

# Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 6/2018

## 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, e-Swabs for bacterial culture, Cary-Blair for stool culture, VTM for viral and Chlamydia cultures, and urine boric acid transport for bacterial urine cultures). **See attached collection containers pages 29 and 30.** Check expiration date before inoculating collection device.
4. Whenever possible, collect specimens prior to administration of antimicrobial agents.
5. Properly label the specimen (a minimum two patient identifiers are required) 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 and swabs will not be accepted.

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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. Alternatively, 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 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 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. If a Gram stain is needed, collect two swabs.
5. Note requests to rule out *Actinomyces* sp., *Cutibacterium* (previously *Propionibacterium*) *acnes* sp. or *Nocardia* sp. on the requisition/EPIC.

## Amniocentesis

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

## 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.
2. Arthropod specimens should be submitted to the laboratory live (if possible).
3. 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.
4. Testing is performed at a reference laboratory.

## Blood

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.

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[https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix\\_101712.pdf](https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101712.pdf)

1. Gather necessary supplies.
  - a. If the patient is not on antibiotics, a routine culture set consists of a standard aerobic bottle and an anaerobic culture bottle.
  - b. If the 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.
  
2. Explain the procedure to the patient and/or their significant other if present.
  - a. Verify the patient's identification by using two patient identifiers per policy.
  
3. Perform hand hygiene per hospital approved policy (IFC 001 Follow Standard Precautions for all patients). Don appropriate protective equipment such as gloves, masks, and/or face shields.
  
4. Assemble necessary equipment before preparation of the patient's skin.
  - a. Mark culture bottles with a pen or marker, indicating 10 mL fill point
  - b. Remove dust caps from culture bottles.
  - c. Scrub culture bottle top surface with alcohol wipe(s). Do not use iodine.
  - d. Leave the alcohol wipe on the bottle top during skin preparation.
  - e. Remove alcohol wipe just prior to inoculating the bottles - do not use iodine.
  - f. Create a sterile field with the inside of the outer sterile glove package.
  - g. Open supplies and place on the field.
  
5. Apply tourniquet to the extremity and identify the phlebotomy site.
  
6. Preparation of the phlebotomy site:
  - a. Preferred: Use Chloraprep® One-Step Frepp
    - i. Cleanse and scrub the site with 2-3 alcohol swabs. Allow it to dry completely.
    - ii. Don sterile gloves.
    - iii. Squeeze the handle of the Chloraprep® Frepp to break the ampule and scrub the skin back and forth and up and down with the foam surface using a scrubbing motion for 30 seconds.
    - iv. A 10 cm area of the skin shall be disinfected.
    - v. Allow the site to dry at least 30 seconds before venipuncture.
  - b. If unable to utilize Chloraprep® One-Step Frepp, use 1% tincture of iodine frepp.
  
7. Povidone-iodine swabs permit more contamination than either chlorhexidine or 1% iodine frepps.
  - a. Using the 1% iodine frepp, cleanse a 10 cm area using circular motion starting at the site and working outward.
    - i. Using the 1% iodine frepp, cleanse a 10 cm area using circular motion starting at the site and working outward.
    - ii. Allow to dry for a at least 30 seconds to allow antiseptic effect
    - iii. If using iodine product, clean patient's skin with alcohol to remove excess iodine (to prevent iodine burns).
  - b. If unable to use Chloraprep® One-Step Frepp or iodine (e.g., allergic):
    - i. Use alcohol pad to cleanse the patient's skin, using a circular motion starting at the site and moving outward.
    - ii. Repeat times two.

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- iii. 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.
    - a. Insert the needle into the vein.
    - b. Try to keep the dominant hand sterile.
    - c. Remove the alcohol pad from the top of the culture bottles.
    - d. 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.
    - e. 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 A of the JHH Interdisciplinary Clinical Practice Manual Infection Control Blood Culture Policy and Procedure.
  
  11. For units ordering through the electronic medical record (EMR):
    - a. Label the culture bottles with an EMR generated label in the presence of the patient. Indicate the time that the specimen was obtained.
    - b. Do not place label over bar-coded area of the bottle.
    - c. If using a vascular access device (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 (e.g. left subclavian triple lumen).
    - d. Barcode scan specimens.
  
