Overview

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

Basic Concepts for Specimen Collection

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

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Abscess

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

2. Collect purulent material aseptically from an undrained abscess using a sterile needle and syringe. Open miliary abscesses with a sterile scalpel and collect the expressed material with a sterile needle and syringe.

3. Transfer 5-10 ml of the aspirated material to an anaerobic transport vial. Transport immediately. *Anaerobic transport media is not recommended for AFB culture. If requesting AFB culture, transfer at least 1 ml of the aspirated material into a sterile container.*

4. Swabs are a poor choice because they dry easily and because of the limited amount of material obtained. Swabs are not optimal for fungal 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., *Propionibacterium* 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 should be collected using tweezers to remove or extract from the skin if attached. Place into a vial or container with or without a small volume of alcohol and secure the lid well to prevent leaking.

Blood

1) Determine the type of culture bottles to utilize, as indicated per physician's order (aerobic and anaerobic or resin bottles and anaerobic bottles), or other types as specified below. If necessary, discuss timing of cultures, sites, need for any special instructions on the lab slip, etc., with the physician. Please also refer to the JHH Interdisciplinary Clinical Practice Manual Blood Culture Procurement Policy and Procedure.

   [https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101712.pdf?_=0.490010783007](https://hpo.johnshopkins.edu/hopkins/policies/39/4282/appendix_101712.pdf?_=0.490010783007)

2) 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.

3) Explain the procedure to patient and/or their significant other if present.
   a) Verify the patient's identification by using two patient identifiers per policy.

4) Perform hand hygiene per hospital approved policy (IFC 001Follow Standard Precautions for all patients. Don appropriate protective equipment such as gloves, masks, and/or face shields.

5) 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.

6) Apply tourniquet to the extremity and identify the phlebotomy site.

7) 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. Allow the prep to fully dry (at least 30 seconds).
      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.
      i) Povidone-iodine swabs permit more contamination than either chlorhexidine or 1% iodine frepps.
      ii) Using the 1% iodine frepp, cleanse a 10 cm area using circular motion starting at the site and working outward.
      iii) Allow to dry for at least 30 seconds to allow antiseptic effect.
      iv) If using iodine product, clean patient's skin with alcohol to remove excess iodine (to prevent iodine burns).
   c) 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.
      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 - 7510).
   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.

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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.

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 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).
      1. Isolator media is light sensitive and is stored in brown paper bags.
      2. Inoculate isolator tube with 10 ml of blood.
      3. Send culture in brown paper bag directly to Microbiology Lab.

f) **Viral blood cultures (heparin tube not acceptable for PCR; please see Molecular testing guidelines)**
   i) Obtain one 3 ml 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) Draw 3 ml of blood in a lavender top (EDTA) vacutainer tube.
   ii) Deliver the tube to the Microbiology lab within 2 hours of collection.
   iii) Indicate patient's travel history.

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 would be 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.

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16) **Antibiotic Activity (Serum)**

   i) Requires authorization by Infectious Disease, Microbiology Faculty or a Laboratory Medicine Resident.

   ii) Collect 2-5 ml of blood in a red top tube pre and one hour post infusion of the antimicrobial agents.
ii) Include: patient isolate, culture number, specimen collection time, dosage time, and antibiotic therapy.
iii) Process requirements: aliquot 2 ml of serum into a plastic vial and freeze.

17) **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

18) **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.

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

**Bone Marrow**

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

6. Transport specimens immediately at ambient temperature.

**Bordetella pertussis**

**Culture and PCR**

1. Obtain collection system from Microbiology lab, Meyer 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:
   - 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. Descriptions of the methodology are readily available in the literature.

2. Transport in a sterile container immediately at ambient temperature.

3. Aspergillus galactomannan antigen (Bronchial lavage)
   - Please send 1-2mL for galactomannan testing (minimum 1mL).
Bullae, Cellulitis, Vesicles

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

Cellulitis

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

Vesicle Fluids and Scrapings

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

Cerebrospinal Fluid

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

Hair

1. Scrape the scalp with a blunt scalpel.
2. Place specimen in a dry sterile container.
3. Transport at ambient temperature.
4. The following specimens are also acceptable:
   1. Hair stubs
   2. Contents of plugged follicles
   3. Skin scales
   4. Hair plucked from the scalp with forceps

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

Nails

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

Skin

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

Ear

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

Eye

1. Cleanse the skin around the eye with a mild antiseptic.
2. Purulent conjunctivitis: Collect purulent material with a regular cotton swab.
   a. Place the swab into appropriate 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 placed in 3 ml of viral transport media. **Do not use Aptima Collection Kit.**
3. Corneal infections:
   a. Obtain Cornea Pack from the Microbiology Laboratory, Meyer B-171, 5-6510 option #1.
b. Swab the conjunctiva as described above.
c. 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.
d. Transport at ambient temperature.
e. Gram stain is not routinely performed.

