



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Overview

The proper collection of a specimen 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/detecting the causative organism(s) and/or result in the recovery of contaminating organisms.

Basic Concepts for Specimen Collection

1. Collect the specimen from the actual site of infection, avoiding contamination from adjacent tissues or secretions.
2. Collect the specimen at optimal times (for example, early morning sputum for AFB culture).
3. Collect a sufficient quantity of material. Use appropriate collection devices: sterile, leak-proof specimen containers. Use appropriate transport media (anaerobe transport vials, eSwabs for bacterial culture, Cary-Blair for bacterial stool nucleic acid testing (NAT) testing, (viral transport media (VTM) for viral and *Chlamydia* testing, and urine boric acid transport for bacterial urine cultures). Check expiration date before inoculating collection device. For more information, see [Specimen Collection Containers](#) link or the Epic Procedure Catalogue, which has collection device pictures and SAP numbers for ordering.
4. Whenever possible, collect specimens prior to administration of antimicrobial agents.
5. Properly label the specimen in the presence of the patient (a minimum of two patient identifiers are required) and order appropriately in EPIC (Refer to HPO Policy SPEC001: "Laboratory Specimens, Patient Identification and Specimen Labeling").
 - The specific specimen type is required in EPIC. Example: Specimen type-Wound.
 - To document completely, add a specimen source as well. Example: Specimen source-Left Foot.
6. Send the specimen to the laboratory as soon as possible after collection.
7. If appropriate, decontaminate the skin surface as per hospital guidelines. Allow a contact time of two minutes to maximize the antiseptic effect.
8. For orders with more than one test, ensure that the proper transport is utilized. For example, anaerobic culture requests need to be submitted in anaerobic transport media; bacteriology requests should **not** be in viral media; Mycobacteriology requests should **not** be in anaerobic transport media and swabs will not be accepted.
9. Table 1 contains a summary of specimen rejection criteria.

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



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

Table 1: Microbiology Specimen Rejection Criteria

Specimen	Comment
Sputum specimen with > 25 squamous epithelial cells per low powered field	Most likely saliva; submit a new sample
Induced sputum submitted for routine bacterial culture	Diluted specimen unlikely to provide clinically meaningful results
Swabs for Mycobacterial Culture	Submit tissue or aspirate
Specimen in anaerobe transport for Mycobacterial cultures	
Unpreserved urine > 24 hours old	
Urine catheter tip	
Specimen received in formalin	
Specimens submitted in ThinPrep for <i>Chlamydia trachomatis</i> and/or <i>Neisseria gonorrhoeae</i>	
Central venous catheter tip without concomitant blood culture within 24 hours	
Nasopharyngeal (NP) swabs for bacterial culture	May not represent infection in the lower respiratory tract
Formed stool for <i>C. difficile</i> and any enteric pathogen testing	Patient unlikely to have clinical disease; positive results may reflect colonization
Bacterial stool panel testing for inpatients hospitalized more than 3 days	
Repeat stool testing within 7 days for routine enteric testing	
Stool for fecal lactoferrin, <i>H. pylori</i> antigen testing, or protozoan pathogen panel submitted in Cary-Blair transport medium	
Mislabeled/unlabeled specimen	Patient safety issue
Specimens that have leaked in transit	Potential specimen contamination
Duplicate test requests	Wasteful practices



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Contents: By Specimen Type

Abscess	3
Amniocentesis	4
Arthropods	4
Blood.....	4
Body Fluids, Sterile (except urine and cerebrospinal fluid)	6
Bone Marrow	7
<i>Bordetella pertussis</i>	7
Bullae, Cellulitis, Vesicles	8
Cerebrospinal Fluid.....	9
Cutaneous (Fungal only)	10
Ear	10
Eye.....	11
Gastric Biopsy	11
Genital Sources	12
Molecular Testing	16
Nares Surveillance.....	19
Prostate	20
Rectal Swabs (Surveillance Cultures Only).....	20
Respiratory Specimens	21
Stool, Feces.....	25
Throat	28
Tissue	29
Urine for Bacterial, Fungal, AFB, Parasite and Viral Cultures	30
Viral Transport Media (VTM)	32
Worm ID	33
Wounds.....	34

Abscess

1. Collect purulent material aseptically:
 - From an undrained abscess: use a sterile needle and syringe after appropriate surface decontamination.
 - For large abscesses: open with a sterile scalpel and collect the expressed material with a sterile syringe.
2. Transfer 5-10 ml of the aspirated material to the appropriate transport container based on the test being requested. See [Test Directory](#) for specific guidelines for each test.