  12. For units that do not have access to electronic ordering:
    - a. 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.
    - b. Fill out Pathology 5 (Microbiology-routine) lab slip.
    - c. Indicate the site from which blood was collected using comment section. If using a VAD to draw the culture, you must indicate type and site of VAD in the comments section (e.g., left subclavian triple lumen).
    - d. Select appropriate test (i.e., Blood Culture).
    - e. Indicate suspected diagnosis, if necessary (required for rule out endocarditis).
    - f. Include date and time of collection.
    - g. Document that cultures were obtained on appropriate nursing form, if applicable.
- [https://hpo.johnshopkins.edu/hopkins/policies/39/4282/policy\\_4282.pdf?\\_af=0.083803167459](https://hpo.johnshopkins.edu/hopkins/policies/39/4282/policy_4282.pdf?_af=0.083803167459)
13. Send specimens to the laboratory as soon as possible (utilize pneumatic tube). Never refrigerate blood culture specimens. Send specimens directly to the Microbiology Lab.

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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:
  - a. **Suspected catheter sepsis**
    - i. Draw two blood culture sets.
    - ii. One set is obtained from the suspected catheter.
    - iii. At the same time, a second set must be from a separate, peripheral site.
    - iv. Time of collection should be noted for both specimens.
    - v. 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.**
  - b. **Acute endocarditis**
    - i. Draw 2-3 culture sets from separate sites within 30 minutes of each other and before beginning antimicrobial therapy.
    - ii. Begin therapy after cultures are obtained.
  - c. **Subacute endocarditis**
    - i. Draw 2-3 blood culture sets on day 1, spaced 30-60 minutes apart. This may help to document a continuous bacteremia.
    - ii. If all are negative, additional sets can be drawn on days 2 and 3 (no more than 4 sets in a 24 hour period).
    - iii. Immediate antibiotics are less important than establishing a specific microbial diagnosis.
  - d. **Mycobacterial blood cultures (AFB)**
    - i. 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 option #1) during evening hours. Pneumatic tube can be used to obtain culture media.
    - ii. Prepare skin as for routine blood cultures.
    - iii. Inoculate 5 ml of blood into a BD Bactec Myco/F Lytic culture bottle (**do not exceed 5 ml of blood**). Do not place patient label over bottle bar code label.
    - iv. Deliver the blood bottle in the *brown bag* promptly to the Microbiology Lab. Pneumatic tube can be used as long as the sample is sent directly to the Microbiology Lab in the brown paper bag.
    - v. An authorized prescriber must have specifically placed an order for Mycobacterial blood culture.
  - e. **Fungal Cultures**
    - i. **Candida spp.** - If a physician orders fungal cultures, follow routine procedure for bacterial cultures as described above.
    - ii. **Cryptococcus, Histoplasma, or other filamentous organisms**--obtain an Isolator tube from the Microbiology Lab (call 5-6510 option #1).
      - a. **Adults:**
        - i. Isolator media is light sensitive and is stored in brown paper bags.
        - ii. Inoculate isolator tube with 10ml of blood.
        - iii. Send culture in brown paper bag directly to Microbiology Lab.
      - b. **Pediatric From children < 8 kg body weight**
        - i. Isolator media is light sensitive and is stored in brown paper bags.

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- ii. Inoculate Pediatric isolator tube with 1.5 ml of blood.
    - iii. Send culture in brown paper bag directly to Microbiology Lab as soon as possible.
  - f. **Viral blood cultures (heparin tube not acceptable for PCR; please see [Molecular testing guidelines](#))**
    - i. Draw 3 ml of blood in a green top tube (heparinized) after preparing the skin as above.
    - ii. Deliver the tube promptly to the Microbiology Lab. Pneumatic tube can be used as long as sample is sent directly to the Microbiology Lab.
  - g. **Malaria, Babesia or other blood parasites**
    - i. The submission of a single blood specimen does NOT rule out malaria (especially in immunologically naïve patients); submit additional bloods every 6-8 hours for up to 3 days if malaria remains a consideration.
      - 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).
  - h. **When rare organisms such as *Brucella*, *Campylobacter* or *Bartonella* are suspected:**
    - i. An ID physician shall be consulted.
    - ii. The Microbiology laboratory shall be consulted to advise which type of specimen is most likely to support the suspected organism.
  - i. **Pediatric Blood cultures:**
    - i. 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.
  - j. [https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix\\_101714.pdf?\\_af=0.921568357146](https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101714.pdf?_af=0.921568357146)
16. **Aspergillus galactomannan antigen**
- i. Collect 3-5 mL blood in a serum separator tube (SST) without anticoagulants.
  - ii. Transport the specimen to the lab as soon as possible.
17. **1, 3-beta-D-glucan test**
- i. Collect 3-5 mL blood in a serum separator tube (SST) without anticoagulants or a gold top tube.
  - ii. Transport the specimen to the lab as soon as possible.
18. **HIV Serology**
- i. Collect 6 mL blood in a pink top tube with EDTA
  - ii. 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 cultures](#).
2. Collect the fluid using a sterile needle and syringe.
3. For aerobic and anaerobic organisms, submit 10 ml in a sterile container.

