c. Transfer 2 ml of urine in test-specific transport media.

4. **Suprapubic aspiration:**
   a. This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in the literature.
   b. 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:**
   a. Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
   b. Clean the catheter with an alcohol pad.
   c. Use BD urine vacutainer luer-lock access device. Aspirate the urine into the grey top vacutainer tube with boric acid.
   d. Urine catheter tip cultures are not acceptable.

NOTE: For additional information on CAUTI (Catheter Associated Urinary Tract Infection) prevention protocol, see HPO policy GEN016.

6. **Transplant patient bacterial urine culture:**
    a. Follow instructions above for collection.
    b. When ordering in EPIC choose the following:
        - Specimen Type: Urine, other
        - Specimen Source: Transplant

7. **Parasitic examination:** Collect in a sterile container without preservatives. The time of collection is dependent on the suspect pathogen:
    a. **Filariasis:** Microfilariae may be detected in urine of patients with chyluria, of patients with heavy filarial infections, and of patients treated with diethylcarbamazine. Collect specimens as first-voided urine in a sterile container without preservatives.
    b. ***S. haematobium*:** Collection of a midday urine specimen in a sterile container without preservatives is recommended. Peak egg excretion occurs between noon and 3 p.m.
    c. ***Microspordia:*** Microsporidial spores may be detected in concentrated urine of patients who are immunosuppressed, including those with AIDS. First-voided specimen is preferred.

Specimen handling: Once sample is collected, label the container immediately.

Notes:

- AFB culture: Minimum volume is 40 ml.

Viral Transport Media (VTM or UVTM)

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

- Tissue and biopsy material can be placed directly into the viral transport media (VTM). Each sample need not be more than 1-2 cm in diameter.
- 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 VTM.
- CSF should be submitted in a sterile container.
- Urine should be submitted in a sterile container.
- Rectal swab (Dacron only) should be submitted in VTM.
- Blood for viral culture should be submitted in a heparin tube.
- 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.
- For CMV colitis, culture of biopsy tissue is preferred. Stool is frequently toxic to cultured cells and virus is infrequently recovered from this source.

Worm ID – Macroscopic

Macroscopic worms should be submitted to the laboratory live (if possible) and without preservative to permit complete study in a sterile container.

Wounds

1. For closed wounds, refer to Abscess and Bullae, Cellulitis, Vesicles.
2. For open wounds:
    a. 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.
    b. Attempt to culture the base or edges of the wound to avoid collecting "normal microbiota" organisms.
    c. The following are preferred specimens for sinus tracts:
        i. Aspiration of material obtained by needle or catheterization.
        ii. Curettings from the lining of the sinus tract.

d. 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.
e. 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.