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

Gastric Biopsy

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

Genital Sources

Routinely processed only for 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. Skin prep as for regular skin sites.
2. Aspirate material from Bartholin gland abscess.
3. Send to lab in anaerobic transport medium.

Cervix (Endocervix) for Culture

1. Place the patient in the lithotomy position.
2. Prepare the speculum, avoiding the use of a lubricant other than warm water.
3. Insert the speculum and visualize the cervical os.
4. Remove excess mucus with a cotton ball.
5. Insert a Dacron swab into the cervical os, rotate gently, and allow to remain for 10 to 30 seconds.
6. Remove swab and place in bacterial transport medium.
7. Transport at ambient temperature.

Cervix (Endocervix) for HPV DNA Testing

1. Samples are referred to microbiology from the Johns Hopkins Cytopathology laboratory.
Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 6/2016

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

<table>
<thead>
<tr>
<th></th>
<th>Cervix</th>
<th>Vagina</th>
<th>Rectum (male/female)</th>
<th>Pharynx (male/female)</th>
<th>Urine</th>
<th>Urethra (male only)</th>
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<tbody>
<tr>
<td>Chlamydia trachomatis</td>
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<td>Neisseria gonorrhoeae</td>
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<td>Trichomonas vaginalis</td>
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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 Aptima Urine Collection tube using a transfer pipet (provided) until the liquid level rises to **between** the 2 black lines on the tube.
6. Cap and label the tube with patient ID and date.
7. Transport the specimen to the lab as soon as possible.

**Female cervical**

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

**Female vaginal**

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

**Male Urethral**

1. Obtain collection pack from Medical Distribution Center, item number ESI85958.
2. Instruct patient not to urinate at least 2 hours prior to sampling.
3. Insert the **blue**-stemmed swab 2-to 4 cm into the urethra. Rotate 3 to 5 sec and withdraw.
4. Transfer the specimen swab to the Aptima Combo swab collection tube and break off the swab at the scored line. **Swab must remain in the transport tube**.
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