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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:
  - a. Bacterial culture requests, inoculate into a blood culture bottle - do not send in a Heparin tube.
  - b. 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).
  - c. Viral test into a Heparin (green top) tube.
  - d. 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 will 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:
  - o Seat the patient comfortably. Tilt the head back.

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- 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.

Analyte	SOFT Test ID	EPIC Test ID	Specimen types
<i>Bordetella pertussis</i>	MBOPE	LAB21238	Swab

### Bronchial Brush/Wash/Lavage

1. This technique should be performed by an experienced individual. Descriptions of the methodology are available in the literature.
2. Transport in a sterile container immediately at ambient temperature.
  - 40 mL of BAL fluid is needed if the immunocompromised host protocol is ordered.
3. 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 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**

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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.

### **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.
  - a. Insert the needle. Collect the fluid into three sterile leak-proof tubes. Collect an adequate volume of fluid as recommended below:
    1. bacterial culture 1-5 ml
    2. fungal culture 5-10 ml
    3. molecular 1.5-2 ml
    4. mycobacterial culture 5-10 ml
    5. viral culture 1.5-2 ml
4. 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.

### **Amoeba Culture: CSF**

Please contact the Microbiology Laboratory at 410-614-0148 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:

#### CSF for *Acanthamoeba* and *Naegleria* species

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. Keep at room temperature.

### **Cutaneous (Fungal only)**

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## 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:
  - a. Hair stubs
  - b. Contents of plugged follicles
  - c. Skin scales
  - d. 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:
  - a. Clippings from any discolored or brittle parts of the nail
  - b. 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.

## 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).
  - a. Place the swab into the eSwab transport media and transport at ambient temperature.
  - b. This is **NOT** an acceptable specimen for anaerobe culture.
  - c. 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:
  - a. Obtain Cornea Pack from the Microbiology Laboratory, Meyer B-171, 5-6510 option #1.

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- b. 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.
  - c. Transport at ambient temperature.
  - d. Gram stain is not routinely performed.
4. Intraocular fluid:
- a. Collect fluid by surgical needle aspiration.
  - b. Transport cultures at ambient temperature.

### **Amoeba Culture: Contact lens, contact lens solution, corneal scrapings/tissue**

Please contact the Microbiology Laboratory at 410-614-0148 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 strep 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](#).

### **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.

### **Cervix (Endocervix) for Culture**

1. Place the patient in the lithotomy position.

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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.

### Cervix (Endocervix) for HPV DNA Testing

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

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

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

### *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.

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## Female vaginal

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

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

## 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.
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.

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## Urethra

1. Instruct patient not to urinate at least 2 hours prior to sampling.
2. Insert the swab 2-to 4 cm into the urethra. Rotate 3 to 5 sec and withdraw.
3. 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.

For Bacterial Vaginosis Detection collect vaginal secretions from the posterior vaginal area using a sterile dacron swab. Avoid using lubricant prior to collection. A quantitative interpretation is performed using the Nugent Score system. This is only offered Monday through Friday from 8 AM to 10 PM.

For 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.

Analyte	SOFT Test ID	EPIC Test ID	Specimen types
Group B <i>Streptococcus</i>	NSTGB	LAB45303	Swab