Note: Anaerobic transport media is not recommended for Mycobacterial culture. If requesting Mycobacterial culture, transfer at least 1 ml of the aspirated material into a sterile container.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

3. Transport immediately.
4. Note requests to rule out *Actinomyces* sp., *Cutibacterium* (formerly *Propionibacterium*) *acnes* sp. or *Nocardia* sp. in the Epic order or on the requisition.

Amniocentesis

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

Arthropods

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

Blood

A. Blood Culture

Determine the type of culture bottles to utilize, as indicated per physician's order (aerobic and anaerobic or resin bottles and anaerobic bottles), or other types as specified below. Please refer to the **JHH Interdisciplinary Clinical Practice Manual (ICPM): Patient Care - Blood Cultures: Ordering, Procurement and Transport Policy.**

1. Adult Blood cultures:

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

If **absolutely necessary** to draw from a central catheter site, utilize the site that has been most recently inserted (unless ruling out catheter sepsis). Follow the procedure as outlined in the JHH ICPM Patient Care policy Blood Cultures: Ordering, Procurement and Transport



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

- Send second set of blood cultures using the same procedure as above. If a different peripheral site is possible, the second set may be drawn immediately. If using the same site, wait at least 10 minutes for the second set, and if possible (i.e. not waiting to give antibiotics) draw a third set 1-3 hours later.
- For complete procedures for blood culture collection see the ICPM policy (PAT063) Blood cultures: ordering, procurement and transport.

2. Pediatric Blood cultures:

For pediatric patients, 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.

3. Mycobacterial blood cultures (AFB)

- Use a Mycobacterial blood culture bottle (BD BACTEC Myco/F Lytic culture bottle). Because the media is unstable, bottles must be obtained from the Microbiology Lab (5-6510 option #1). Pneumatic tube can be used to obtain culture media. When returning the sample to the lab, make sure it is returned in the brown paper bag it is sent in to avoid exposure to light. See Appendix D of the ICPM policy (PAT063) Blood cultures: ordering, procurement and transport.

4. Fungal Cultures

- **Candida spp.** - If a physician orders fungal cultures, follow routine procedure for collection of bacterial cultures as described above.
- **Cryptococcus, Histoplasma, or other filamentous organisms**--obtain a Myco/F lytic culture bottle from the Microbiology Lab (call 5-6510 option #1).
 - **Adult and Pediatric Patients:**
 - Inoculate Myco/F lytic culture bottle. Because the media is unstable, bottles must be obtained from the Microbiology Lab (5-6510 option #1). Pneumatic tube can be used to obtain bottle. When returning the sample to the lab, make sure it is returned in the brown paper bag it is sent in to avoid exposure to light. See Appendix D of the ICPM Policy (PAT063) Blood cultures: ordering, procurements, and transport.

5. When rare organisms such as *Brucella*, *Campylobacter* or *Bartonella* are suspected:

- An ID physician shall be consulted.
- The Microbiology laboratory shall be consulted to advise which type of specimen is most likely to support the suspected organism.

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

1. Draw 3 ml of blood in a lavender top (EDTA) vacutainer tube using the standard venipuncture procedure.



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

2. Deliver the tube to the Microbiology lab immediately or within 2 hours of collection.
3. Indicate patient's travel history (if available) and suspected pathogen (i.e., rule-out *Loa loa* filariasis).

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

C. *Aspergillus galactomannan antigen*

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

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

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

Body Fluids, Sterile (except urine and cerebrospinal fluid)

1. Prepare the skin as for Blood culture.
2. Collect the fluid using a sterile needle and syringe and place in transport container based on test being requested:
 - For aerobic organisms submit 10 ml in a sterile container (30 mL for pleural fluid).
 - For aerobic and anaerobic organisms, submit 10 ml in anaerobe transport (30 mL for pleural fluid).
 - If mycobacterial or fungal infections are suspected, collect a minimum of **5 ml** of fluid into a sterile container.
 - If testing for multiple labs, add up the volume needed, based on the above volumes, collect in a sterile container and deliver to the lab within 1 hour.
3. Transport immediately.

******Do not send Sterile Body Fluids on swabs.**



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Bone Marrow

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



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

tube for swab transport containing black medium into which the swab should be placed once the specimen has been collected.

- ONE 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 Universal Transport Medium (UTM) and a flocked swab.
 - 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 10 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 flocked swab from the package and repeat collection steps above after inserting the swab into the alternate nares. Place the swab into the UTM. Label this tube with the patient's name and identification number. Leave swab in the UTM.