**Pharyngeal (Male/Female)**

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

**Rectal (Male/Female)**

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

**Gonorrhea Culture**

1. Obtain charcoal swab from microbiology, Meyer 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.
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 yeast 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. A quantitative interpretation is performed using the Nugent Score system. This assay is only offered Monday through Friday from 8 AM to 10 PM.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>New Test Code</th>
<th>SOFT Test ID</th>
<th>Specimen types</th>
<th>Volume Requested</th>
<th>Analytical Sensitivity†</th>
<th>Cut off: Must Receive By</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QUALITATIVE NAT (VIRUSES)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>8696</td>
<td>MADVE</td>
<td>Conjunctiva&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.0 mL</td>
<td>1 copy/rxn</td>
<td>3:00 PM Mon – Fri</td>
</tr>
<tr>
<td></td>
<td>8711</td>
<td>MADVU</td>
<td>Urine</td>
<td></td>
<td></td>
<td>3:00 PM Mon, Wed, Fri</td>
</tr>
<tr>
<td>BK Virus</td>
<td>8712</td>
<td>MBKVU</td>
<td>Urine</td>
<td></td>
<td>10 copies/rxn</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>8617</td>
<td>MCMVC</td>
<td>CSF</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8618</td>
<td>MCMVE</td>
<td>Vitreous</td>
<td></td>
<td>20–200 copies/rxn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8619</td>
<td>MCMVA</td>
<td>Amniotic fluid</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EBV</td>
<td>8713</td>
<td>MEBVC</td>
<td>CSF</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6–60 copies/rxn</td>
<td></td>
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<tr>
<td>Enterovirus</td>
<td>8620</td>
<td>MENTC</td>
<td>CSF</td>
<td></td>
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<tr>
<td></td>
<td>8621</td>
<td>MENTP</td>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8622</td>
<td>MENTS</td>
<td>Stool</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8623</td>
<td>MENTR</td>
<td>Rectal swab&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8624</td>
<td>MENTA</td>
<td>NP aspirate&lt;sup&gt;‡&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8697</td>
<td>MENTT</td>
<td>Throat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8698</td>
<td>MENTN</td>
<td>NP swab&lt;sup&gt;‡&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV 1+2</td>
<td>8625</td>
<td>MHSVC</td>
<td>CSF</td>
<td></td>
<td>10 copies/rxn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8626</td>
<td>MHSVE</td>
<td>Vitreous</td>
<td></td>
<td>100 copies/rxn</td>
<td></td>
</tr>
<tr>
<td>JCV</td>
<td>7901</td>
<td>MJCVC</td>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>8633</td>
<td>MVZVC</td>
<td>CSF</td>
<td></td>
<td>10 copies/rxn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8634</td>
<td>MVZVV</td>
<td>Vesicle fluid&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8640</td>
<td>MVZVE</td>
<td>Vitreous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>8627</td>
<td>MB19P</td>
<td>Plasma</td>
<td></td>
<td>100 copies/rxn</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8628</td>
<td>MB19A</td>
<td>Amniotic fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resp Virus NAT</td>
<td>8647</td>
<td>MRVBL</td>
<td>BAL</td>
<td></td>
<td>1.0 mL in sterile container</td>
<td>312:00 AM Sun, Tues, Thurs, Fri (Wed during respiratory season)</td>
</tr>
<tr>
<td></td>
<td>8648</td>
<td>MRVBB</td>
<td>Bronchial brush</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8676</td>
<td>MRVBW</td>
<td>Bronchial wash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8677</td>
<td>MRVNS</td>
<td>NP swab</td>
<td></td>
<td>1.0 mL in VTM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8678</td>
<td>MRVNW</td>
<td>NP wash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>7125</td>
<td>HIVP</td>
<td>Whole blood</td>
<td></td>
<td>1 copies/rxn</td>
<td>8:00 AM Thurs</td>
</tr>
<tr>
<td>HSV-1+2</td>
<td></td>
<td>MHS12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td>MVZV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1+2/VZV</td>
<td></td>
<td>MH12V</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
## Johns Hopkins Medical Microbiology
### Specimen Collection Guidelines – Updated 6/2016

<table>
<thead>
<tr>
<th>Analyte</th>
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<th>Specimen types</th>
<th>Volume Requested</th>
<th>Analytical Sensitivity†</th>
<th>Cut off: Must Receive By</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV High Risk</td>
<td>8693</td>
<td>MHPVC</td>
<td>Cervix</td>
<td>Cell pellets post-SurePath processing</td>
<td>8:00 AM Tues &amp; Thrus Requests must go through Cytopathology</td>
<td></td>
</tr>
</tbody>
</table>

### SENT OUT TESTS (QUALITATIVE NAT VIRUSES)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>New Test Code</th>
<th>SOFT Test ID</th>
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<th>Volume Requested</th>
<th>Analytical Sensitivity†</th>
<th>Cut off: Must Receive By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvovirus B19</td>
<td>5143</td>
<td>Q0931</td>
<td>Bone marrow</td>
<td>1.0 mL</td>
<td>Send to Customer Service for testing by Quest Diagnostics</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>5144</td>
<td>Q0932</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>5145</td>
<td>Q0933</td>
<td>BAL</td>
<td></td>
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</tr>
<tr>
<td>VZV</td>
<td>5146</td>
<td>Q0934</td>
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### QUANTITATIVE NAT