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Analyte	SOFT Test ID	EPIC Test ID	Specimen types	Volume Requested	Analytical Sensitivity <sup>†</sup>	Cut off: Must Receive By
<b>QUALITATIVE NAT (VIRUSES)</b>						
Adenovirus	MADVE	LAB21370	Conjunctiva*	1.2 mL  *Conjunctiva, vesicle fluid, and respiratory sources must be sent in 2.0 mL viral transport media (VTM). Recommended that rectal swabs be sent in VTM.	1 copy/rxn	3:00 PM Mon – Fri
	MADVU	LAB21373	Urine		3:00 PM Mon, Wed, Fri	
BK Virus	MBKVU	LAB1375	Urine		10 copies/rxn	
CMV	MCMVC	LAB21345	CSF		20–200 copies/rxn	8:00 AM Mon – Sat
	MCMVE	LAB21346	Vitreous			
	MCMVA	LAB21347	Amniotic fluid			
EBV	MEBVC	LAB21374	CSF		6–60 copies/rxn	
Enterovirus	MENTC	LAB1302	CSF		0.1 pfu/mL	
	MENTP	LAB21348	Plasma			
	MENTS	LAB21349	Stool			
	MENTR	LAB21350	Rectal swab*			
	MENTA	LAB21351	NP aspirate*			
	MENTT	LAB21371	Throat*			
HSV 1+2	MHSVC	LAB1343	CSF	10 copies/rxn		
	MHSVE	LAB21352	Vitreous			
JCV	MJCVC	LAB21292	CSF	100 copies/rxn		
VZV	MVZVC	LAB21355	CSF	10 copies/rxn		
	MVZVV	LAB21356	Vesicle fluid*			
	MVZVE	LAB21357	Vitreous			
Resp Virus NAT	MRVBL	LAB45382	BAL	1.0 mL in sterile container		
	MRVBB		Bronchial brush			
	MRVBW		Bronchial wash			
	RVCET	LAB45384	Endonasotrach	1.0 mL in VTM		
	MRVNS	LAB45380	NP swab			
	MRVNW	LAB45381	NP wash			
HSV-1+2	MHS12	LAB44940			8:00 AM Mon-Sun	
VZV	MVZV	LAB44941				
HSV-1+2/VZV	MH12V	LAB44939				
HPV High Risk + genotyping	MHPVD	LAB46433	Cervix	Original SurePath Vial		Mon, Wed, Fri Requests <b>must</b> go through Cytopathology

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Analyte	SOFT Test ID	EPIC Test ID	Specimen types	Volume Requested	Analytical Sensitivity <sup>†</sup>	Cut off: Must Receive By
FluA/B/RSV NAT	RVSNS	LAB45386	NP Swab	N/A		N/A
<b>SENT OUT TESTS (QUALITATIVE NAT VIRUSES)</b>						
Parvovirus B19	Q0931		Bone marrow	1.0 mL	Send to Customer Service for testing by Quest Diagnostics	
	MB19P		Plasma			
	MB19A		Amniotic fluid			
CMV	Q0932		BAL			
HSV	Q0933					
VZV	Q0934					
JCV	MJCVQ		Plasma			
HIV	HIVP	LAB878	Whole Blood	3 mL in lavender top EDTA or yellow top ACD		

Analyte	SOFT Test ID	EPIC Test ID	Specimen types	Volume Requested	Analytical Sensitivity <sup>†</sup>	Cut off: Must Receive By
<b>QUANTITATIVE NAT</b>						
Adenovirus	MADVQ	LAB21301	Plasma in PPT (pearl top) tube	1.0 mL	15 copies/mL, measures 3.1-8.0 log <sub>10</sub> copies/mL	3:00 PM Mon, Wed, Fri
BK Virus	MBKVQ	LAB1374		5.0 mL	32 IU/mL, measures 2.4-8.0 log <sub>10</sub> copies/mL	8:00 AM Tues, Fri
CMV	MCMVQ	LAB913		5.0 mL	34.5 IU/mL, measures 2.1-6.95 log <sub>10</sub> IU/mL	12 Noon Mon-Thurs 3:00 PM Friday
EBV	MEBVQ	LAB1373		3.0 mL	200 copies/mL, measures 1.7-7 log <sub>10</sub> copies/10 <sup>5</sup> lymphs	8:00 AM Mon, Thurs
HBV	MHBVQ	LAB951		5.0 mL	29 IU/mL, measures 1.46-8.04 log <sub>10</sub> IU/mL	8:00 AM Thurs