Bullae, Cellulitis, Vesicles

1. Cleanse the skin as for Blood culture.
2. Aspirate the fluid/purulent material using a sterile needle and syringe.
 - If an aspirate is obtained, place in appropriate viral or bacterial transport tube or vial.
 - If no material is obtained, unroof vesicle or bullous lesion and use a flocked swab to collect cells from the base of the lesion (do not use sharps to unroof). Place in appropriate viral or bacterial transport media.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

A. Cellulitis

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

B. Vesicle Fluids and Scrapings for Viral Testing

Select a fresh vesicle, wipe gently with alcohol, dry thoroughly with sterile gauze. Using a tuberculin syringe with a small needle (26 g x 1/2 inch) aspirate vesicular fluid; transfer the fluid to the viral transport medium by filling the remainder of the syringe with the medium, then flush the solution into the transport tube or by swabbing the vesicle and breaking the swab off into a tube of viral transport media. **USE STERILE TECHNIQUE AT ALL TIMES.**

Cerebrospinal Fluid

1. Physicians should wear gowns, masks, and gloves to collect the specimen. Because an open tube is held to collect the fluid, other personnel should stand away or wear masks in order to avoid respiratory contamination.
2. Decontaminate the skin with 1-2% tincture of iodine, followed by 70-90% alcohol using an increasingly outward circular movement.
3. Drape sterile linen over the skin surrounding the puncture site.
4. Insert the needle. Collect the fluid into three sterile leak-proof tubes. Collect an adequate volume of fluid as recommended below:
 - bacterial culture 1-5 ml
 - fungal culture 5-10 ml
 - molecular 1.5-2 ml
 - mycobacterial culture 5-10 ml
5. Cap the tubes tightly. Submit the third tube for culture to reduce the possibility of contamination due to skin-microbiota. Transport immediately at ambient temperature.

A. CSF for cryptococcal antigen testing

1. Collect at least 300 μ l aseptically following accepted procedures.

B. CSF for beta-D-glucan testing

1. Collect at least 300 μ l aseptically following accepted procedures.

C. CSF for galactomannan testing

1. Collect at least 400 μ l aseptically following accepted procedures.



Johns Hopkins Medical Microbiology

Specimen Collection Guidelines – Updated 11/2024

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

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

Cutaneous (Fungal only)

A. Hair

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

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

B. Nails

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

C. Skin

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

Ear

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



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

EYE

1. Cleanse the skin around the eye with a mild antiseptic.
2. Purulent conjunctivitis: Collect purulent material with an eSwab (green cap-mini tip).
 - Place the swab into the eSwab transport media and transport at ambient temperature.
 - This is **NOT** an acceptable specimen for anaerobe culture.
 - Nucleic acid testing – *Chlamydia* only: collect specimen with rayon or Dacron swab and place in 3 ml of viral transport media. **Do not use Cobas Collection Kit.**
3. Corneal infections:
 - Obtain Cornea Pack from the Microbiology Laboratory, Meyer B-171, 5-6510 option #1.
 - Collect multiple corneal scrapings and inoculate directly onto bacterial agar media (chocolate agar, sheep blood agar, and Schaedler's broth), mycology media (inhibitory mold agar with gentamicin), and/or viral transport media.
 - Transport at ambient temperature.
 - Gram stain is not routinely performed.
4. Intraocular fluid:
 - Collect fluid by surgical needle aspiration.
 - Transport cultures at ambient temperature.

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

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

Contact lens and corneal scraping or corneal tissue

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

Contact lens solution

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

Gastric Biopsy

Appropriate for *Helicobacter pylori* culture only. Contact the Microbiology Laboratory at (410)-955-6510 for appropriate transport media. Must be transported to the Core Laboratory within one hour of collection. This is sent to a reference laboratory for culture and antimicrobial



Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024
susceptibility testing (if recovered).

Genital Sources

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

For sexually transmitted diseases testing, refer to Chlamydia/Gonorrhea.

A. Bartholin's Glands

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

B. Cervix (Endocervix) for Culture

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



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

C. Cervix (Endocervix) for HPV DNA Testing

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

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

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

• *Chlamydia trachomatis*/*Neisseria gonorrhoeae*/*Trichomonas vaginalis*/*Mycoplasma genitalium* 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**



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

1. **Carbomer-containing products/lubricants should not be used, as they interfere with test results.**
2. Wipe exocervix with the white-stemmed sterile swab, removing the excess mucus. **Discard this swab.**
3. Insert the **flocked swab** into the endocervical canal. Rotate 10-30 seconds and withdraw.
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

➤ **Female vaginal**

1. Obtain collection pack from Consolidated Service Center (CSC), SAP number 233352.
2. Insert the **woven swab** about 2 inches past the introitus and gently rotate the swab for 10-30 seconds. Ensure the swab touches the walls of the vagina.
3. Carefully withdraw the swab without touching the skin.
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.
6. Transport the specimen to the lab as soon as possible.