<table>
<thead>
<tr>
<th>Analyte</th>
<th>New Test Code</th>
<th>SOFT Test ID</th>
<th>Specimen types</th>
<th>Volume Requested</th>
<th>Analytical Sensitivity†</th>
<th>Cut off: Must Receive By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>7955</td>
<td>MADVQ</td>
<td></td>
<td>1.0 mL</td>
<td>100 copies/mL, measures 3.1-8.0 log10 copies/mL</td>
<td>3:00 PM Mon, Wed, Fri</td>
</tr>
<tr>
<td>BK Virus</td>
<td>7951</td>
<td>MBKVQ</td>
<td></td>
<td>5.0 mL</td>
<td>28 copies/mL, measures 2.4–8.0 log10 copies/mL</td>
<td>8:00 AM Tues, Fri</td>
</tr>
<tr>
<td>CMV</td>
<td>7929</td>
<td>MCMVQ</td>
<td></td>
<td>5.0 mL</td>
<td>137 IU/mL, measures 2.1–6.95 log10 IU/mL</td>
<td>12 Noon Mon-Thurs 3:00 PM Friday</td>
</tr>
<tr>
<td>EBV</td>
<td>7927</td>
<td>MEBVQ</td>
<td>Plasma</td>
<td>2 x 3.0 mL</td>
<td>50 copies/10^5 lymphs, measures 1.7–7 log10 copies/10^5 lymphs</td>
<td>8:00 AM Mon, Wed, Thurs</td>
</tr>
<tr>
<td>HBV</td>
<td>6046</td>
<td>MHBVQ</td>
<td></td>
<td>5.0 mL</td>
<td>29 IU/mL, measures 1.46-8.04 log10 IU/mL</td>
<td>8:00 AM Thurs</td>
</tr>
<tr>
<td>HCV</td>
<td>2915</td>
<td>MHCVQ</td>
<td></td>
<td>5.0 mL</td>
<td>43 IU/mL, measures 1.6 to 7.8 log10 IU/mL</td>
<td>8:00 AM Mon, Tues, Wed, Fri</td>
</tr>
<tr>
<td>JCV</td>
<td>7305</td>
<td>MJCVQ</td>
<td></td>
<td>1.0 mL</td>
<td>400 copies/mL, measures 2.6-7.5 log10 copies/mL</td>
<td>3:00 PM Mon, Wed, Fri</td>
</tr>
</tbody>
</table>
# Johns Hopkins Medical Microbiology
## Specimen Collection Guidelines – Updated 6/2016

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<tr>
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<th>Volume Requested</th>
<th>Analytical Sensitivity†</th>
<th>Cut off: Must Receive By</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QUALITATIVE NAT (BACTERIA/OTHER)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>7111</td>
<td>MBOPE</td>
<td>Swab</td>
<td>N/A</td>
<td>0.2 cfu/rxn</td>
<td>7:00 PM Mon-Fri</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>7079</td>
<td>MCDIF</td>
<td>Stool</td>
<td></td>
<td>10 copies/rxn</td>
<td>10:00 AM Mon-Sun and 4:00 PM Mon-Fri</td>
</tr>
<tr>
<td>Chlamydia trachomatis/Neisseria gonorrhoeae (CT/NG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8820</td>
<td>CTNGC</td>
<td>Cervix</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8821</td>
<td>CTC</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8822</td>
<td>NTC</td>
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<tr>
<td></td>
<td>8823</td>
<td>CTNGM</td>
<td>Urethra</td>
<td>Swab in Aptima collection tube</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8824</td>
<td>CTM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8825</td>
<td>NGM</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8826</td>
<td>CTNGV</td>
<td>Vagina</td>
<td></td>
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<tr>
<td></td>
<td>8827</td>
<td>CTV</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8828</td>
<td>NGV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8829</td>
<td>CTNGU</td>
<td>Urine</td>
<td>Urine transferred into Aptima collection tube</td>
<td></td>
<td>CT: 5 IFU/mL (urine), 7.25 IFU/mL (swab)</td>
</tr>
<tr>
<td></td>
<td>8830</td>
<td>CTU</td>
<td></td>
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<tr>
<td></td>
<td>8831</td>
<td>NGU</td>
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<td></td>
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<tr>
<td></td>
<td>8832</td>
<td>CTNGR</td>
<td>Rectal swab</td>
<td>Swab in Aptima collection tube</td>
<td></td>
<td>NG: 250 cells/mL (urine), 362 cells/mL (swab)</td>
</tr>
<tr>
<td></td>
<td>8833</td>
<td>CTR</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>8834</td>
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<tr>
<td></td>
<td>8835</td>
<td>CTNGT</td>
<td>Throat</td>
<td>Swab in Aptima collection tube</td>
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<tr>
<td></td>
<td>8836</td>
<td>CTT</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8837</td>
<td>NGT</td>
<td></td>
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<tr>
<td></td>
<td>8838</td>
<td>CTE</td>
<td>Conjunctiva</td>
<td>Swab in 2.0 mL VTM</td>
<td></td>
<td>8:00AM Mon-Fri</td>
</tr>
<tr>
<td>n/a</td>
<td>NGCON</td>
<td>Bacterial isolate</td>
<td></td>
<td>Swab in Aptima collection tube</td>
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### Trichomonas vaginalis (TV)