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HCV	MHCVQ	LAB887			15 IU/mL, measures 1.6 to 7.8 log <sub>10</sub> IU/mL	8:00 AM Mon, Tues, Wed, Fri
HIV	HIVQC	LAB21303	Plasma	10 mL in EDTA (pink top) tube	20 copies/mL	8:00 AM Mon – Fri
<b>VIRAL GENOTYPING</b>						
Adenovirus	MADGI	LAB21359	Isolate	1.0 mL		Call lab at 5-2642 for more information
	MADGP	LAB21358	Plasma			
CMV	MCMGP	LAB21360	Plasma	1.0 mL	<b>1000 copies/mL</b>	
	MCMGT	LAB21361	Tissue			
	MCMGB	LAB21362	BAL			
	MCMGI	LAB21363	Isolate			
HCV	MHCGP	LAB915	Plasma	1.0 mL	4,000 IU/mL	8:00 AM Mon

Analyte	SOFT Test ID	EPIC Test ID	Specimen types	Volume Requested	Analytical Sensitivity <sup>†</sup>	Cut off: Must Receive By
<b>QUALITATIVE NAT (BACTERIA/OTHER)</b>						
<i>Chlamydia trachomatis/Neisseria gonorrhoeae (CT/NG)</i>	CTNGC	LAB1376	Cervix	Swab in Cobas Dual Swab collection tube	<b>CT: 5 IFU/mL (urine), 7.25 IFU/mL (swab)  <b>NG: 250 cells/mL (urine), 362 cells/mL (swab)</b></b>	8:00AM Mon-Fri
	CTC	LAB21375				
	NGC	LAB262				
	CTNGV	LAB21379	Vagina			
	CTV	LAB21380				
	NGV	LAB21381				
	CTNGU	LAB21382	Urine			
	CTU	LAB21383				
	NGU	LAB21384				
	CTNGR	LAB21385	Rectal swab			
	CTR	LAB21386				
	NGR	LAB21387				
	CTNGT	LAB21388	Throat			
	CTT	LAB21389				
	NGT	LAB21390				
CTE	LAB21391	Conjunctiva	Swab in 2.0 mL VTM			
NGCON	N/A	Bacterial isolate	Swab in Cobas Dual Swab collection tube			

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<i>Trichomonas vaginalis (TV)</i>	TRICC	LAB921	Cervix	Swab in Cobas Dual Swab collection tube	0.1 TV/mL	8:00AM Mon/Wed/Fri
	TRICV	LAB21260	Vagina			
	TRICU	LAB21261	Urine	Urine transferred into Cobas collection tube		

†Analytical sensitivity for purified DNA may or may not correlate with clinical sensitivity. For culturable viruses, sensitivity is generally similar to or higher than that of cell culture. “Measures” indicates measurable range for quantitative assays.

**Methodologies employed:**

- Genotyping tests for ADV, CMV and HCV are sequenced-based.
- CT/NG is Real-Time PCR and TV is Real-Time PCR with melt analysis.
- Remaining assays are based on Real-Time PCR.
- HSV-1+2/VZV is helicase dependent amplification.

**Molecular Microbiology Contact Information**

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**Nares Surveillance**

**Instructions for Proper Nares Cultures Technique:**

When obtaining a Swab (SAP 173665 – Adult), (SAP 173666- Peds) 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 place swab in collection container and snap off shaft of swab. Make sure the cap is securely fastened.
4. Label the tube with the patient's name, specimen or specimen bar-code (nares culture) and date.

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5. Send to microbiology lab with a requisition slip.
6. Nares swabs are only acceptable for MSSA/MRSA surveillance, not routine culture.
7. Routine hospital surveillance for MRSA is performed by culture using chromogenic media.
8. The chart below refers to the pre-surgical screening MRSA/MSSA NAAT test.

Analyte	SOFT Test ID	EPIC Test ID	Specimen types
Methicillin Resistant <i>Staphylococcus aureus</i> /Methicillin Susceptible <i>Staphylococcus aureus</i>	EPMNS	LAB24297	Nasal Swab

### Nasopharyngeal

**Nasopharyngeal swabs for *Bordetella pertussis* Culture and PCR – see [Bordetella Pertussis section](#).**

#### Swab (Flocked swab for 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.
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/>

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**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.**

**Parasitology Specimen Collection and Testing:**

**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.

**Test menu, acceptable specimen types, testing schedule, test names and anticipated TAT for medical parasitology testing.**