➤ **Pharyngeal (Male/Female)**

1. **Use the woven swab for collection.**
2. Swab area between the tonsillar pillars and the region posterior to the pillars.
3. 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.**
4. Close the transport tube securely, label and date.
5. 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 woven swab for collection.**
4. Place swab sample into collection tube provided in the Cobas Dual Swab collection kit and break off the swab at the scored line. **Swab must remain in the transport tube.**
5. Close the transport tube securely, label and date.



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

6. Transport the specimen to the lab as soon as possible.

E. Gonorrhea Culture

- Collect using ESwab (regular or mini-tip). Must transport to the laboratory in <24 hours.

➤ **Endometrium**

- Place the patient in the lithotomy position.
- Insert speculum and visualize the cervical os.
- Place a narrow-lumen catheter within the cervical os.
- Insert the tip of an Eswab through the catheter and collect the endometrial specimen. This method prevents touching the cervical mucosa and reduces the chance for contamination.
- Place the Eswab into its container and transport at ambient temperature to the laboratory in <24 hours.

➤ **Urethra**

- Instruct patient not to urinate at least 2 hours prior to sampling.
- Insert the Eswab 2-to 4 cm into the urethra. Rotate 3 to 5 sec and withdraw.
- Place the Eswab into its container and transport at ambient temperature to the laboratory in <24 hours.

➤ **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* table. If yeast infection is suspected, a fungal culture should be ordered rather than a routine culture.

- **Group B *Streptococcus* screening:** collect combined vaginal and rectal swab using a culturette. Testing is performed using Lim broth enrichment followed by a nucleic acid testing for detection.
- **Vaginosis/Vaginitis:** Collect vaginal swab specimens from **symptomatic** women prior to the speculum examination. **Note:** No lubricant should be used for the sample technique. If lubricant must be used for speculum insertion, the lubricant should be used sparingly and applied only to the exterior sides of the speculum blades, avoiding contact with the tip of the speculum.
 - Use the BD Molecular Swab Collection Kit (available from the CSC: SAP # 280684) and follow the instructions. Hold the collection swab by the cap and insert it into the vaginal opening about 2 inches (5 cm) gently rotating the swab for 10-15 sec. Make



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

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

Molecular Testing

Virus	Sources	Specimen
Qualitative NAT		
Adenovirus	<ul style="list-style-type: none"> ▪ Conjunctiva ▪ Urine ▪ Respiratory (Bronchoalveolar lavage (BAL), NP swab ▪ Stool (included in Enteric Viral Pathogen Panel) 	<ul style="list-style-type: none"> ▪ Swab in 2.0 mL viral transport media (VTM or UVTM) ▪ Sterile container ▪ Sterile container or mini flocced swab in viral transport for NP swab. ▪ Cary-Blair transport media
CMV	<ul style="list-style-type: none"> ▪ CSF ▪ Amniotic fluid, urine ▪ Sterile Eye ▪ Saliva (neonates <21d) 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3 ▪ Sterile container ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM) ▪ Flocced swab of neonate saliva in viral transport media (VTM or UVTM).
EBV	<ul style="list-style-type: none"> ▪ CSF 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3
Enterovirus	<ul style="list-style-type: none"> ▪ CSF ▪ Plasma 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3 ▪ Lavender top (EDTA) tube or pearl top (PPT/EDTA) tube ▪ Viral transport media (VTM or UVTM) ▪ Dacron or flocced swab in viral transport media (VTM or UVTM)
HSV 1+2	<ul style="list-style-type: none"> ▪ CSF ▪ Sterile eye ▪ Lesion ▪ BAL ▪ Plasma 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3 ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM) ▪ Dacron or flocced swab in viral transport media (VTM or UVTM) ▪ Sterile container ▪ Lavender top (EDTA) tube or pearl top (PPT/EDTA) tube