<table>
<thead>
<tr>
<th>Code</th>
<th>Method</th>
<th>Source</th>
<th>Reagent</th>
<th>Volume</th>
<th>Time</th>
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<tr>
<td>7307</td>
<td>TRICC</td>
<td>Cervix</td>
<td>Swab in Aptima collection tube</td>
<td>0.1 TV/mL</td>
<td>8:00AM Mon/Wed/Fri</td>
</tr>
<tr>
<td>7308</td>
<td>TRICV</td>
<td>Vagina</td>
<td>Urine transferred into Aptima collection tube</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7309</td>
<td>TRICU</td>
<td>Urine</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

†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 for ADV, CMV and HCV are sequenced-based.
- CT/NG and TV are transcription-mediated amplification (TMA).
- Remaining assays are based on Real-Time PCR.

**Molecular Microbiology Contact Information**

Director: Dr. Alexandra Valsamakis, MD PhD  
Phone: (410) 955-5077  
Pager: (410) 283-0221

Technical Specialist: Michael Forman  
Phone: (410) 955-2642

Lead Medical Technologist: Melissa Geahr  
Phone: (410) 955-2642
Nares Surveillance

Instructions for Proper Nares Cultures Technique:

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

1. Grasp the swab cap with fingers. (Be careful to avoid contacting the swab or stick with your fingers).

2. Withdraw the swab; sweep around the interior surface of the anterior nares. (Do both sides with one swab.)

3. Carefully 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.

5. Send to microbiology lab with a requisition slip.

6. Nares swabs are only acceptable for MSSA/MRSA surveillance, not routine culture.

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

For video demo of the procedure and additional education, click here: http://www.copanusa.com/downloads/education/

Nose

1. Collect anterior nares culture with a regular cotton swab. In small children, use a nasopharyngeal swab to facilitate collection.

2. Transport at ambient temperature.

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

Parasitology Specimen Collection and Testing

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

Please contact the Parasitology Laboratory at 410-955-2653 or 5-2653 to coordinate amoeba culture. Special media is required and prepared fresh for amoeba culture. The Parasitology laboratory requires 24 hours’ notice to have the media available for bedside inoculation. In special circumstances the media can be prepared within an hour. Culture media will be provided to the requesting healthcare provider to inoculate the specimens at bedside (optimal for recovery). If beside 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.

**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.

**Arthropod ID**

1. Arthropod specimens should be submitted to the laboratory live (if possible) and without preservative to permit complete study.
2. For scabies, submit skin scrapings on glass microscope slide. Cover with a clean slide and fasten down to transport. Rubber bands work well. Transport slides in a clean, dry container.
3. Submit arthropods (ticks, lice, nits, bed bugs, etc.) in a clean, dry container without preservative.
Cryptosporidium/Cystoisospora/Cyclospora 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 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 once a day Monday to Friday.

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.

Blood: Malaria, Babesia or other blood parasites
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).

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.

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. Please wear gloves while collecting the specimen.
2. Hold the paddles by the cap and remove it from the tube.
3. Using gentle pressure, press the sticky side of the paddle against the skin around the rectum 3 or 4 times.
4. Insert the paddle into its protective tube and tighten the lid.
5. Keep at room temperature.

*Pneumocystis jirovecii*, Direct Immunofluorescence stain
1. Induced sputum (submitted on ice), bronchial washings and bronchoalveolar lavage specimens submitted in a sterile container are acceptable for testing.
2. Induced sputum is collected by Pulmonology and nursing staff on OSL-8.
3. Induced sputum specimens will only be accepted on weekdays (M-TH) until 3 PM and Fridays until 7 PM. **No** induced sputum specimens will be accepted/tested on weekends or holidays.
4. Testing is performed once daily on weekdays and weekends. A second run is performed on Friday's at 7:00 pm.

**Stool: Ova and Parasite Examination and Giardia Antigen ELISA:**

Ova and Parasite Examination
Optimally, 3 fecal specimens collected over a 7 to 10 day period must be submitted in a Total-Fix 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 on weekdays.

*Giardia* Antigen ELISA:
Testing of two separate stool specimens (collected at least one day apart) is recommended before the patient is considered negative for *Giardia*.
1. Fresh unpreserved stool collected in a sterile container or stool collected in the Total-Fix vial is acceptable for testing.
2. Refrigerate the unpreserved specimen. Keep stool in Total-Fix at room temperature.
3. Testing is performed Tuesday and Friday mornings.