Test	Specimens	EPIC Test Code	SOFT ID	Testing schedule	Turn-around time
Amoeba Culture ( <i>Acanthamoeba</i> or <i>Neogleria</i> )	Contact lens, contact lens solution, cornea scrapping/tissue, CSF/brain tissue	LAB21328	PAAMO	CSF microscopic exam: STAT	CSF microscopic exam: STAT
				Culture: Once a day on weekdays	Culture: Read daily for 7 days with a 14 day read
Arthropod ID	Fleas, lice, bedbugs, ticks, mites and fly larvae	LAB247	PAART	Send out to Quest	1-5 days
<i>Cyclospora/ Isospora</i> Staining	Stool, intestinal aspirates and respiratory specimens	LAB46513	QGCIE	Send out to Quest	1-5 days
Malaria, <i>Babesia</i> , microfilariae, trypanosomes	Blood	LAB31446	PARMG (rapid antigen and smears)	STAT	Rapid antigen: 30 minutes
		LAB88331	PAMAT (smears only- monitoring)		Smears: Up to 8 hours
Microsporidia stain	Stool, intestinal aspirates, respiratory specimens and ocular specimens	LAB21329	PAMIC	8:00 am on weekdays	1-3 days
Ova and Parasite Exam – <u>is discouraged as a first line diagnostic for intestinal parasites</u>	Stool and other miscellaneous sources* (i.e., respiratory	LAB46511 (stool, urine, sputum)	QGOAP	Send out to Quest	1- 5 days

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(see above Stool Enteric Protozoan Panel or Comprehensive Enteric Parasite Panel)	specimens, bone marrow, Aspirates and Tissues, Urine, muscle, ocular,	LAB46512 (Fluids, BAL, etc.)	QGFOP		
Pinworm exam	Perianal tape prep	LAB248	PATAP	Once a day on weekdays	1-3 days
<i>Pneumocystis jirovecii</i> , Direct Immunofluorescence stain	Induced sputum, bronchial washings and bronchoalveolar lavage	LAB906	AGPCP	3:00 am-3:00 pm on weekdays and 2:00 pm on weekends for bronchoalveolar lavages and bronchial washings only. A second run is performed at 7:00 pm on Friday for bronchoalveolar lavages and bronchial washings.	1 day
Stool Enteric Protozoan Panel –molecular detection of <i>Giardia duodenalis</i> , <i>Cryptosporidium</i> species and <i>Entamoeba histolytica</i>	Total-Fix preserved stools or unpreserved stool	LAB46514	PSEPP	Monday-Friday	1-3 days
Stool Comprehensive Enteric Parasite Panel	Total-Fix preserved stools	JHMI inpatients: O217225  JHMI outpatient: O21747		Weekdays for the Enteric Protozoan Panel and microsporidia stains O&P: send out to Quest	1-3 days for Enteric Protozoan Panel and microsporidia stains  O&P: 1-5 days
Worm ID - Macroscopic	Worm submitted in a sterile container	LAB46515	PWORM	Weekdays	1-3 days

\*Please note the suspected pathogen as differential microscopy and/or staining techniques may be required.

**Prostate**

1. Cleanse the glans with soap and water.
2. Obtain prostate fluid by digital massage through the rectum.

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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.

### **Sputum**

1. Assure patient cooperation to get an adequate specimen.
2. Instruct the patient as follows:
  - a. Rinse mouth with tap water to remove food particles and debris.
  - b. Have patient breathe deeply and cough several times to achieve a deep specimen.
  - c. Patient should expectorate into dry, sterile container.
  - d. 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 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. 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**

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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.
  - a. For Norovirus testing, unpreserved stool in a sterile container is required. Testing is performed 7 days per week, 24 hours a day.
  - b. For bacterial pathogens (not including *C. difficile*), transfer specimen into a Cary-Blair transport medium container for routine bacterial stool pathogen detection or the appropriate container for other tests as listed below.
  - c. For *C. difficile* and fecal lactoferrin testing, place stool in sterile container. For ova and parasite, use Total-Fix transport media.
2. 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.
- 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.
- For *H. pylori* stool antigen testing, collect stool in a sterile air tight container and transport to the laboratory immediately. Stools sent in transport media, swabs or preservatives are not acceptable specimens. Testing is performed at 8:00 am on Mondays and Thursdays.

### ***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.

### **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).
4. Testing is performed once a day Monday to Friday.

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### **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 adhesive tape exams are required prior to considering the patient negative for pinworm.

Transparent adhesive tape method (Scotch- tape test, cellulose-tape slide test)

1. Please wear gloves while collecting the specimen.
2. A length of transparent adhesive tape, about 2 ½ inches long, is applied (using a tongue depressor), sticky side down, to the anal area skin around the rectum 3 or 4 times.
3. The tape is removed and applied, sticky side down, to a glass microscope slide and pressed firmly into place.
4. Place slide into a slide holder or a sterile container.
5. Keep at room temperature.