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

	<ul style="list-style-type: none"> ▪ Vesicle fluid 	<ul style="list-style-type: none"> ▪ Viral transport media (VTM or UVTM)
JCV	<ul style="list-style-type: none"> ▪ CSF 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3
VZV	<ul style="list-style-type: none"> ▪ CSF ▪ Sterile eye ▪ Lesion ▪ Vesicle fluid ▪ Plasma 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3 ▪ 0.5 mL Vitreous/Aqueous humor in sterile tube/syringe; corneal scrapings in viral transport media (VTM or UVTM) ▪ Dacron or flocked swab in viral transport media (VTM or UVTM) ▪ Viral transport media (VTM or UVTM) ▪ Lavender top (EDTA) tube or pearl top (PPT/EDTA) tube
Parechovirus	<ul style="list-style-type: none"> ▪ CSF 	<ul style="list-style-type: none"> ▪ CSF collection container, tube #3
Orthopoxvirus	<ul style="list-style-type: none"> ▪ Lesion 	<ul style="list-style-type: none"> ▪ Dacron or flocked swab in viral transport media (VTM or UVTM)
Respiratory Pathogen Panel: Flu A/B, RSV, Para flu 1/2/3/4, Rhino/Enterovirus, Metapneumo, Adenovirus, <i>Mycoplasma</i> , Endemic Coronaviruses SARS-CoV-2	<ul style="list-style-type: none"> ▪ BAL ▪ NP swab 	<ul style="list-style-type: none"> ▪ Sterile container ▪ Mini flocked swab in viral transport
SARS-CoV-2	<ul style="list-style-type: none"> ▪ NP swab ▪ Bilateral Nasal Mid-Turbinate (NMT) swab ▪ BAL 	<ul style="list-style-type: none"> ▪ Mini flocked swab in viral transport for NP collection ▪ Regular flocked swab in viral transport for NMT collection ▪ Sterile container



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

HPV High Risk + genotyping	<ul style="list-style-type: none">▪ Cervix▪ Anal▪ Vaginal	<ul style="list-style-type: none">▪ Original ThinPrep vial
-----------------------------------	---	--



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

Quantitative NAT		
Adenovirus	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Lavender top EDTA tube or Pearl top PPT tube.
BK virus	<ul style="list-style-type: none"> ▪ Plasma ▪ Urine 	<ul style="list-style-type: none"> ▪ Pearl top tube (PPT) ▪ Sterile container
CMV	<ul style="list-style-type: none"> ▪ Plasma ▪ BAL 	<ul style="list-style-type: none"> ▪ Pearl top tube (PPT) ▪ Sterile container
EBV	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Lavender top EDTA tube or Pearl top PPT tube.
HBV	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Pearl top tube (PPT)
HCV	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Pearl top tube (PPT)
HIV	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Pink top tube (EDTA)
HHV-6	<ul style="list-style-type: none"> ▪ Plasma ▪ CSF 	<ul style="list-style-type: none"> ▪ Pearl top tube (PPT) ▪ CSF collection container, tube #3
Send out Tests (Qualitative NAT Viruses)		
Parvovirus B19	<ul style="list-style-type: none"> ▪ Bone marrow, Amniotic fluid, Plasma 	<ul style="list-style-type: none"> ▪ Send to Customer Service for testing by Quest Diagnostics
VZV	<ul style="list-style-type: none"> ▪ BAL 	<ul style="list-style-type: none"> ▪ Send to Customer Service for testing by Quest Diagnostics
JCV	<ul style="list-style-type: none"> ▪ Plasma 	<ul style="list-style-type: none"> ▪ Send to Customer Service for testing by Quest Diagnostics
HIV genotyping	<ul style="list-style-type: none"> ▪ Whole blood 	<ul style="list-style-type: none"> ▪ Send to Customer Service for testing by Quest Diagnostics
SARS-CoV-2	<ul style="list-style-type: none"> ▪ Endo/Trach Suction ▪ Sputum 	<ul style="list-style-type: none"> ▪ Send to Customer Service for testing by Quest Diagnostics

Nares Surveillance

Instructions for Proper Nares Cultures Technique:

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

- Grasp the swab cap with fingers. Be careful to avoid contacting the swab or stick with your fingers.
- Withdraw the swab; sweep around the interior surface of the anterior nares.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

- Perform on both nares with one swab.
- Carefully place swab in collection container and snap off shaft of swab. Make sure the cap is securely fastened.
- Label the tube with the patient's name, specimen or specimen barcode (nares culture) and date.
- Send to microbiology laboratory.
- Nares swabs are only acceptable for MSSA/MRSA surveillance, not routine culture.

A. Pre-op MRSA/MSSA Nasal Screen:

1. Collect anterior nares culture with an eSwab
2. Transport at ambient temperature.

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

Prostate

1. Cleanse the glans with soap and water.
2. Obtain prostate fluid by digital massage through the rectum.
3. Collect fluid using a sterile swab.
4. Transport at room temperature.
5. Alternatively, a urine specimen obtained immediately before and after massage may be submitted for culture. If this is done, please indicate "pre" or "post" massage when ordering the urine culture.
 - Specimen type: Urine, other
 - Specimen source:
 - Pre-Prostatic Massage
 - Post-Prostatic Massage

Rectal Swab (Surveillance Cultures Only)

Vancomycin-resistant enterococci (VRE), multidrug-resistant gram-negative bacilli and carbapenem-resistant gram-negative organism surveillance cultures.