**Urine**

Urine specimens for parasitic examination should be collected in a sterile container without preservatives. The time of collection is dependent on the suspect pathogen:
1. **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.
2. **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.
3. Microsporidia
**Microsporidial spores may be detected in concentrated urine of patients who are immunosuppressed, including those with AIDS. First-voided specimen is preferred.**

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimens</th>
<th>Routinely Code</th>
<th>SOFT ID</th>
<th>Testing schedule</th>
<th>Turn-around time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoeba Culture</strong></td>
<td>Contact lens, contact lens solution, cornea scraping/tissue, CSF/brain tissue</td>
<td>8425</td>
<td>PAAMO</td>
<td>CSF microscopic exam: STAT</td>
<td>CSF microscopic exam: STAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Culture: Once a day on weekdays</td>
<td>Culture: Read daily for 7 days with a 14 day read</td>
</tr>
<tr>
<td><strong>Arthropod ID</strong></td>
<td>Fleas, lice, bedbugs, ticks, mites and fly larvae</td>
<td>7374</td>
<td>PAART</td>
<td>Weekdays</td>
<td>1-3 days</td>
</tr>
<tr>
<td><strong>Cryptosporidium/ Cystoisospora/ Cyclospora Staining</strong></td>
<td>Stool, intestinal aspirates and respiratory specimens</td>
<td>8428</td>
<td>PACRY</td>
<td>8:00 am on weekdays</td>
<td>1-3 days</td>
</tr>
<tr>
<td><strong>Giardia Antigen ELISA</strong></td>
<td>Fresh or Total-Fix Vial stools</td>
<td>8461</td>
<td>AGGIA</td>
<td>8:00 am on Tuesday and Friday</td>
<td>1-4 days</td>
</tr>
<tr>
<td><strong>Malaria, Babesia, microfilariae, trypanosomes</strong></td>
<td>Blood</td>
<td>8429</td>
<td>PARMG (rapid antigen and smears)</td>
<td>STAT</td>
<td>Rapid antigen: 30 minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8525</td>
<td>PAMAT (smears only)</td>
<td></td>
<td>Smears: Up to 8 hours</td>
</tr>
<tr>
<td><strong>Microsporidia stain</strong></td>
<td>Stool, intestinal aspirates, respiratory specimens and ocular specimens</td>
<td>8430</td>
<td>PAMIC</td>
<td>8:00 am on weekdays</td>
<td>1-3 days</td>
</tr>
<tr>
<td><strong>Ova and Parasite Exam</strong></td>
<td>Stool and other miscellaneous sources* (i.e., respiratory specimens, bone marrow, Aspirates and Tissues, Urine, muscle, ocular,</td>
<td>8410</td>
<td>PAOP</td>
<td>8:00 am on weekdays</td>
<td>1-3 days</td>
</tr>
<tr>
<td><strong>Pinworm exam</strong></td>
<td>Perianal tape prep</td>
<td>7071</td>
<td>PATAP</td>
<td>Once a day on weekdays</td>
<td>1-3 days</td>
</tr>
<tr>
<td><strong>Pneumocystis jirovecii, Direct</strong></td>
<td>Induced sputum, bronchial</td>
<td>8460</td>
<td>AGPCP</td>
<td>3:00 pm on weekdays and 2:00 pm on weekends.</td>
<td>1 day</td>
</tr>
</tbody>
</table>
**Immunofluorescence** washings and bronchoalveolar lavage

A second run is performed at 7:00 pm on Friday.

*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.
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:
   1. Rinse mouth with tap water to remove food particles and debris.
   2. Have patient breathe deeply and cough several times to achieve a deep specimen.
   3. Patient should expectorate into dry, sterile container.
   4. *Tuberculosis patients should expectorate sputum in the early morning, into a sterile container with lid sealed tightly. Leaking specimens may be cancelled. Collection containers are to be obtained from Central Storage. Microbiology lab does not supply collection containers.*
3. Transport immediately at ambient temperature. Refrigerate if a delay of more than one hour is anticipated.
4. 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. 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 Pulmonology and nursing staff on OSL-8. Induced sputum is acceptable for Legionella culture, *Pneumocystis jiroveci* (PCP) (on ice)*, fungal, and AFB testing. Not acceptable for viral cultures or routine bacterial cultures.
Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 6/2016

* For PCP testing only - induced sputum specimens will only be accepted on weekdays (M-TH) until 3 PM and Fridays until 8 PM. No induced sputum specimens will be accepted/tested on weekends or holidays.