Pinworm paddle kit (a paddle coated with adhesive material)

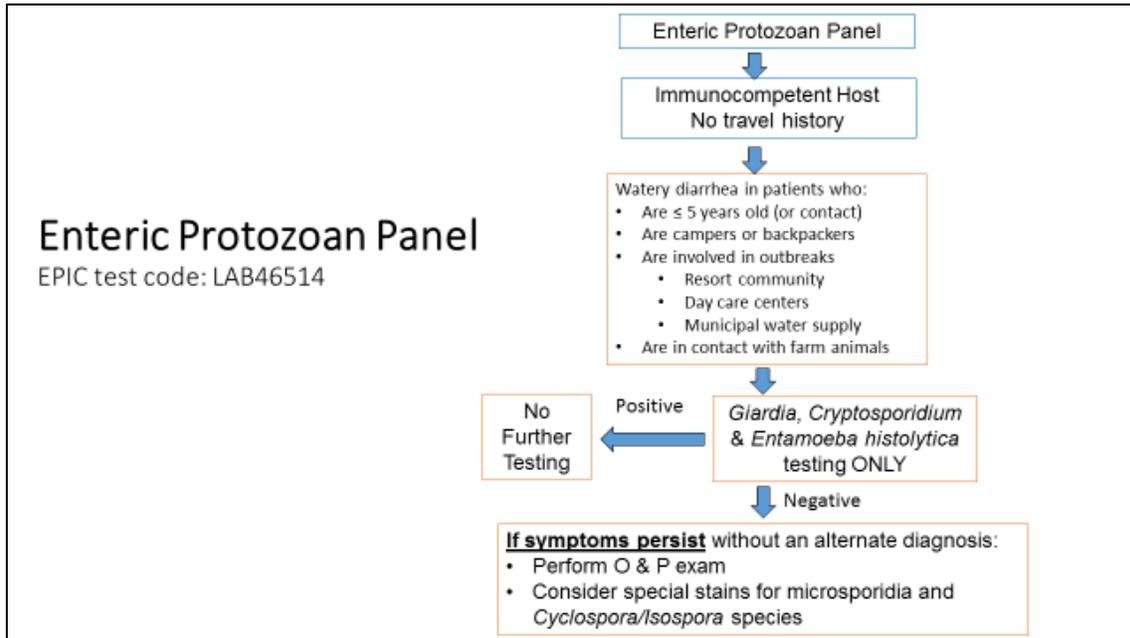
1. These can be obtained from the Microbiology laboratory. Please call 410-955-6510.
2. Please wear gloves while collecting the specimen.
3. Hold the paddles by the cap and remove it from the tube.
4. Using gentle pressure, press the sticky side of the paddle against the skin around the rectum 3 or 4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

### **Stool Enteric Protozoan Panel (EPIC test code: LAC46514)**

The Stool Enteric Protozoan Panel is a rapid and sensitive molecular test for the detection of *Giardia duodenalis*, *Cryptosporidium* species and *Entamoeba histolytica* from Total-Fix fecal specimens. 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 (see algorithm below). 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 are acceptable.
2. Fill the Total-Fix vial 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.

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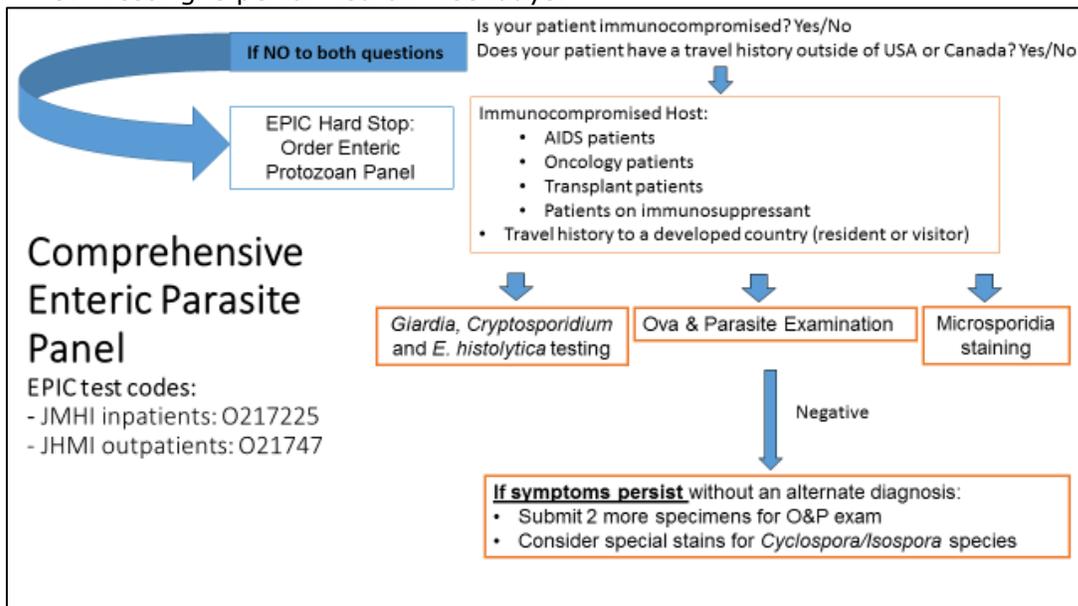