1. Collect rectal swab with a white-cap E-swab
 - Remove swab from package and collect sample
 - Insert swab 1-1.5 inches into anus
 - Gently rotate swab 10 seconds
 - Remove screw cap from tube and set aside
 - Insert swab at the bottom of the tube
 - Break the applicator shaft at the breakpoint



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

- Screw cap tightly to prevent leakage
- Apply patient label to tube

Respiratory

Bronchial Brush/Wash/Lavage

1. This technique should be performed by an experienced individual. See HPO procedure: Bronchoscopy and Associated Procedures (ENDO628).
 - Transport in a sterile container immediately at ambient temperature
(NOTE: RULE OUT TB SAMPLES SHOULD BE HAND CARRIED TO THE MICROBIOLOGY LABORATORY. THESE SAMPLES SHOULD NEVER BE SENT THROUGH THE PNEUMATIC TUBE SYSTEM.)
 - **Immunocompromised host protocol:** 40 mL of BAL fluid is required
 - ***Aspergillus galactomannan antigen*** (Bronchial lavage): Please send 1-2mL for galactomannan testing (minimum 1mL).
 - ***Pneumocystis jirovecii (PJP), PCR***
 - Submit bronchial washings and bronchoalveolar lavage for PJP testing in a sterile container. PCR testing is performed once daily, 7 days per week. Samples must be received by 7:00 AM for same day testing to occur. Minimum volume required for testing is 1 mL.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Endonasotracheal Aspirates

1. Follow instructions in the JHH Respiratory Care Manual in HPO for specimen collection.
2. Transport as soon as possible to the laboratory. Refrigerate samples if transport is delayed by more than 2 h.
3. Rejection criteria are applied in a fashion similar to expectorated sputum (see below).

Nasopharyngeal

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

B. Nasopharyngeal swabs for *Corynebacterium diphtheriae* culture:

- Collect one nasopharyngeal swab from the inflamed areas in the nasopharynx.
- If membranes are present and can be removed, a swab from beneath the membrane should also be collected.
- Collection from several areas can increase sensitivity.
- Swabs can be cotton- or polyester-tipped. Standard ESwabs are also acceptable. Routine swab collection systems, such as Amies or Stuart medium, are acceptable. Dry swabs are not acceptable.

C. Viral Respiratory Testing – Flocked Swab

- 1 Copan® brand flexible **flocked sterile swab applicator**
 - 1 **Viral Transport Medium** tube
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 then a secondary bag (double bag specimen) and send to lab via the pneumatic tube.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

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

For bilateral nasal mid-turbinate (NMT) collection, click here:
<https://www.youtube.com/watch?v=DSrWjVyxEeg>

Paranasal Sinuses

1. Tissues or aspirates are the most desirable sample type.
 - a. Submit for aerobic/anaerobic culture not CSF, urine or blood.
 - b. If transport time exceeds two hours, place in anaerobic transport media.
2. If swabs are obtained, use an Eswab with mini tip and insert the swab into the ostia during the endoscopic procedure if drainage is visible.
 - a. Submit for aerobic/anaerobic culture not CSF, urine or blood.
 - b. Submit to the laboratory within 24 h.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Sputum, Expecterated

1. Assure patient cooperation to get an adequate specimen.
2. Instruct the patient as follows:
 - Rinse mouth with tap water to remove food particles and debris.
 - Have patient breathe deeply and cough several times to achieve a deep specimen.
 - Patient should expectorate into dry, sterile container.
 - Patients suspected of having tuberculosis should expectorate sputum in the early morning, into a sterile container with lid sealed tightly. Leaking specimens may be cancelled.
3. Transport immediately at ambient temperature. Refrigerate if a delay of more than two hours is anticipated.
4. Expecterated sputum is acceptable for bacterial, mycobacterial, and fungal cultures. It is 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. In patients with clinical and chest x-ray findings compatible with tuberculosis, collect 3 first morning sputum specimens (on 3 separate days) for AFB culture.