Stool, Feces

- 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.
  - For Norovirus testing, unpreserved stool in a sterile container is required. Testing is performed 7 days per week, day shift only.
  - Transfer specimen into a Cary-Blair transport medium container for routine bacterial stool culture or the appropriate container for other tests as listed below.
    - For C. difficile and fecal lactoferrin testing, place stool in sterile container. For ova and parasite, use Total-Fix transport media.
    - Transport at ambient temperature within two hours of collection.

Notes:

- Stool samples collected on patients hospitalized longer than 3 days prior to collection are not acceptable for routine enteric culture.
- Only loose or diarrheal stools are recommended for routine bacterial and C. difficile cultures and PCR. 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 culture:
  1. A swab of rectal mucus, or
  2. A rectal swab inserted one inch into the anal canal (not acceptable for Rotavirus/Adenovirus EIA or C difficile testing).
- 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 O & P examination, optimally 2 to 3 fecal specimens collected over a 7 to 10 day period must be submitted in a Total-Fix. It is not acceptable to send more than one specimen collected on any given day.
- 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.

Throat

1. Use a cotton, Dacron (for viral culture), calcium alginate swab, or eSwab.

2. Use a tongue blade and a good light source to ensure good visualization.

3. Reach behind the uvula and swab:
   1. both tonsillar fauces, and
   2. the posterior pharynx, and
3. any ulceration, exudate, lesion, or area of inflammation.

4. Place the swab into the appropriate transport media and transport at ambient temperature.

Bacterial culture requests are only tested for Beta-hemolytic streptococci and *Arcanobacterium hemolyticum*.

**Tissue**

1. Tissue collection is an invasive procedure and requires surgery by a trained physician.
2. Collect tissue aseptically. Include material from both the center and the edge of the lesion.
3. Place the specimen in a sterile container on sterile gauze moistened with sterile nonbacteriostatic saline.
4. Transport in less than an hour at ambient temperature, in a manner to ensure recovery of anaerobic organisms. For virology cultures, do not allow the tissue to dry and transport in viral transport media (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, Parasite and Viral Cultures**

1. Instructions for female patients to collect midstream urine for bacterial culture:
   1. Remove undergarments.
   2. Wash hands thoroughly with soap and water, rinse them, and dry them on a disposable paper towel or shake off excess water.
   3. Spread labia, with one hand, and keep them continuously apart.
   4. Open the WASH PACK and wash the urinary opening and the surrounding area. Discard the cloth in the waste basket.
   5. Take the open sterile cup in the other hand without touching the rim or inner surface of the cup or lid.
   6. Void 20 to 25 ml into the toilet and catch a portion of the rest of the urine in the container without stopping the stream. Do not touch the legs, vulva, or clothing with the cup.
   7. Place the lid securely on the cup.
   8. Immediately transfer to the urine bacterial culture transport media.
   9. Low volume urine (less than 3 mL) send in sterile cup.

2. Instructions for male patients to collect midstream urine for bacterial culture:
   1. Wash hands.
   2. Retract the foreskin completely.
   3. Wipe head of penis in a single motion with first towelette. Repeat with second towelette. If not circumcised, hold foreskin back before cleansing.
4. Void 20 to 25 ml into the toilet and catch a portion of the remaining urine in the cup without stopping the stream. Do not touch the cup with the penis.
5. Place the lid on the cup securely.
6. Immediately transfer to the urine bacterial culture transport media.
7. 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).
   1. Patient must not have urinated during the previous two hours.
   2. Collect the first 10 to 30 ml of the urine stream in a clean, empty plastic cup.
   3. Transfer 2 ml of urine in test-specific transport media.

4. Suprapubic aspiration:
   1. This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in the literature.
   2. Faculty approval required for anaerobic culture, call 5-5077. Specimen should be submitted in an anaerobic environment if an anaerobic culture is approved.

5. Indwelling catheter urine:
   1. Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
   2. Clean the catheter with an alcohol pad.
   3. Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
   4. Transfer the urine to a sterile specimen container or appropriate transport media.
   5. Urine catheter tip cultures are not acceptable.

6. Specimen handling:
   1. Label the container immediately.

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

Viral Transport Media (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.

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