6.

### Comprehensive Stool Enteric Parasite Panel (EPIC test codes for JHMI inpatients: O217225 and JHMI outpatients: O21747):

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 (see algorithm below). 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. Unpreserved stools are 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.



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### 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. Unpreserved stools are 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.

Analyte	SOFT Test ID	EPIC Test ID	Specimen types	Volume Requested	Cut off: Must Receive By
<i>Clostridium difficile</i>	MCDIF	LAB21231	Stool	> 1 mL	8:00 AM Mon-Sun and 5:00 PM Mon-Sun
Norovirus	NNORV	LAB46019	Stool	> 1 mL	N/A
<b>Enteric Bacterial Panel:</b> <i>Salmonella</i> spp., <i>Campylobacter</i> spp. (jejuni and coli), <i>Shigella</i> spp./enteroinvasive <i>E. coli</i> (EIEC), stx1/stx2 found in Shiga toxin-producing <i>E. coli</i> , <i>Plesiomonas shigelloides</i> , <i>Vibrio</i> ( <i>V. vulnificus</i> , <i>V. parahaemolyticus</i> , and <i>V. cholera</i> ), enterotoxigenic <i>Escherichia coli</i> (ETEC) and <i>Yersinia enterocolitica</i>	NSTL	LAB46216	Stool in Cary-Blair Transport Media	Fill to line on the container	8:00 AM Mon-Sun

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<b>Enteric Protozoan Panel:</b> <i>Giardia duodenalis</i> , <i>Cryptosporidium</i> species and <i>Entamoeba histolytica</i>	PSEPP	LAB46514	Stool in Total Fix Transport	Fill to line on the container	4:00 AM Mon-Fri
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**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:
  - a. both tonsillar fauces, and
  - b. the posterior pharynx, and
  - c. any ulceration, exudate, lesion, or area of inflammation.
4. Place the swab into the eSwab collection tube provided and transport at ambient temperature.

Beta 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*.

Analyte	SOFT Test ID	EPIC Test ID	Specimen types
<i>Rapid Strep Group A</i>	MGASN	LAB48203	Swab
<i>Rapid Strep Group A, Reflex Culture</i>	MGASC	LAB48204	Swab

**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 (VTM).

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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.**

### **Biopsy Specimens for Tissue Parasites:**

Biopsy specimens submitted to the laboratory for the examination of tissue parasites should be placed in a sterile container and transported to the microbiology laboratory as soon as possible. If delayed transport is expected, place a small amount of sterile saline in the container.

## **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.

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- 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 a sterile needle and syringe to puncture the tubing or use the BD urine vacutainer. Aspirate the urine directly from the tubing.
- d. Transfer the urine to a sterile specimen container or appropriate transport media.
- e. Urine catheter tip cultures are not acceptable.

### 6. Specimen handling:

- a. 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.

Urine specimens for parasitic examination should be collected in a sterile container without preservatives. The time of collection is dependent on the suspect pathogen:

#### 6. 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 specimen in a sterile container without preservatives.

#### 7. *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.

#### 8. Microsporidia

Microsporidial spores may be detected in concentrated urine of patients who are immunosuppressed, including those with AIDS. First-voided specimen is preferred.

## **Viral Transport Media (VTM)**

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.

- 1. 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.
- 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 VTM.

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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 VTM.
6. Blood for viral culture 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.

### **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.