*****NOTE: SAMPLES OBTAINED FROM PATIENTS WITH SUSPECTED TUBERCULOSIS SHOULD BE HAND CARRIED TO THE MICROBIOLOGY LABORATORY. THESE SAMPLES SHOULD NEVER BE SENT THROUGH THE PNEUMATIC TUBE SYSTEM*****



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

Induced Sputum

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

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

Submit induced sputum for PJP testing in a sterile container on ice. Induced sputum specimens will only be accepted on weekdays (M-F) and must be received by 12 PM for same day testing to occur. **No** induced sputum specimens will be tested on weekends or holidays. Testing is performed once daily on weekdays. Samples received after 12 PM on Fridays will be tested the following Monday.

Stool, Feces

1. Collect specimen in a clean bedpan or use plastic wrap placed between the toilet seat and the bowl. Do not submit feces contaminated with urine or toilet water.

- **Unpreserved stool in a sterile container:**

- *C. difficile*
- Fecal lactoferrin testing
- *H. pylori* stool antigen testing

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

- **Cary-Blair transport medium:**

- Bacterial pathogens (not including *C. difficile*): *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., Enterotoxigenic *E. coli*, *Yersinia enterocolitica*, *Vibrio* sp., Shiga-toxin producing *E. coli*, *Plesiomonas shigelloides*, *Aeromonas* sp.
- Viral Pathogens: Adenovirus F40/41, Human Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus genotype I, II, IV, and V.

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



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

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

Transfer specimen into a Total Fix transport medium container. Transport at ambient temperature within two hours of collection.

Notes:

- Stool samples collected on patients hospitalized longer than 3 days prior to collection are not acceptable for routine enteric testing.
- Repeat testing within the same diarrheal episode or within 7 days of a previous result are not acceptable for bacterial enteric testing.
- Only loose or diarrheal stools are recommended for routine bacterial and *C. difficile* cultures and PCR. There is a limit of one sample per week for *C. difficile* testing. Minimum testing volume is 1 mL.
- Place the specimen in an appropriate stool preservative or transport media, immediately after collection.
- If a stool specimen is not available, the following are suitable alternatives for testing:
 1. A swab of rectal mucus, or
 2. A rectal swab inserted one inch into the anal canal (not acceptable for Rotavirus/Adenovirus EIA or *C difficile* testing).
- For CMV colitis, culture of biopsy tissue is preferred. Stool is frequently toxic to cultured cells and virus is infrequently recovered from this source.

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

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

Optimally, 3 fecal specimens collected over a 7 to 10 day period must be submitted in a Total-Fix container as intestinal parasites are shed intermittently. It is not acceptable to send more than one specimen collected on any given day.

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

B. Microsporidia Stain

Testing is performed once per day (M-F). 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.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

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

C. Pinworm Exam, Perianal

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

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

Pinworm paddle kit (a paddle coated with adhesive material)

1. These can be obtained from the Core laboratory.
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.
7. This is a send-out test.

D. Stool Enteric Protozoan Panel:

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

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.

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

1. Stool in Total-Fix preservation system is preferred. Unpreserved stool specimens and Para Pak (10% Formalin (Pink Top) are acceptable.
2. Fill the Total-Fix or Para Pak 10% Formalin vial to the fill line. Please 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.

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



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

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

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

F. Stool Ova and Parasite Examination:

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

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

1. Stool in Total-Fix preservation system is required. Unpreserved stool is unacceptable.
2. Fill the vials to the fill line. Please DO NOT overfill.
3. Keep at room temperature.
4. Indicate patient's travel history and if the patient is immunocompromised or not (if applicable).
5. Testing is performed at Quest Diagnostics.

Throat

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



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

2. Use a tongue blade and a good light source to ensure good visualization.
3. Reach behind the uvula and swab:
 1. both tonsillar fauces, and
 2. the posterior pharynx, and
 3. any ulceration, exudate, lesion, or area of inflammation.
4. Place the swab into the eSwab collection tube provided and transport at ambient temperature.

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

Tissue

1. Tissue collection is an invasive procedure and requires surgery by a trained physician.
2. Collect tissue aseptically. Include material from both the center and the edge of the lesion.
3. Place the specimen in a sterile container on sterile gauze moistened with sterile **non-bacteriostatic** saline.
4. Transport in less than an hour at ambient temperature, in a manner to ensure recovery of anaerobic organisms. For larger tissues, transport within 2-3 hours is acceptable. 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.**
7. If culture for anaerobic bacteria and *Mycobacterium* are required, please divide the tissue specimen and place some tissue in an anaerobe transport tube and another piece in a sterile container. Alternatively, if the tissue cannot be divided, transport in a sterile container within 1 h of collection.



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

Urine for Bacterial, Fungal, AFB and Parasitology

- 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.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

- d. Open the WASH PACK and wash the urinary opening and the surrounding area. Discard the cloth in the waste basket.
- e. Take the open sterile cup in the other hand without touching the rim or inner surface of the cup or lid.
- f. Void 20 to 25 ml into the toilet and catch a portion of the rest of the urine in the container without stopping the stream. Do not touch the legs, vulva, or clothing with the cup.
- g. Place the lid securely on the cup.
- h. Immediately transfer to the urine bacterial culture transport media (gray top tubes).
- i. Low volume urine (less than 3 mL) send in sterile cup.

2. Instructions for male patients to collect midstream urine for bacterial culture:

- a. Wash hands.
- b. Retract the foreskin completely.
- c. Wipe head of penis in a single motion with first towelette. Repeat with second towelette. If not circumcised, hold foreskin back before cleansing.
- d. Void 20 to 25 ml into the toilet and catch a portion of the remaining urine in the cup without stopping the stream. Do not touch the cup with the penis.
- e. Place the lid on the cup securely.
- f. Immediately transfer to the urine bacterial culture transport media.
- g. Low volume urine (less than 3 mL) send in sterile cup.

3. First void urine for nucleic acid amplification tests – males and females (Chlamydia/ Gonorrhea).

- a. Patient must not have urinated during the previous two hours.
- b. Collect the **first 10 to 30 ml** of the urine stream in a clean, empty plastic cup.
- c. Transfer 2 ml of urine in test-specific transport media.

4. Suprapubic aspiration:

- a. This is not a routine technique and is best performed by an experienced individual. Descriptions of the method are readily available in the literature.
- b. Faculty approval required for anaerobic culture, call 5-5077. Specimen should be sent to the laboratory within 2 h of collection.

5. Indwelling catheter urine:

- a. Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
- b. Clean the catheter with an alcohol pad.
- c. Use BD urine vacutainer luer-lock access device. Aspirate the urine into the grey top vacutainer tube with boric acid.
- d. Urine catheter tip cultures are not acceptable.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

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

6. Transplant patient bacterial urine culture:

- a. Follow instructions above for collection.
- b. When ordering in EPIC choose the following:
 - Specimen Type: Urine, Transplant

7. Parasitic examination: Collect in a sterile container without preservatives. The time of collection is dependent on the suspect pathogen:

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

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

Notes:

- AFB culture: Minimum volume is 40 ml.

Viral Transport Media (VTM or UVTM)

Some samples can be submitted without utilizing a transport media with a reasonable expectation of virus viability. Specimens in this category include, sterile fluids such as cerebrospinal fluid, pleural fluid, blood, urine, as well as some nonsterile specimens such as bronchoalveolar lavage, and feces. Whenever there is a question of stability, the specimen should be placed in a suitable virus transport medium such as UVTM. Refer to specific test in the alphabetical test list of this User's Guide for more information.



Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 11/2024

- 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. This is a send out test.
- Abscess material, bullae, pustules, vesicles, lesions, and skin scrapings can be collected on a Dacron swab and placed directly into viral transport media. If the material has been aspirated, place no more than 3 ml (equal to the amount of transport media) in the vial of VTM.
- CSF should be submitted in a sterile container.
- Urine should be submitted in a sterile container.
- Rectal swab (Dacron only) should be submitted in VTM.
- Blood for viral culture should be submitted in a heparin tube. This is a send out test.
- Swabs that are made of calcium alginate and wood are known to interfere with the recovery of some viruses. These can also act as PCR inhibitors and are not appropriate for this type of testing.
- For CMV colitis, culture of biopsy tissue is preferred. This is a send out test.
- **Neonatal CMV - saliva:**
 - Obtain a BD kit: mini-tip flocced swab & viral transport media
 - Confirm that the baby was breastfed > 1 hour prior to collection of the sample.
 - Wash hands and put gloves on, then proceed to opening the sterile flocced swab.
 - Introduce the swab between the cheek and the gum in one side of the mouth. Allow to sit for 10-15 seconds.
 - Move the swab tip to the other side of the mouth for another 10-15 seconds. Make sure the swab appears moistened when removed.
 - Remove the swab from the mouth, place in Universal Viral Transport Medium collection tube, appropriately labeled, and transport to the lab. The sample is stable 48 hrs at room temperature or 7 days refrigerated.
 - Note about testing neonatal urine for CMV: Urine samples will have to be either transported at room temperature to the lab within 2 hours or transported refrigerated within 8 hours.

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. This test is sent out to a reference laboratory.



**Johns Hopkins Medical Microbiology
Specimen Collection Guidelines – Updated 11/2024**

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 a wound is the preferred specimen for anaerobes, mycobacteria and fungi.